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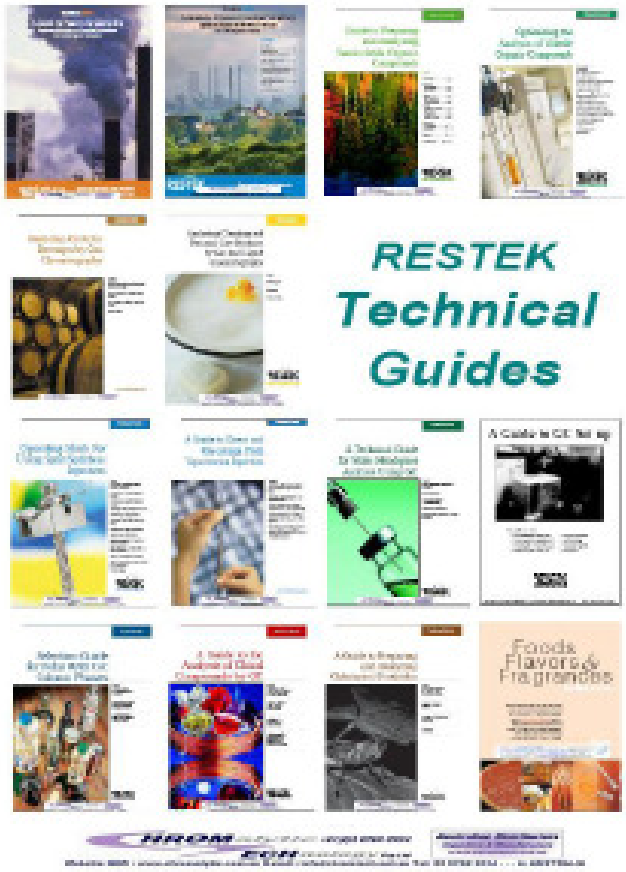

Applications Note

Improve Moisture Dry-Down and Corrosion Resistance

Restek treated tubing and system components offer improved analytical reliability and longer lifetimes.

Introduction

Gas transfer systems serving the semiconductor industry often require low moisture content and retention, and high resistance to corrosion. The current substrate of choice in semiconductor manufacturing, electropolished VIM/VAR (vacuum induction melt/vacuum arc melt) 316L stainless steel, frequently is insufficient in these capacities, increasing periodic maintenance, prolonging equilibration times, and allowing system contamination and inaccurate analytical results. In contrast, surface treatments available through the Restek Performance Coatings Group provide significant added value to conventional stainless steel substrates, by greatly accelerating wet-up and dry-down times and dramatically improving corrosion resistance.

Restek surface treatments deposit an amorphous silicon based layer onto, and into, the steel surface through a chemical vapor deposition (CVD) process in vacuum at 400°C. There are no line of sight limitations; all exposed surfaces are coated. Layer depth is well controlled and, for corrosion resistance, is optimized at 5 to 10 microns. The amorphous silicon layer can be further functionalized using the patented Siltek® process (US Patent #6,444,326), which has been optimized to reduce moisture hold-up and improve surface inertness.

Data for wet-up and dry-down experiments, measuring the relative response time for moisture content change in treated electropolished stainless steel tubing, untreated electropolished stainless steel tubing, and standard 316L stainless steel tubing, demonstrate a significant advantage in treated versus untreated substrates.[1] Tubing used in the wet-up / dry-down experiments was supplied by Cardinal UHP (St. Louis, MO). All tubing was tested as 100 foot coils of 1/4" OD x 0.020" wall 316L stainless steel. Electropolished tubing had a surface roughness of 10 to 15 microinches. Siltek® treated tubing (Restek Performance Coatings, Bellefonte, PA) is finished with 5µm of amorphous silicon, followed by a surface functionalization that increases inertness and hydrophobicity.

Wet-up curves for Siltek® treated electropolished, electropolished, and standard tubing are compared in Figure 1. Treated electropolished tubing reached the 98% saturation limit in 30 minutes, compared to 60 minutes for electropolished tubing. Standard tubing could only achieve a 96% uptake, after 180 minutes.

Figure 1 Restek treated electropolished tubing stabilizes at 1ppm moisture much faster than conventional surfaces.[1]

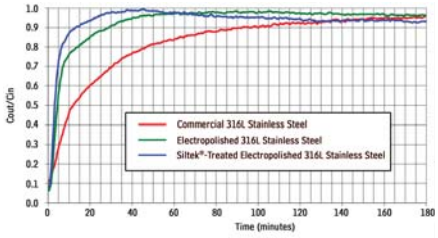

Figure 2 Restek treated electropolished tubing dries much faster than conventional surfaces.[1]

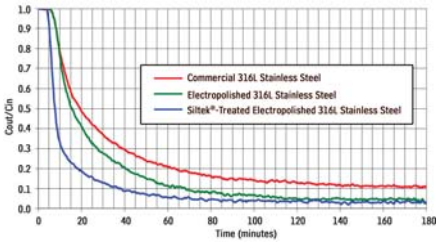

Table I Restek treated electropolished tubing provides the shortest drying times.[1]

Moisture Concentration		Time Required to Detect Change (min.)		
From	To	Treated Electropolished Tubing	Untreated Electropolished Tubing	Standard Tubing
10ppm*	5ppm	4	5	13
5ppm	1ppm	22	46	71
1ppm	500ppb	40	63	96
500ppb	100ppb	80	103	153
100ppb	50ppb	98	121	---

*Initial moisture concentration.

Economical solutions for ultra-high purity streams

Restek surface treatment extends the lifetimes of steel and steel alloy systems, while maintaining high dimensional tolerances, high temperature capability, and leak-free conditions.

- Up to three times faster wet-up or dry-down than electropolished or conventional surfaces.
- Corrosion resistance improved tenfold, or more—increases component lifetime and maintains pure product stream.
- Custom services: can be applied to existing equipment.

for **more** info

For more information about the anti-corrosive properties of Silcosteel®-CR treatment, request packet 59048, or visit us online.

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After the tubing was stabilized with 1ppm of moisture, dry-down properties were measured. Moisture dry-down curves for the three tubing treatments show treated electropolished tubing achieved dry-down in 35 minutes, electropolished tubing required 65 minutes, and standard tubing required 175 minutes (Figure 2). Table 1 compares time to various dry-down levels for tubing saturated with 10ppm of moisture.

In addition to rapid wet-up and dry-down, the other key advantage of Restek treatment for 316L stainless steel is a dramatic improvement in corrosion resistance. The amorphous silicon layer is insoluble in many of the acidic environments encountered in the semiconductor industry. Figures 3, 4, and 5 briefly summarize the results of corrosion testing by ASTM methods. Comparisons between treated and untreated test samples illustrate the improvements in corrosion resistance offered by Silcosteel®-CR treatment. For more information about corrosion resistance, request information packet 59048., or visit our website.

When moisture considerations and corrosion concerns arise in transfer of ultra-high purity gas streams, Restek treated tubing and system components will dramatically improve dry-down, reduce contamination from moisture carryover, and extend periodic maintenance cycles.

Figure 3 In chloride environments, Silcosteel®-CR treated stainless steel outperforms untreated metal by an order of magnitude (ASTM G 48, Method B).

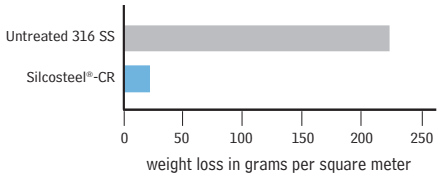
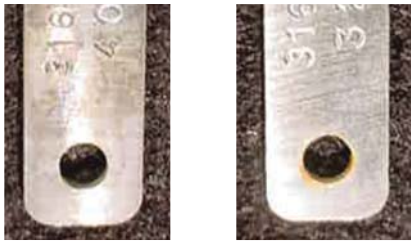


Figure 4 Silcosteel®-CR treated 316L stainless steel coupons show no sign of attack after 4000-hour salt spray exposure, per ASTM B117.



Silcosteel®-CR treated untreated

Reference
1. *Relative Response Time of True Tube™ when Measuring Moisture Content in a Sample Stream* Test Report, Haritec Scientific & Engineering Support, Calgary, Alberta, Canada, May 2004.
Reference courtesy of O'Brien Corporation, available on request from Restek.

Figure 5 Silcosteel®-CR treated 316L stainless steel coupons show no crevice corrosion and only slight pitting corrosion after 72-hour exposure to ferric chloride; untreated coupons exhibit severe crevice corrosion.

Silcosteel®-CR treated untreated

free literature

Learn more about our precisely applied, highly durable surface treatments: request our brochure lit. cat.# 59493.

Siltek®- and Silcosteel®-CR-Treated Electropolished Tubing

- Exceptional inertness.
- Improved reliability and reproducibility; longer lifetime.
- Use with treated fittings for the most inert sample pathway available.

Siltek®-Treated Electropolished Tubing

ID	OD	cat.#	Price-per-foot 5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085"	1/8"	22538				
0.180"	1/4"	22539				

Silcosteel®-CR-Treated Electropolished Tubing

ID	OD	cat.#	Price-per-foot 5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085"	1/8"	22536				
0.180"	1/4"	22537				

Coiled Siltek®- and Silcosteel®-CR-Treated Seamless 316L Grade Stainless Steel Tubing

Siltek®-Treated 316L Tubing

ID	OD	cat.#	Price-per-foot 5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22896				
0.180" (4.57mm)	1/4" (6.35mm)**	22897				

Silcosteel®-CR Treated 316L Tubing

ID	OD	cat.#	Price-per-foot 5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22508				
0.180" (4.57mm)	1/4" (6.35mm)**	22509				

*1/8" OD: 5 ft. to 100 ft. in one continuous coil; 1/4" OD: 5 ft. to 300 ft. in one continuous coil. Longer lengths will be more than one coil.
**0.035" wall thickness

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Applications Note

Sulfur Compound Sampling, Storage, and Transfer Considerations

More accurate results and faster cycle times, using Sulfinert® treated components

Introduction

Accurate analyses for parts-per-million to parts-per-billion levels of sulfur-containing compounds in petrochemical streams are critical to meeting new regulations for lower levels of sulfur in diesel fuel and gasoline. Many organo-sulfur compounds—hydrogen sulfide, methyl mercaptan, and ethyl mercaptan among them—adsorb strongly to metal surfaces. Adsorption of sulfur compounds in sampling, storage, and/or transfer apparatus can cause prolonged analysis cycle times as well as inaccurate, falsely low values.

In the studies described here, active sulfur gases were sampled, stored, and transferred in control (untreated) and Sulfinert® treated static (storage) and flow-through system components, to determine quantitative losses of the sulfur gas species. Concentrations of the sulfur gases spanned the low parts-per-million to low parts-per-billion range.

Sulfur Compound Storage: Sulfinert® Treated vs. Untreated Sample Cylinders

Figure 1a depicts results from a comparison in which a gas containing 17ppbv of hydrogen sulfide was stored for 7 days in untreated or in Sulfinert® treated stainless steel sample cylinders. The response ratio for hydrogen sulfide, relative to a stable reference material, dimethyl sulfide, is steady at approximately 1:1 for at least seven days in Sulfinert® treated cylinders. The data show a Sulfinert® treated system will reliably store ppb levels of the active sulfur-containing compound during transport from the sampling site to the analytical laboratory. In contrast, hydrogen sulfide degraded rapidly in the untreated cylinder, and was lost totally within 24 hours.

In a similar study in which gas containing 18.8ppbv methyl mercaptan was stored for 60 hours in Sulfinert® treated sample cylinders, recovery of the active sulfur compound was equally high relative to the stable reference material, dimethyl mercaptan, as shown in Figure 1b.

Sample Transfer: Adsorption of Sulfur Compounds to Tubing Surfaces

Comparison of the transport properties of Sulfinert® treated electropolished stainless steel tubing (TrueTube™ EPS tubing, surface roughness average (RA): 5-10, O'Brien Corporation, St. Louis, MO), untreated electropolished stainless steel tubing (TrueTube™ EP tubing, RA 5-10, O'Brien Corporation), and raw commercial grade stainless steel tubing (RA 23-27) show only Sulfinert® treated electropolished stainless steel has the inertness necessary for quantitatively transferring sulfur compounds at low ppmv to low ppbv concentrations in sample streams. An experiment was designed to confirm whether a sulfur-containing stream passing through stainless steel tubing would passivate active sites on the steel surface, through adsorption of the active sulfur species. The amount of time elapsed before a representative sample, containing a stable and accurate sulfur content, exited the tubing was the measured indicator of surface activity in the tubing.

Avoid downtime, save money

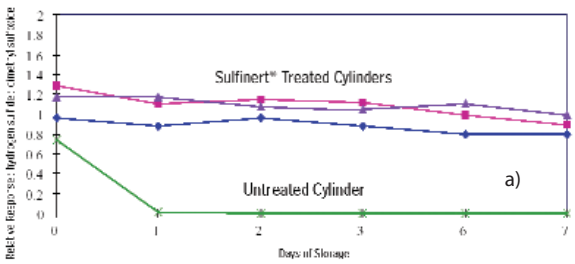
Sulfinert® treatment adds value to your process by ensuring:

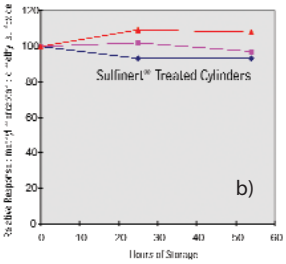
- Accurate results
- Improved yields
- Faster cycle times

Avoid these losses!
A 1-hour delay can cost:[1]

- 800,000 tpy ethylene plant: $50,000
- 250,000 tpy LDPE unit: $36,000
- 250,000 tpy EBSM styrene plant: $33,000
- 200,000 tpy anti-freeze process: $3,600

Figure 1 Sulfur compounds are stable in Sulfinert® treated stainless steel systems
a) 17ppbv hydrogen sulfide in 500mL cylinders
b) 18.8ppbv methyl mercaptan in 300mL cylinders





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Figures 2 and 3 compare the transport properties of 100-foot (30.5-meter) lengths of the three types of seamless 316L stainless steel tubing, 1/8" OD x 0.020" wall, using a gas stream containing 0.500ppmv methyl mercaptan in helium as the test material. Tests were performed at room temperature, using a gas flow rate of 40cc/minute.

Figure 2 demonstrates uptake of the sulfur compound by the three surfaces. The performance of the Sulfinert® treated, electropolished surface is quite dramatic in comparison to that of untreated electropolished tubing. Sulfinert® treated electropolished tubing did not adsorb methyl mercaptan to any measurable extent, delivering a representative sample with no delay. The untreated electropolished tubing, in contrast, totally adsorbed methyl mercaptan for more than 75 minutes, and the sulfur gas level did not stabilize until approximately 130 minutes. Conventional 316L seamless tubing totally adsorbed methyl mercaptan for more than 90 minutes, and the sulfur gas level did not stabilize until approximately 140 minutes.

Closely correlated to the adsorption of sulfur compounds by system components is the subsequent release of the adsorbed compounds. When adsorption of sulfur-containing compounds is prolonged, desorption from the surface also is slow. This "memory" of adsorbed active compounds can cause long delays in equilibrating a sample stream. Figure 3 demonstrates the memory effects of the three types of tubing used to transfer streams containing sulfur compounds. The Sulfinert® treated tubing shows less retention of sulfur compounds by several orders of magnitude, indicating very high inertness.

Figure 2 Sulfinert® treated electropolished stainless steel tubing (red) does not adsorb methyl mercaptan (500ppbv). Blue—untreated electropolished tubing, violet—commercial grade tubing.

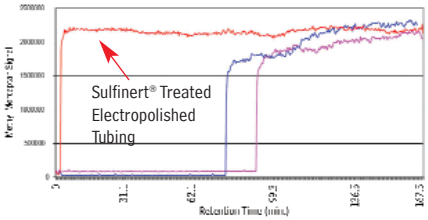
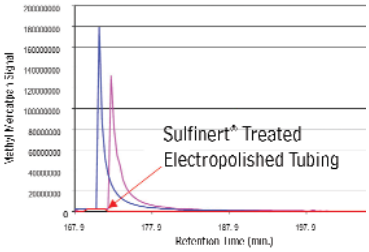


Figure 3 Sulfur memory is prolonged in raw commercial grade stainless steel tubing (violet). Red-Sulfinert® treated electropolished tubing; blue-untreated electropolished tubing (500ppbv methyl mercaptan in helium).



Value of an Inert Pathway

The value of surface treated sampling and transfer equipment is twofold: more accurate results and faster cycle times. Improved accuracy and reliability of data for sulfur, achieved using Sulfinert® treated transfer and sampling equipment, mean downstream processes can be more precisely controlled, with associated cost savings. Shorter cycles translate directly into more samples collected and analyzed in a given period of time. Typical savings can be calculated by looking at the average per-hour cost of operating a process that relies on accurate quantification of sulfur compounds.[1] Example monetary values are reported on the front of this note.

In Summary

We obtained accurate data, with no delay between samples, by using Sulfinert® treated electropolished tubing in the sampling-storage-transport system. In contrast, we obtained significantly less accurate data, even with delays of more than two hours between samples, by using untreated tubing. Analysts charged with monitoring sulfur levels in process streams can significantly improve profitability by using Sulfinert® treated system components and Sulfinert® treated electropolished tubing transport lines.

Reference
1. *Application of TrueTube™ in Analytical Measurement* Cardinal UHP August 2004
Available at www.restekcoatings.com or by contacting us at 800-356-1688, ext. 4. Request lit. cat.# 59088.
Acknowledgement
The authors thank the staff of the Shell Research and Technology Centre, Amsterdam, for data used in evaluating sulfur gas uptake and memory effects of tubing substrates.

Sulfinert®-Treated Electropolished Tubing

ID	OD	cat.#	Price-per-foot 5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085"	1/8"	22538				
0.180"	1/4"	22539				

Coiled Sulfinert®-Treated Seamless 316 Grade Stainless Steel Tubing

ID	OD	cat.#	Price-per-foot 5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22508				
0.180" (4.57mm)	1/4" (6.35mm)**	22509				

Sulfinert®-Treated Sample Cylinders

D.O.T. rated to 1800psi at room temperature.

Size	qty.	cat.#	price
75cc	ea.	24130	
150cc	ea.	24131	
300cc	ea.	24132	
500cc	ea.	24133	
1000cc	ea.	24134	
2250cc	ea.	21394	

Restek offers many **Sulfinert®-treated fittings, valves, sample loops.** For more information and ordering, call us or visit us online.

please note

An extra charge is applied for cutting Sulfinert® tubing, calculated from the total number of pieces produced for each line item:

of Pieces
5 to 15
16 to 30
31 to 75
76 to 99
100 to 200

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Learn more about our precisely applied, highly durable surface treatments: request our brochure lit. cat.# 59493.

**0.035" wall thickness
1/8" OD: 5 ft. to 100 ft. in one continuous coil; 1/4" OD: 5 ft. to 300 ft. in one continuous coil. Longer lengths will be more than one coil.
Note: (required length in meters) x (3.2808) = length in feet.

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Test Report

Study of 6N HCl Corrosion on Commercial 316 SS, Hastelloy C-22 and TrueTube™ Variants

April 2004

Testing Conducted by:

Bellefonte PA 16823

St. Louis MO 63114

Test Report

Testing was conducted to determine the relative corrosion resistance of Hastelloy C-22, commercial 316L SS, commercial tube with fused silica coating, electropolished TrueTube EP, and electropolished TrueTube EPS with a deactivated layer of bonded amorphous silicon applied via CVD technology (Siltek™) provided by Restek Corporation.

Corrosion Test Protocol and Results

The test specimens were ½" OD tubing with 0.049" wall thickness. They were cut, faced and deburred to approximately 8" in length. Each sample was rinsed in DI water and blown dry using 0.003 µm filtered N_2 gas. The samples where weighed and one end was capped using LDPE Tube Caps. The samples where then placed in a tube holder and filled with 6N HCl. The other end of the tube was then capped using a tube cap that had a pinhole in it to let the sample breath. The samples where exposed to the HCl for 72 hours. During the exposure the samples where agitated using vibration every 8 hours. After the 72-hour period the samples where drained rinsed and dried. The final weights where then recorded. The tubing that was tested is listed as follows:

Hastelloy C-22
Commercial Grade 316L Tubing
Commercial Grade 316L Tubing with Fused Silica Coating
Electropolished TrueTube EP Tubing
Electropolished TrueTube EP Tubing with a Siltek Coating

Table 1 Corrosion Rates for Tubing Tested

Material	Weight Loss (g)	Corrosion Rate (g/hr cm^2)	MPY (mils per year)	Sample Variance
Hastelloy C-22	0.0075	1.69E-06	0.6733	0.001622
Commercial Grade 316L Welded Tubing	0.3085	6.93E-05	29.9400	0.640267
Commercial Grade 316L Tubing with Fused Silica Coating	0.0492	1.1E-05	4.7567	13.19002
Electropolished TrueTube EP Tubing	0.1669	3.6E-05	16.5733	0.795022
TrueTube EP with Siltek Coating (TrueTube EPS)	0.0031	6.65E-07	0.2867	0.000622

Determination of Test Data

The corrosion rate was determined using the ASTM equation established in ASTM G31.

$$CorrosionRate = \frac{(K \cdot W)}{(A \cdot D \cdot T)}$$

Where: K is a constant =3.45 E6
W is the weight loss in grams
A is the exposed surface Area cm2
D is the density g/cm2
T is the time in hours

The variance of each sample population was computed to determine a confidence level for the results. The variance was less than 1 for all samples except fused silica coating on commercial tubing. Variance was calculated by the equation: $\frac{\sum(x-\overline{x})}{n}$ where x is the sample mean and n the sample size.

Micrographic Inspection of Test Samples

In addition to the corrosion testing micrographs of the samples where taken at 500x after the exposure to 6N HCl. All the micrographs taken are of the surface after exposure to 6N HCl.

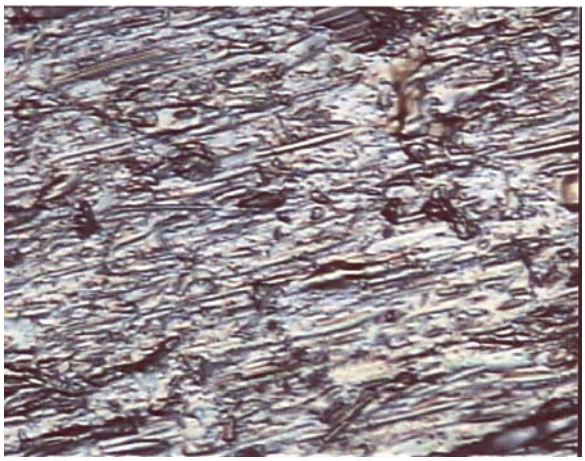

Figure 1: Hastelloy C-22 500x

Figure 1 is the micrograph of Hastelloy C-22. It clearly shows that the 6M HCl has barely touched the surface.

Figure 2 shows the surface of the commercial 316L SS sample. There are several corrosion mechanisms occurring here. The darken lines indicate grain boundary attack. There is also general corrosion due to the irregular surface and some pitting. Comparing the micrographs of the commercial 316L SS tubing and the TrueTube EP tubing illustrates the importance of minimizing nucleation sites in which pitting and general corrosion can occur. In the case of the commercial tubing the nucleation sites are the peaks and valley's on the surface and process contamination. The electropolished TrueTube EP process minimizes these nucleation sites.

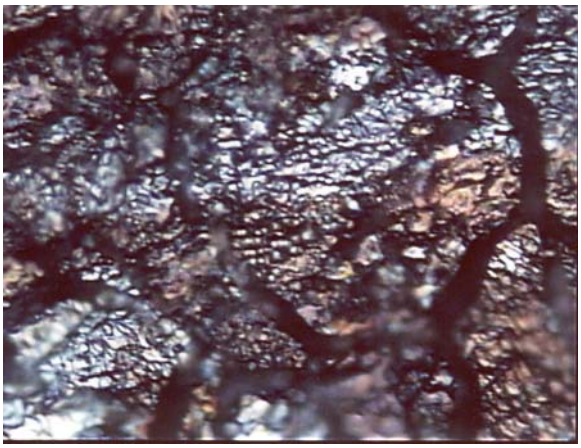

Figure 2: Commercial 316L SS Tubing 500x

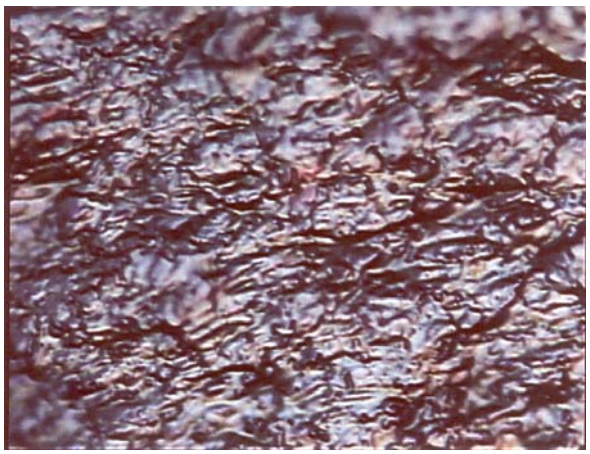

Figure 3: Commercial Grade SS with Fused Silica Coating 500x

Figure 3 is the surface of commercial grade 316L tubing with fused silica coating. Since the coating is transparent the image shows the metallic surface and not the coating surface. Because of the irregular surface roughness of the tubing it is difficult to distinguish any area of preferential attack. Several locations where inspected with no evidence of total coating failure.

Figure 4 shows the surface of the Electropolished TrueTube EP Tubing. Primary corrosion is evident at the grain boundaries with some pitting internal to the grains.

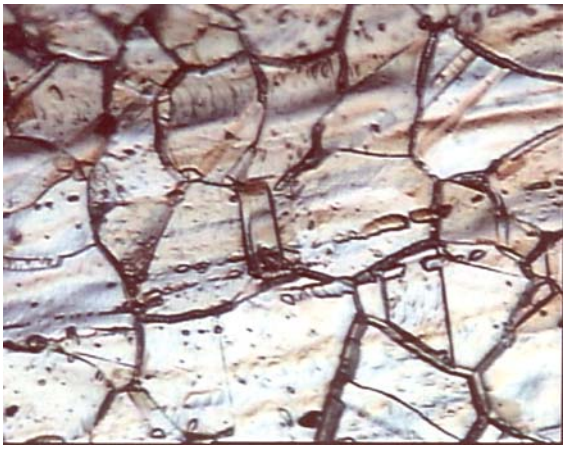

Figure 4: Electropolished TrueTube EP Tubing 500x

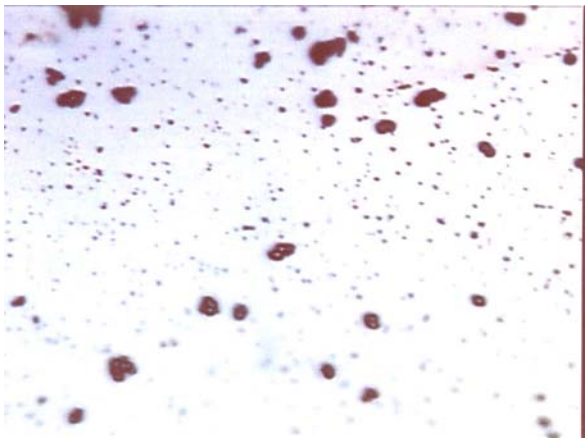

Figure 5: TrueTube EPS – Electropolished Tubing with Siltek Coating 500x

Figure 5 is of the TrueTube EP with Siltek Coating (TrueTube EPS). The micro pitting of the metallic surface is seen. This is the result of the electropolishing and coating process not due to corrosion. The base metal surface appears untouched by the HCl. Comparing the samples commercial grade fused silica (Figure 3) explains the variance in corrosion rate. The discontinuities in the coating for the fused silica coated commercial grade tubing are major sources of weight loss and directly affect the corrosion rate.

Notes on the Resulting Test Data

Commercial 316L SS is used as the base for all relative corrosion resistance rankings.

Hastelloy C-22 is included in the test because it is recognized as the 'material of choice' for HCl service. Test results illustrate a 44 fold improvement in corrosion resistance to 6N HCl over the base commercial 316L SS.

The corrosion rate of the commercial grade fused silica coated tube varied between samples, more than all other tubes tested. The average corrosion rate for commercial grade fused silica was 4.75 mpy or a 6 fold improvement over commercial 316L SS.

Test results clearly illustrate that TrueTube EP improves the corrosion resistance by approximately 2 fold over commercial 316L SS.

Using TrueTube EP as the substrate of the Siltek coating improved the corrosion resistance by 27 fold over commercial grade fused silica coated tubing and 172 fold over commercial 316L SS in the milled condition. The resulting TrueTube EPS showed far less variance then the commercial grade fused silica coated tubing. The average corrosion rate was calculated to be 0.28 mpy. This gives TrueTube EPS a 104 fold advantage over commercial 316L SS material with a very high confidence level.

Conclusion

The corrosion resistance of austenitic stainless steel can be improved by electropolishing or by applying a coating of fused silica or by combining both processes. The combination of O'Brien Corporation electropolished TrueTube EP and the Siltek coating by Restek provided corrosion resistance superior to that of Haselloy C-22.

Surface preparation will affect the ability of any subsequent process to provide additional corrosion protection. This assertion is supported by the variance found in the testing of the fused silica coating on commercial grade tubing. The corrosion rate of these samples varied sixteen times more than all other sample sets. The samples of fused silica applied to commercial tubing showed corrosion resistance changes from a low of 3 fold to a high of 24 fold. While the average corrosion rate for commercial grade fused silica computed to 4.75 mpy or a 6 fold improvement over commercial grade tubing, ranking by the average value belies the uncertainty of predicable performance.

While very different in their values both TrueTube EP and TrueTube EPS provided constant results and enhanced corrosion protection.

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Applications Note

Prevent Adsorption of Mercury, Sulfur, and Nitrogen Compounds in Stack and Monitoring Equipment

Improve analytical reliability and prevent corrosion, using Siltek®/Sulfinert® treated components

Mercury has a significant impact on human health, and mercury contamination in the environment is a growing concern. Coal-fired power generators are one of the major sources of mercury emissions into the environment.[1] The United States is actively developing regulations, limits, and control measures for mercury emissions from coal-fired power generators.

As regulations and guidelines for monitoring and controlling mercury emissions are developed and implemented, proper equipment will be needed for accurate sampling and analysis. A typical coal-fired plant may spend up to $62 million to meet emission regulations (Table I).[2] In addition, in many stack environments common to coal-fired power generators, monitoring equipment is exposed to sulfuric and hydrochloric acids, which leads to corrosion and damage. Regular upkeep and preventive maintenance of testing equipment is costly. Also, analytical testing costs can be substantial. Recent studies have shown a per-test cost of $100-$640 U.S. dollars (Table II).[3] Finally, the costs of inaccurate analysis could have broad financial and environmental repurcussions.

Table I Typical costs of adding air pollution controls to a 1969 vintage 75MW cyclone fired boiler (U.S. dollars, millions).[2]

Item	Wet Scrubber	Dry Scrubber
SCR	23.8	23.8
PJFF and scrubber	22.4	16.1
ACI	—	0.7
ID fans	2.6	2.6
Stack	2.8	2.8
Balanced draft conversion	7.7	7.7
Auxiliary power upgrades	0.9	0.85
Subtotal capital cost	60.2	51.75
Asbestos abatement	2.0	2.0
Total capital cost	**62.2 million**	**53.75 million**

Table II Typical costs of various sampling methods (U.S. dollars).[3]

Method	Approx. Cost of Analysis
US EPA 29	300
US EPA 101A	100
ASTM D6784-02	250
US EPA 324	430
FAMS	640

In flue streams from coal-fired power generators, mercury exists in three forms: elemental, the +2 oxidation state (Hg^{++}), and attached to particulate matter. In many instances, Hg^{++} will react with sulfur compounds, nitrogen, chlorine, and/or oxygen, to produce sulfurous, nitrous, chloride, and oxide mercury species. Elemental and oxidized mercury easily can be lost to reactions and adsorption on the inner surfaces of monitoring equipment. In order to accurately sample and quantify mercury in all forms, it is important to use inert sample pathways. Laboratory testing and field results have proven that Sulfinert® treated sampling and testing equipment is essentially inert to active molecules[4], and customer field testing has shown Sulfinert® treatment to greatly reduce interactions of steel components with mercury.

Siltek®/Sulfinert® treatment applies an inert barrier coating over the entire surface of a steel component, regardless of its geometry. It can be applied to many of the components in a mercury sampling stream, including probe tubing, impingers, fittings, filters, housings, and transfer tubing. A typical sampling train schematic is shown in Figure 1. Application of Siltek®/Sulfinert® treatment to all of the components of a stack or continuous emission monitoring system will greatly improve analytical reliability and sensitivity, and will be needed as regulations are brought on line and emission quotas are enforced.

Similarly, a Siltek®/Sulfinert® treated sampling system will improve the reliability of data for sulfurous and nitrous oxides (SOx and NOx). Emissions of SOx and NOx compounds from coal-fired power generators are stringently regulated, and there currently are many systems in the field to monitor these compounds. As with mercury, it is difficult to reliably transfer these compounds through untreated sampling equipment.

In addition to preventing adsorption of reactive compounds in sampling pathways, Siltek®/Sulfinert® treatment will act as a barrier, protecting the steel and prolonging the lifetime of the treated equipment. The value of Siltek®/Sulfinert® treated monitoring equipment

Improve reproducibility, protect equipment

Siltek®/Sulfinert® treatment adds value to your process:

- Improve analytical sensitivity and reliability for mercury, SOx, or NOx compounds.
- Eliminate costly retests.
- Maximize scrubber performance.
- Protect against corrosion—increase component lifetime.
- Apply to existing equipment; will withstand temperatures to 400ºC.

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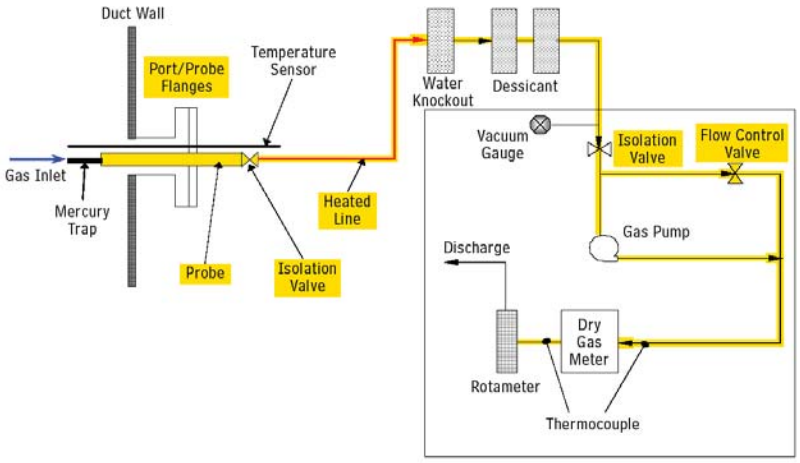
Figure 1 Highlighted components of a mercury sampling train,[5] and all tubing in the system, can be Siltek®/Sulfinert® treated.



Product Listing

Restek offers a complete line of tubing, fittings, and valves not listed here, as well as many air sampling products such as passive air sampling kits, air sampling canisters and miniature air canisters, sample loops, and more. For more information, request our catalog or visit us online.

Siltek®/Sulfinert®-Treated Electropolished Tubing*

- Exceptional inertness.
- Improved reliability and reproducibility; longer lifetime.
- Use with treated fittings for the most inert sample pathway available.

			Price-per-foot			
ID	OD	cat.#	5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085"	1/8"	22538				
0.180"	1/4"	22539				

Coiled Siltek®/Sulfinert®-Treated Seamless 316 Grade Stainless Steel Tubing*

			Price-per-foot			
ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22508				
0.180" (4.57mm)	1/4" (6.35mm)**	22509				

Sulfinert®-Treated Sample Cylinders

D.O.T. rated to 1800psi at room temperature.

Size	qty.	cat.#	price
75cc	ea.	24130	
150cc	ea.	24131	
300cc	ea.	24132	
500cc	ea.	24133	
1000cc	ea.	24134	
2250cc	ea.	21394	

Sulfinert®-Treated Sample Cylinder Valves

- All "wetted" parts are Sulfinert®-treated to make these valves inert and compatible with our inert sample cylinders.
- Stable storage of samples containing low concentrations of sulfur compounds.
- Maximum pressure rating, 5000psi.

Description	qty.	cat.#	price
1/4" NPT Exit, Kel-F® Stem Tip	ea.	24127	
1/4" Compression Exit, Kel-F® Stem Tip	ea.	24128	
1/4" Female NPT Outlet (built-in rupture disc)	ea.	21395	

*1/8" OD: 5 ft. to 100 ft. in one continuous coil; 1/4" OD: 5 ft. to 300 ft. in one continuous coil. Longer lengths will be more than one coil.
**0.035" wall thickness
Note: (required length in meters) x (3.28) = length in feet.

is twofold. First, it will materially increase the accuracy of the analysis: fast and accurate completion of testing, without re-work, can save a great deal of time and money. Second, the protective nature of Siltek®/Sulfinert® treatment will increase the longevity and decrease the upkeep of sampling equipment.

References

1. Pottinger, M., S. Stecklow, and J.J. Fialka, *Invisible Export, A Hidden Cost of China's Growth: Mercury Migration* The Wall Street Journal Online, Dec. 17, 2004.
2. *Proposed National Emissions Standards for Hazardous Air Pollutants: and in the Alternative, Proposed Standards of Performance for New and Existing Stationary Sources, Electric Utility Steam Generating Units;* Comments from the American Public Power Association: Washington, D.C. 2005. p.10.
3. Serne, J.C. *An Overview and Comparison of Available Mercury Emission Test Methods for Boilers;* Symposium on Air Quality Measurement; Methods and Technology 2005, San Francisco, CA; Air & Waste Management Association. paper no. 439, pg. 9.
4. Barone, G., M. Higgins, D. Smith, S. Rowan, W.J. Gross, and P. Harris, *The Surface for Sulfurs* Hydrocarbon Engineering, Dec. 2004 (pages 47–50).
5. Proposed Method 324. *Determination of Vapor Phase Flue Gas Mercury Emissions from Stationary Sources Using Dry Sorbent Trap Sampling.* United States Environmental Protection Agency. Washington, D.C. p. 5.

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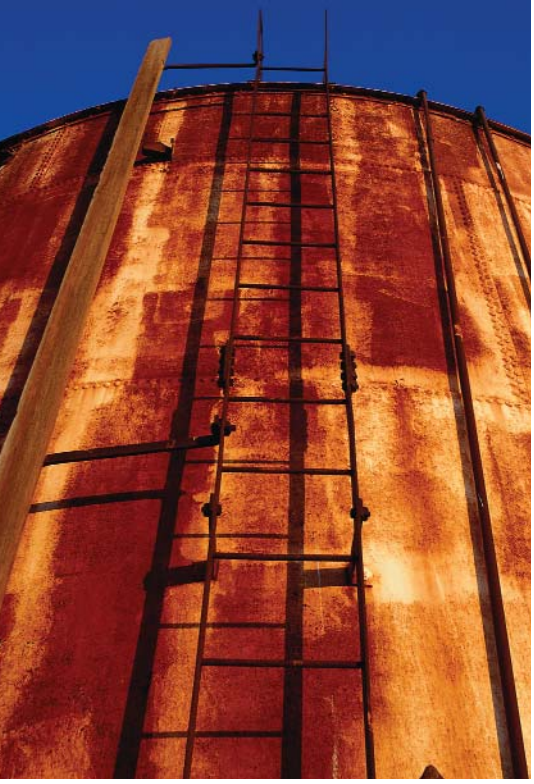
Applications Note



Improve Corrosion Resistance of Stainless Steel Tenfold, or More

Reduce downtime and extend system lifetime with Silcosteel®-CR treatment

Economical protection against corrosion

Silcosteel®-CR treatment extends the lifetimes of steel and steel alloy systems, while maintaining high dimensional tolerances, high temperature capability, and leak-free conditions, making it an ideal treatment for:

- process tubing, fittings, valves, and reactors
- gas transfer and delivery systems
- nozzles
- stack gas monitors
- analytical testing equipment

Silcosteel®-CR is highly effective protection for equipment exposed to:

- hydrochloric, nitric, or sulfuric acid
- marine environments

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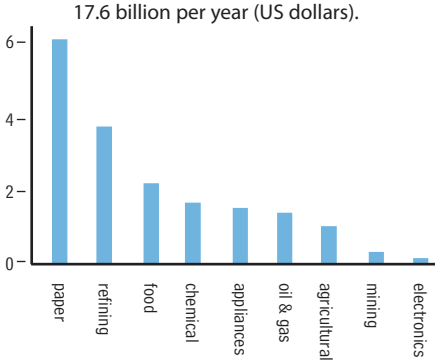
Introduction

The annual cost of metallic corrosion in the United States totals $276 billion[1], or more than 3% of the US gross domestic product. Industry is especially susceptible to high corrosion costs, due to exposure of facilities and equipment to both aggressive process chemicals and ambient weather conditions. The annual direct cost of corrosion to US industry is $17.6 billion[1].

Figure 1 Corrosion costs US industries over 17.6 billion per year (US dollars).



Methods available to control industrial corrosion are limited to corrosion-resistant alloys, barrier coatings, cathodic protection, and corrosion inhibitors. When properly applied, each method can be effective in slowing corrosion, but each has limitations as well. For example, some coatings are inexpensive, but require rigorous inspection and/or frequent reapplication. Corrosion-resistant alloys can provide exceptional protection, but can be prohibitively expensive. Some alloys require significant process redesign, increase operating cost, or generate hazardous waste.

Silcosteel®-CR is a new class of performance coating, offering an order of magnitude improvement in corrosion resistance, or greater, relative to existing processes. Silcosteel®-CR maintains high dimensional tolerances, demonstrates extreme heat capability, and exhibits leak-tight system performance in steel, stainless steel, and alloy systems. This study presents laboratory corrosion test results and potential cost savings of applying this alternative corrosion-resistant barrier coating technology.

Silcosteel®-CR is a proprietary (patent pending), multilayer silicon, chemical vapor-deposited (CVD) coating, specifically designed to improve corrosion resistance of steel, stainless steel, alloys, glass, and ceramics. The unique non line-of-sight CVD process produces a flexible amorphous silicon layer that diffuses into the metal lattice. The layer will conform to the most intricate surface while maintaining high dimensional tolerances. Silcosteel®-CR will deform with tubing surfaces, allowing leak-free seals or radius bends.

Independent Laboratory Testing

Corrosion testing of Silcosteel®-CR treated 316L stainless steel and untreated 316L steel according to ASTM G 48, Method B[2] (72-hour ferric chloride pitting and crevice corrosion testing), shows corrosion of the treated stainless steel is reduced by an order of magnitude, as measured by weight loss (Figures 2 and 3).

Figure 2 Silcosteel®-CR treated stainless steel outperforms uncoated metal by an order of magnitude (ASTM G 48, Method B).

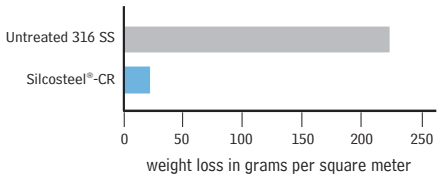


Figure 3 Silcosteel®-CR treated 316L stainless steel coupons show no crevice corrosion and only slight pitting corrosion; untreated coupons exhibit severe crevice corrosion.

Silcosteel®-CR treated

untreated

Testing of Silcosteel®-CR treated coupons in neutral, acidic, and basic chloride solutions, according to ASTM G 61,[2] shows Silcosteel®-CR treatment reduces corrosion rates by an impressive 50x, compared to untreated 316L stainless steel.

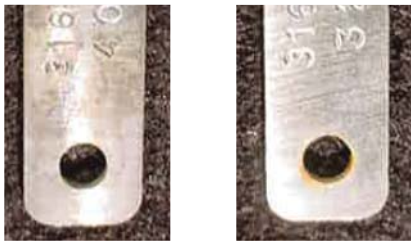
Improved Performance in Marine or Acidic Environments

Silcosteel®-CR treatment is effective in acidic or salt corrosive environments, in which the user demands extended service life for an existing process without using high-priced alloys.

4000-hour salt spray testing (salt spray accelerated weathering test ASTM B117) shows Silcosteel®-CR treated stainless steel coupons exhibit no surface corrosion, while untreated coupons shows surface corrosion and accelerated corrosion at the coupon hole (Figure 4). Neither coupons developed pitting over the test period.[2]

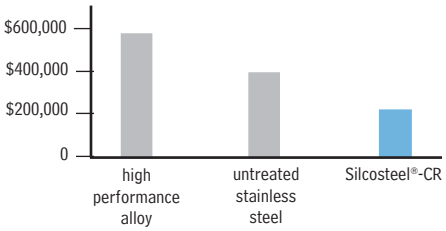
Figure 5, a comparison of lifetime costs in a typical process system, shows Silcosteel®-CR treatment can reduce the overall lifetime cost of the system by hundreds of thousands of dollars. While the initial cost of an unprotected stainless steel system is lower than that of a comparable Silcosteel®-CR system, the overall lifetime cost, considering replacement cost due to corrosion, is nearly double that of a Silcosteel®-CR treated system. Conversely, high performance alloy systems offer superlative corrosion performance, but the initial material cost can be up to six times that of a comparable stainless steel system.

Figure 4 Silcosteel®-CR treated 316L stainless steel coupons show no sign of attack after 4000-hour salt spray exposure, per ASTM B117.



Silcosteel®-CR treated | untreated

Figure 5 Silcosteel®-CR demonstrates significant cost savings, compared to untreated stainless steel or alloys (US dollars).



Silcosteel®-CR-Treated Electropolished Tubing

- Exceptional inertness.
- Improved reliability and reproducibility; longer lifetime.
- Use with treated fittings for the most inert sample pathway available.

ID	OD	cat.#	Price-per-foot 5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085" *	1/8"	22536				
0.180" *	1/4"	22537				

Coiled Silcosteel®-CR -Treated Seamless 316L Stainless Steel Tubing

ID	OD	cat.#	Price-per-foot 5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22896				
0.180" (4.57mm)	1/4" (6.35mm)**	22897				

*1/8" OD: 5 ft. to 100 ft. in one continuous coil; 1/4" OD: 5 ft. to 300 ft. in one continuous coil. Longer lengths will be more than one coil.
**0.035" wall thickness

Silcosteel®-CR treatment has extended the life of process systems in oil and gas production, oil refining, petrochemical processing, aerospace equipment, food and beverage processing, and laboratory testing.

Silcosteel®-CR treated tubing and treated Swagelok® or Parker fittings are available from stock. Custom treatment is available for other fittings, or for your process-specific components.

Summary

Test data show that Silcosteel®-CR treatment is effective in extending the corrosion resistance of stainless steel process systems while reducing overall system maintenance cost. Because Silcosteel®-CR treatment can be applied to a majority of existing process components, process equipment life is extended without significant re-engineering.

To learn more about how Silcosteel®-CR treatment can improve the corrosion performance of your process system, visit Restek Performance Coatings on the web at www.restekcoatings.com or contact our technical service group at 814-353-1300 or 800-356-1688, ext. 4.

References

1. G. Koch; M. Brongers; N. Thompson; Y. Virmani; J. Payer; Report # FHWA-RD-01-156, *Corrosion Cost and Preventive Strategies in the United States*; Office of Infrastructure Research and Development, Federal Highway Administration, McLean, VA. 2001

2. M. Zamanzadeh; G. Bayer; G. Rhodes; D. Smith; M. Higgins; *Laboratory Corrosion Testing of a Chemical Vapor Deposited Amorphous Silicon Coating*; Matco Associates, Inc. Pittsburgh, PA; Restek Corporation, Bellefonte, PA. 2005

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RESTEK environmental

Applications note

GC Analysis of US EPA Method 619 Triazine Herbicides Using the Rtx®-CLPesticides and Rtx®-CLPesticides2 Columns

Triazine herbicides have risen in usage due to their lower toxicity and less persistance in the environment as compared to the chlorophenoxy herbicides (US Environmental Protection Agency Method 8151). The US EPA Method 619 is a test commonly performed by environmental laboratories for the analysis of triazine herbicides in wastewater. Although this method was written for use with packed columns, capillary columns have all but replaced them in modern laboratories performing this method.

Table I

Compounds Determined by US EPA Method 619

Peak	Compound	CAS No.
1	prometon	1610-18-0
2	simazine	122-34-9
3	atraton	1610-17-9
4	propazine	139-40-2
5	atrazine	1912-24-9
6	terbuthylazine	5915-41-3
7	secbumeton	26259-45-0
8	simetryn	1014-70-6
9	ametryn	834-12-8
10	prometryn	7287-19-6
11	terbutryn	86-50-0

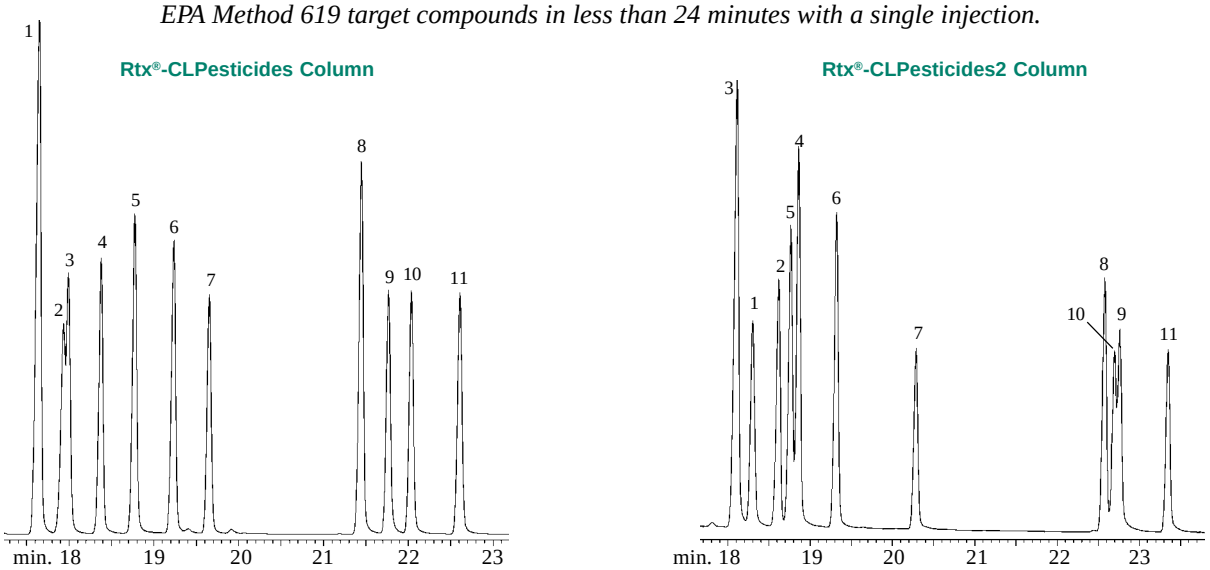
Even when using capillary columns, separation of the 11 target compounds listed in Method 619 can be difficult due to their similar structures. Most common capillary column stationary phases do not have adequate selectivity to resolve these compounds, and a confirmation column of different selectivity can be difficult to find.

Restek specifically designed the Rtx®-CLPesticides and Rtx®-CLPesticides2 columns for the analysis of the organochlorine pesticides. These stationary phases are highly selective for compounds that contain electronegative substituents and, correspondingly, work well for the analysis of chlorophenoxy herbicides, PCBs, and the analytes listed in US EPA Method 619 (Table 1).

Figure 1 shows the analyses of Method 619 target compounds on the Rtx®-CLPesticides and Rtx®-CLPesticides2 columns, connected in parallel in the same GC oven using a 'Y' Press-Tight® connector. This configuration is beneficial because both the primary and confirmation analysis is performed under the same conditions, at the same time, using a single injection port. The combination of the Rtx®-CLPesticides and Rtx®-CLPesticides2 columns resolves all target compounds in less than 24 minutes. These stationary phases are thermally stable to

Figure 1

The Rtx®-CLPesticides and Rtx®-CLPesticides2 columns, connected in parallel, resolve all EPA Method 619 target compounds in less than 24 minutes with a single injection.



30m, 0.32mm ID, 0.50µm Rtx®-CLPesticides (cat.# 11139) and 30m, 0.32mm ID, 0.25µm Rtx®-CLPesticides2 (cat.# 11324) with a 5m, 0.32mm ID guard column (cat.# 10044) and a 'Y' Press-Tight® connector (cat.# 20403). Direct injection using a Uniliner® sleeve (cat.#21303) and adaptor for an HP 5890 (cat.# 20964). **On-column concentration:** 50pg each compound. **Oven temp.:** 100°C to 250°C @ 4°C/min. (hold 5 min.); **Inj./det. temp.:** 250°C/NPD @275°C; **Carrier gas:** hydrogen, 9.65psi constant pressure; **GC:** HP 6890 with purged packed injection port.

environmental

330°C, allowing for "bake-out" of any high-boiling contaminants, which extends column lifetime and minimizes baseline instability.

Restek's Rtx®-CLPesticides and Rtx®-CLPesticides2 columns provide the complete solution to analyze chlorinated pesticides, herbicides, PCBs, and now US EPA Method 619 compounds. If your laboratory is analyzing samples using these methods, and would like more information, please contact Restek's Technical Service Team at 800-356-1688 or 814-353-1300, ext 4.

Product Listing

Rtx®-CLPesticides Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.25	340°C	11120	11123
0.32mm	0.50	340°C	11136	11139
0.53mm	0.50	340°C	11137	11140

ID	df (µm)	Stable to	10m	20m
0.18mm	0.18	340°C	42101	42102

Rtx®-CLPesticides2 Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.20	340°C	11320	11323
0.32mm	0.25	340°C	11321	11324
0.53mm	0.42	340°C	11337	11340

ID	df (µm)	Stable to	10m	20m
0.18mm	0.14	340°C	42301	42302

Rtx®-CLPesticides Column Kits

These kits include both a CLPesticides and CLPesticides2 column, a Universal Angled 'Y' Press-Tight® Connector, and a 5m guard column. (Note: Columns are not preconnected in these kits.)

Description	cat.#
0.53mm ID Rtx®-CLPesticides Kit	11197
0.32mm ID Rtx®-CLPesticides Kit	11198
0.25mm ID Rtx®-CLPesticides Kit	11199

Universal 'Y' Press-Tight® Connectors

20405, each	20406, 3-pk.

Universal Angled 'Y' Press-Tight® Connectors

20403, each	20404, 3-pk.

5m Phenylmethyl-Deactivated Guard Columns

ID (mm)	cat.#
0.25	10043
0.32	10044
0.53	10045

Uniliner® Direct Injection Sleeves for HP GCs

4mm ID x 6.3mm OD x 78.5mm Length	
20335, each	20336, 5-pk.

Additional Applications Notes from Restek!

Cat.# 59539… ***GC Analysis of US EPA Method 504.1 Organochlorine Pesticides using the Rtx®-CLPesticides and Rtx®-CLPesticides2 Columns.***

Cat. #59547… ***GC Analysis of US EPA Method 8081A Chlorinated Pesticides Using Rtx®-CLPesticides and Rtx®-CLPesticides2 Columns.***

Cat. #59559… ***Optimizing the Analysis of Chlorophenoxy Herbicides.***

RESTEK pharmaceutical

Applications note

European Pharmacopoeia Tests—Newly Revised for Residual Solvents

Residual solvent testing in pharmaceutical formulations can be confusing. The International Conference on Harmonization (ICH) has proposed a set of guidelines that may end the confusion and the European Pharmacopoeia (EP) was the first to revise their regulations for clarity.[1,2] However, these guidelines are challenging, containing over 60 compounds of regulatory interest to manufacturers of active substances, excipients, and medicinal products. The EP methods also allow testing limits based on either a concentration limit in a product, or calculated from the maximum daily dosage of the product and the permissible daily exposure limit of the solvent. These technical challenges will affect the sampling method and capillary column needed to ensure precise and accurate results.

The two most common sampling techniques for residual solvent testing are direct injection and static headspace sampling. While the EP method lists only static headspace sampling, the ICH allows the use of any validated sampling method. Although the majority of the regulated compounds may be successfully tested by either sampling method, six of the Class 2 compounds cannot be tested by headspace. These compounds—formamide, 2-methoxyethanol, N-methylpyrrolidone, sulfolane, 2-ethoxyethanol, and ethylene glycol—are available from Restek in a separate mix at the regulatory limit for EP residual solvent testing to be analyzed by direct injection.

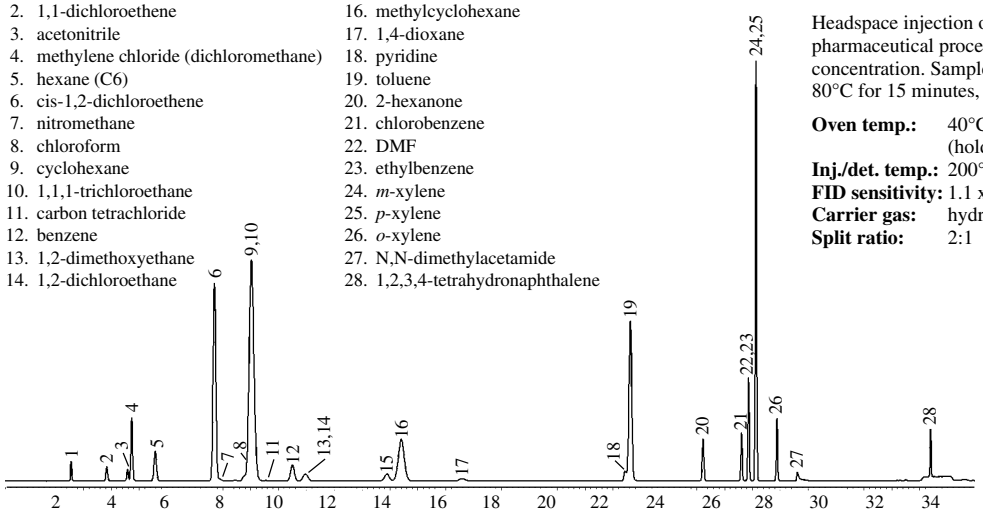
Restek sells EP calibration mixes at the regulatory concentration limit, allowing the same sample:dilutant (1:20) ratio to be used for the calibration material without any further concentration correction back to the sample concentration. *See the product listing for Class 1 and Class 2 residual solvent classifications.*

Restek sells the Class 1 and Class 2 compounds in a mix of water:dimethylsulfoxide (90:10). The use of co-solvents helps precision by limiting the loss of volatile analytes during standard preparation and handling, and product dispersion during sample preparation. Restek can provide custom analytical reference materials in N,N-dimethylformamide (DMF) or 1,3 dimethyl-2-imidazolidinone (DMI) to meet certain EP testing requirements.

The recommended capillary columns for EP residual solvent testing are the Rtx®-1301 and Stabilwax®. We can recommend other columns for customers analyzing abbreviated residual solvent lists. The Rtx®-1301 column shows excellent resolution of most European Pharmacopoeia Class 1 and 2 compounds at the regulation limit concentration (**Figure 1**). The Stabilwax® column makes an excellent confirmation column for the analysis of residual solvents (**Figure 2**). Please call us with your specific compound list and our technical representatives will find the best column for your solvent list and provide you with a price quotation for the custom analytical reference materials you need.

Figure 1: *The Rtx®-1301 column shows excellent resolution of most European Pharmacopoeia Class 1 and Class 2 compounds at the regulation limit concentration.*

1. methanol
2. 1,1-dichloroethene
3. acetonitrile
4. methylene chloride (dichloromethane)
5. hexane (C6)
6. cis-1,2-dichloroethene
7. nitromethane
8. chloroform
9. cyclohexane
10. 1,1,1-trichloroethane
11. carbon tetrachloride
12. benzene
13. 1,2-dimethoxyethane
14. 1,2-dichloroethane
15. trichloroethylene (1,1,2-trichlorethene)
16. methylcyclohexane
17. 1,4-dioxane
18. pyridine
19. toluene
20. 2-hexanone
21. chlorobenzene
22. DMF
23. ethylbenzene
24. *m*-xylene
25. *p*-xylene
26. *o*-xylene
27. N,N-dimethylacetamide
28. 1,2,3,4-tetrahydronaphthalene



30m x .53mm ID x 3.0mm Rtx®-1301 (cat.# 16085-126)

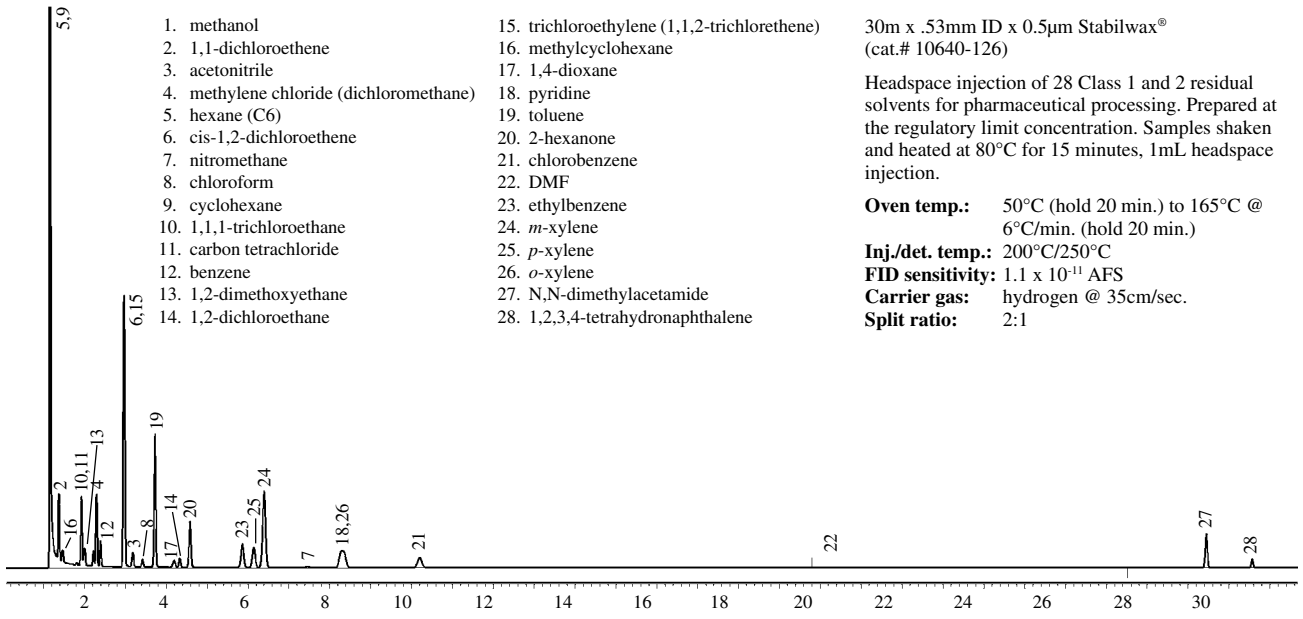
Headspace injection of 28 Class 1 and 2 residual solvents for pharmaceutical processing. Prepared at the regulatory limit concentration. Samples shaken and heated at 80°C for 15 minutes, 1mL headspace injection.

Oven temp.: 40°C (hold 20 min.) to 240°C @ 10°C/min. (hold 20 min.)
Inj./det. temp.: 200°C/250°C
FID sensitivity: 1.1 x 10^{-11} AFS
Carrier gas: hydrogen @ 35cm/sec.
Split ratio: 2:1

To place an order: call 814-353-1300 or 800-356-1688, ext. 3.

References:
1. "ICH Harmonized Tripartite Guideline, Impurities: Guideline for Residual Solvents," The Fourth International Conference on Harmonization, July 17, 1997.
2. European Pharmacopoeia Supplement, January 1999, pp.14-15, 208.

Figure 2: *The Stabilwax® column makes an excellent confirmation column for the analysis of residual solvents.*



1. methanol
2. 1,1-dichloroethene
3. acetonitrile
4. methylene chloride (dichloromethane)
5. hexane (C6)
6. cis-1,2-dichloroethene
7. nitromethane
8. chloroform
9. cyclohexane
10. 1,1,1-trichloroethane
11. carbon tetrachloride
12. benzene
13. 1,2-dimethoxyethane
14. 1,2-dichloroethane
15. trichloroethylene (1,1,2-trichlorethene)
16. methylcyclohexane
17. 1,4-dioxane
18. pyridine
19. toluene
20. 2-hexanone
21. chlorobenzene
22. DMF
23. ethylbenzene
24. *m*-xylene
25. *p*-xylene
26. *o*-xylene
27. N,N-dimethylacetamide
28. 1,2,3,4-tetrahydronaphthalene

30m x .53mm ID x 0.5µm Stabilwax®
(cat.# 10640-126)

Headspace injection of 28 Class 1 and 2 residual solvents for pharmaceutical processing. Prepared at the regulatory limit concentration. Samples shaken and heated at 80°C for 15 minutes, 1mL headspace injection.

Oven temp.: 50°C (hold 20 min.) to 165°C @ 6°C/min. (hold 20 min.)
Inj./det. temp.: 200°C/250°C
FID sensitivity: 1.1 x 10^{-11} AFS
Carrier gas: hydrogen @ 35cm/sec.
Split ratio: 2:1

Product Listing

European Pharmocopoeia/ICH Class 1 Mix

benzene	2µg/mL	1,1-dichloroethene	8µg/mL
carbon tetrachloride	4	1,1,1-trichloroethane	1500
1,2-dichloroethane	5		

Prepared in water:dimethylsulfoxide 90:10, 1mL/ampul

Ea.: 36228 **5-pk.: 36228-510** **10-pk.: 36328**

European Pharmocopoeia/ICH Class 2 Mix A

chlorobenzene	360µg/mL	N,N-dimethylformamide	880µg/mL
cyclohexane	3,880	toluene	890
cis-1,2-dichloroethene	1,870	1,1,2-trichloroethene	80
dichloromethane	600	*m*-xylene	1,302
ethylbenzene	369	*o*-xylene	195
hexane	290	*p*-xylene	304
methylcyclohexane	1,180		

Prepared in water:dimethylsulfoxide 90:10, 1mL/ampul

Ea.: 36229 **5-pk.: 36229-510** **10-pk.: 36329**

European Pharmocopoeia/ICH Class 2 Mix B

acetonitrile	410µg/mL	2-hexanone	50µg/mL
chloroform	60	methanol	3,000
1,2-dimethoxyethane	100	nitromethane	50
N,N-dimethylacetamide	1,090	pyridine	200
1,4-dioxane	380		
1,2,3,4-tetrahydronaphthalene (tetraline)	100		

Prepared in water:dimethylsulfoxide 90:10, 1mL/ampul

Ea.: 36230 **5-pk.: 36230-510** **10-pk.: 36330**

European Pharmocopoeia/ICH Class 2 Mix C

2-ethoxyethanol	160µg/mL	N-methylpyrrolidone	4,840µg/mL
ethylene glycol	620	sulfolane	160
formamide	220		
2-methoxyethanol	50		

Prepared in water, 1mL/ampul

Ea.: 36231 **5-pk.: 36231-510** **10-pk.: 36331**

Rtx®-1301 Columns

Length	ID	df (µm)	cat.#/price
30m	0.32mm	1.5	16069
30m	0.53mm	3.0	16085

Stabilwax® Columns

Length	ID	df (µm)	cat.#/price
30m	0.32mm	0.25	10624
30m	0.53mm	0.50	10640

RESTEK ISO 9001 REGISTERED

RESTEK Tech-Tips

Applications note

Improved GC Analysis of Basic Organic Compounds Using Base-Deactivated Columns and Inlet Liners

The gas chromatographic (GC) analysis of basic organic compounds (e.g., amines, basic drugs, and azo dyes) is a challenge to the analyst, especially when the compounds are at low concentrations. The challenge is mainly caused by acidic silanol groups on the pathway surfaces of GC system (i.e., inlet liner, wool, guard and analytical columns) that remain after standard chemical deactivation. Vaporized basic compounds are adsorbed onto the active sites, causing peak tailing and reduced response.

Because of the interaction between basic compounds in the sample with acidic active sites in the sample pathway, a "priming" effect may be noticed. With priming, the first injection of a basic sample results in compounds adsorbing onto the active sites and covering them. Repeated injections cause more and more sites to be covered until an equilibrium is reached. Response subsequently improves, but reproducibility is poor. The priming effect is temporary because the system will revert back to an adsorptive state after column conditioning or periods of inactivity.

Restek has an entire line of base-deactivated products to aid in the analysis of basic compounds—inlet liners, fused silica wool, guard columns, and the Rtx®-5 Amine analytical column. (Restek's analytical column deactivated specifically for the analysis of organic basic compounds). Base-deactivation is a unique deactivation process that bonds basic functional groups to the analytical surface. These functional groups on the surface greatly reduce adsorption and priming effects, thereby improving peak shape, response, and reproducibility. Figure 1 compares the effect of a base-deactivated liner/wool system with a standard-deactivated liner/wool system on diethanolamine response. In

Figure 1

Base-deactivated liner/wool system provides better diethanolamine response.

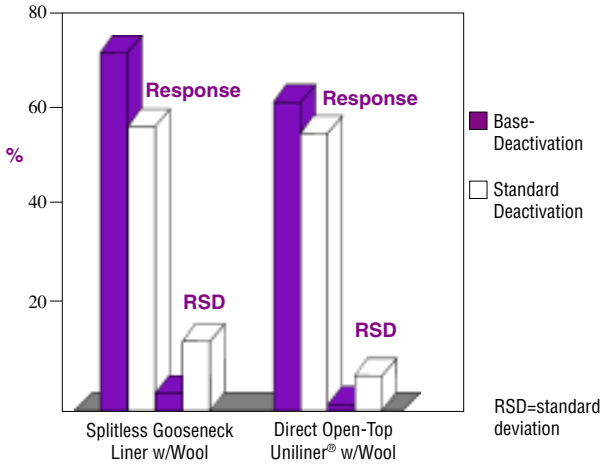
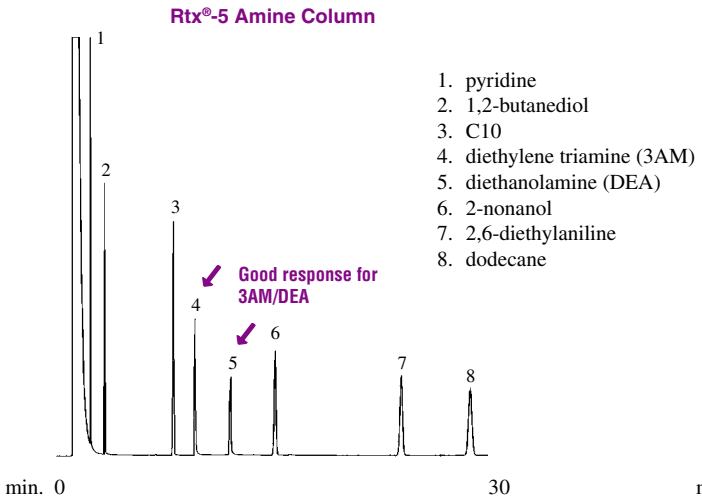
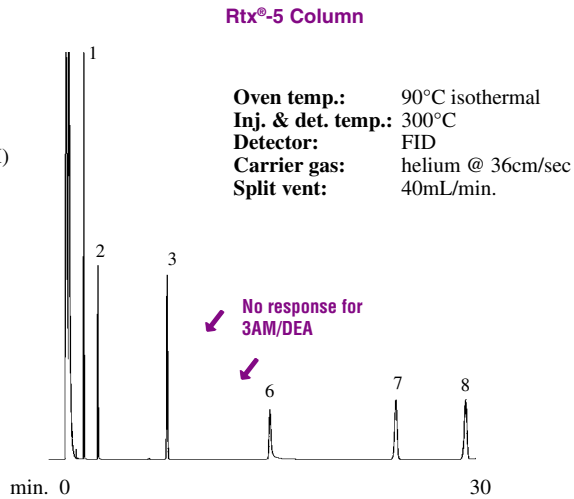


Figure 2

The Rtx®-5 Amine column achieves better response for active basic compounds compared to the standard 5% phenyl column.



30m, 0.32mm ID, 1.0µm Rtx®-5 Amine (cat.# 12354) and a base-deactivated 4mm gooseneck sleeve with base-deactivated wool (cat.# 20798-211.1).



30m, 0.32mm ID, 1.0µm Rtx®-5 (cat.# 10254) and a 4mm gooseneck sleeve with wool (cat.# 20798-200.1).

tech-tips

each case, response and reproducibility improved using the base-deactivation as opposed to standard-deactivation. Using the base-deactivated wool improves response of basic test probes by preventing the vaporized sample from contacting the highly-adsorptive hot metal inlet disc at the bottom of the injection port, and by providing a high-surface area, non-adsorptive surface on which to vaporize the sample.

The inertness of the Rtx®-5 Amine column is obvious when compared to a standard 5% phenyl analytical column (Figure 2). Two of the active basic compounds—diethylene triamine and triethanolamine—are completely adsorbed on the standard 5% phenyl column, yet exhibit good peak shape and response on the Rtx®-5 Amine column. To ensure the integrity of the Rtx®-5 Amine columns, Restek uses a demanding test mixture containing amines and alcohol-amines designed to confirm column inertness to basic probes.

Restek's base-deactivation process is effective for all but highly acidic compounds, and is specially-designed to ensure a highly inert GC pathway for basic compounds, from inlet to analytical column. This deactivation provides a surface that is chemically compatible with basic compounds, thereby greatly reducing priming, peak tailing, and poor reproducibility associated with basic compound analysis on standard-deactivated surfaces.

Product Listing

Rtx®-5 Amine Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.50	340°C	12335	12338
	1.00	340°C	12350	12353
0.32mm	1.00	340°C	12351	12354
	1.50	340°C	12366	12369
0.53mm	1.00	340°C	12352	12355
	3.00	340°C	12382	12385

Base-Deactivated Fused Silica Guard Columns

Nominal ID	Nominal OD	cat.#
0.25mm	0.37 ± 0.04mm	10000
0.32mm	0.45 ± 0.04mm	10001
0.53mm	0.69 ± 0.05mm	10002

Amine Column Test Mix

1,2-butanediol	0.60mg/mL
pyridine	0.60
decane	0.60
diethylenetriamine	1.20
diethanolamine	1.20
2-nonanol	0.60
2,6-dimethylaniline	0.60
dodecane	0.60

In CH_2Cl_2/methanol (50:50), 1mL/ampul. cat.# 35002, each

Base-Deactivated Inlet Liners for HP GCs

Description	each	5-pk.	25-pk.
4mm Split w/wool	20781-211.1	20782-211.5	20783-211.25
Cyclosplitter®	20706-210.1	20707-210.5	——
4mm Splitless Straight	20772-210.1	20773-210.5	——
2mm Gooseneck	20795-210.1	20796-210.5	20797-210.25
4mm Gooseneck	20798-210.1	20799-210.5	20800-210.25

Base-Deactivated Inlet Liners for Varian GCs

Description	each	5-pk.	25-pk.
Splitter with wool	20792-211.1	20793-211.5	——
Frit Splitter	20715-210.1	20716-210.5	——

Restek stocks the most requested liners with base-deactivation for immediate delivery. However, if you do not see the liner you need, orders can be placed on a custom basis for base-deactivated liners by adding the appropriate suffix number: each (-210.1), 5-packs (-210.5), and 25-packs (-210.25). For base-deactivated liners packed with base-deactivated wool: each (-211.1), 5-packs (-211.5), and 25-packs (-211.25).

Base-Deactivated Fused Silica Wool

Quantity	cat.#
10 grams	20999

Restek offers additional deactivation procedures—call Technical Service for details.

RESTEK environmental

Applications note

CarboPrep™ SPE Cleanup of Method 8081A Chlorinated Pesticides

Many chlorinated pesticides have been banned for use because of their short- and long-term toxicity, carcinogenicity, and environmental persistence[1]. An expanded list of these chemicals, some of which are still actively applied in the field, has been included in the updated US Environmental Protection Agency (EPA) Method 8081A. Despite the fact that most of these chlorinated pesticides are now illegal to use, manufacture, and transport in the US, organochlorines are the eighth most common source of pesticide poisoning that results in reportable illness[2]. Although most of these chlorinated pesticides, insecticides, and herbicides have limited water solubility and mobility, they are found to bioaccumulate and persist in the environment. There is an ongoing risk for exposure from a number of sources, so it is still essential to test soils, wastewater, and sediments for their presence.

Many of the additional components included in the EPA Method 8081A update are difficult to analyze by gas chromatography (GC), especially the isomers of the carbamate herbicide diallate. However, these have been shown to separate well using the Rtx®-CLPesticides and Rtx®-CLPesticides2 column pair. For more details on EPA Method 8081A analysis using these columns, refer to Applications Note #59547.

Standard EPA methods for preparation and analysis of pesticide-containing hazardous wastes require initial liquid/liquid extraction with dichloromethane, gel permeation chromatography (GPC) fractionation of higher molecular weight interferences, and a final cleanup of polar contaminants with Florisil® columns or solid phase extraction (SPE) tubes, before analysis with GC-electron capture detection (ECD). Many labs have found that these cleanup precautions are not adequate and still produce high backgrounds in analytical samples, resulting in difficult quantitation and frequent GC/detector maintenance. The addition of a CarboPrep™ SPE tube, after the Florisil® cleanup, will remove additional mid- to non-polar contaminants without compromising the recovery of pesticides. Extracts will have lower backgrounds, producing better chromatograms with fewer interferences and extending the lifetime of inlet sleeves, guard columns, and reducing maintenance of sensitive ECD detectors. Results in Table I below show that recovery levels are excellent for this extended chlorinated pesticides list, when following the method described in Figure 1.

CarboPrep™ SPE tubes contain a nonporous, chromatographic grade, graphitized carbon, optimized for cleanup and concentration of environmental samples. Graphitized carbon has also been shown to be effective in the concentration and extraction of many environmentally significant analytes found in drinking and waste water, such as phenols and triazine herbicides[3,4]. This form of carbon has been useful in multi-residue fractionation of base/neutral compounds from acidic pesticides[5]. The high flow rates, which may be used with this material, allow rapid extraction of 1 to 4 liters of aqueous samples. The tubes have a low background level, especially suitable for pesticides. The high surface area provides maximum capacity with a minimum of bed weight, reducing the volume of solvent used during extraction. These same characteristics make CarboPrep™ SPE tubes an excellent choice for cleanup of solid waste samples.

References:

1. The 8th Report on Carcinogens - 1998, US Dept. of Health & Human Services, Public Health Service, www.ehis.niehs.nih.gov.
2. Recognition and Management of Pesticide Poisonings, US EPA, www.epa.gov.
3. "Trace Determination of Phenols in Natural Waters", A. DiCorcia, A. Bellioni, M. Madbouly, S. Marchese, *Journal of Chromatography A*, 1996, 733, 383-393.
4. "Ultratrace Determination of Atrazine and its Six Major Degradation Products in Water", A. DiCorcia, C. Crescenzi, E. Guerriero, R. Samperi, *ES&T*, 1997, 31, 1658-1663.
5. "Development of a Multiresidue Method for Analyzing Pesticide Traces in Water", C. Crescenzi, A. DiCorcia , E. Guerriero, R. Samperi, *ES&T*, 1997, 31, 479-488.

References not available from Restek.

Table I

Recovery of Chlorinated Pesticides from Solvent Extracts Using CarboPrep™ 90 SPE Cartridge

Analytes*	Percent Recovery	RSD n=6
dibromochloropropane	84.7	11.4
hexachlorocyclopentadiene	100.4	9.9
2,4,5,6 tetrachloro-*m*-xylene (surrogate)	90.6	8.1
cis-diallate	73.0	11.3
hexachlorobenzene	96.8	7.4
trans-diallate	93.0	10.3
α-BHC	93.7	9.7
γ-BHC	97.7	8.7
β-BHC	97.0	7.9
δ-BHC	106.7	6.4
heptachlor	111.6	8.0
aldrin	97.3	8.6
isodrin	98.5	7.8
heptachlor epoxide	101.6	7.0
γ-chlordane	91.2	11.3
α-chlordane	96.1	7.4
endosulfan I	97.0	7.5
4,4' DDE	100.1	6.7
dieldrin	104.9	4.1
endrin/chlorobenzilate	121.9	6.3
4,4' DDD	107.8	2.9
endosulfan II	108.8	4.9
4,4' DDT	103.5	6.5
endrin aldehyde	110.0	2.8
endosulfan sulfate	102.7	6.5
methoxychlor	119.1	6.1
endrin ketone	101.0	3.3
decachlorobiphenyl (surrogate)	96.8	3.5

*Analytes spiked at 80-160ng/mL in hexane.

Figure 1

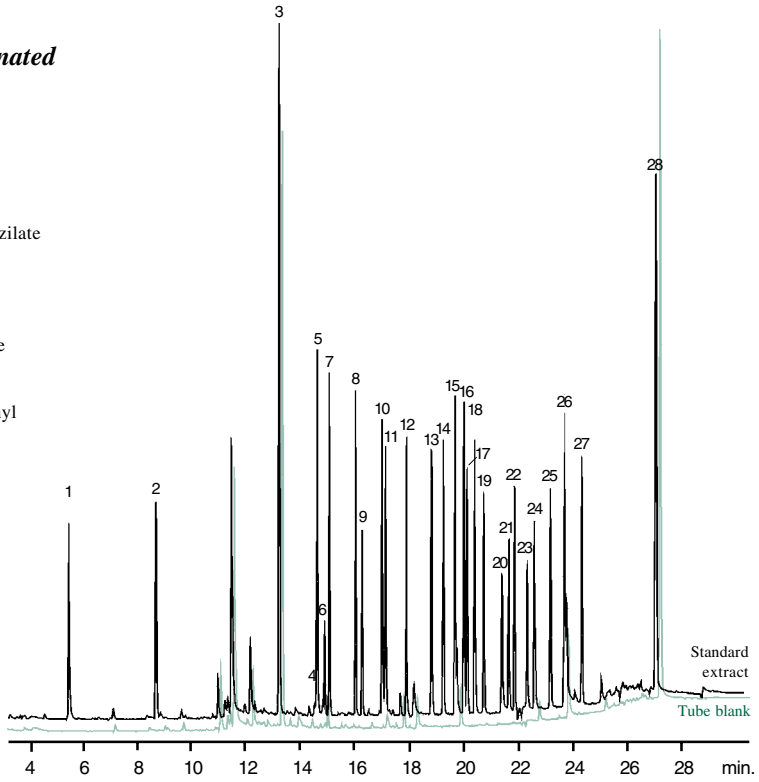
Cleanup method for chlorinated pesticides provides better separation.

Solvent Extracts of Method 8081A Chlorinated Pesticides Using CarboPrep™ 90

1. dibromochloropropane
2. hexachlorocyclopentadiene
3. tetrachloro-*m*-xylene
4. *cis*-diallate
5. hexachlorobenzene
6. *trans*-diallate
7. α-BHC
8. γ-BHC
9. β-BHC
10. δ-BHC
11. heptachlor
12. aldrin
13. isodrin
14. heptachlor epoxide
15. γ-chlordane
16. α-chlordane
17. endosulfan I
18. 4,4'-DDE
19. dieldrin
20. endrin/chlorobenzilate
21. 4,4'-DDD
22. endosulfan II
23. 4,4'-DDT
24. endrin aldehyde
25. endosulfan sulfate
26. methoxychlor
27. endrin ketone
28. decachlorobiphenyl

30m, 0.32mm ID, 0.25µm
Rtx® CLPesticides2 (cat.# 11324).

Oven temp.: 80°C (hold 1 min.) to 300°C @ 9°C/min. (hold 10 min.)
Inj.: 220°C direct injection using Uniliner® sleeve (cat.# 20335).
Det. temp.: ECD 310°C
Carrier gas: helium



Standards:	Organochlorine Pesticide Mix AB #1	cat# 32291
	Pesticide Surrogate Mix	cat# 32000
	Organochlorine Pesticide Mix C #1	cat# 32296
Column:	Rtx®-CLPesticides2, 30m x 0.32mmID, 0.25µm	cat# 11324
Sample:	0.5 - 1.0mL solvent extracted sample exchanged into hexane. Standards and surrogates were spiked into hexane.	
Tube:	3mL, 250mg CarboPrep™ 90	cat# 26091
Tube conditioning:	Apply 2mL CH_2Cl_2:hexane (20:80) and pass through tube.	
Extract clean-up:	Prepare collection rack with vials, place under each tube. Add 0.5-1.0mL of extracted sample to tube and collect all solutions passing through. Do not expose carbon bed to air. Add 20mL of CH_2Cl_2:hexane (20:80) to tube and allow it to elute using gravity feed.	
Extract concentration:	Solvent exchange extract to hexane. Do not allow extracts to dry completely. Concentrate extract to 0.5- 1.0mL. Sample is ready for analysis.	

Product Listing:

Description	cat. #
RTX®-CLPesticides2, 30m, 0.32mm ID, 0.25µm	11324
CarboPrep™ 90 cartridge 3mL, 250mg	26091
Pesticide Surrogate Mix	32000

Description	cat. #
Organochlorine Pesticide Mix AB #1	32291
Organochlorine Pesticide Mix C #1	32296

RESTEK environmental

Applications note

Minimizing Breakdown of Chlorinated Pesticides Using Siltek™-Deactivated GC Accessories

Gas chromatographic (GC) analysis of chlorinated pesticides presents unique challenges to environmental laboratories because these compounds often are at trace levels and susceptible to decomposition. Some analyses, such as the US Environmental Protection Agency (EPA) Methods 8081 and 608, have stringent breakdown and reproducibility criteria. Breakdown occurs when a compound decomposes into related compounds, generally because a thermal or chemical reaction has occurred. Two compounds notorious for exhibiting breakdown are endrin, which breaks down into endrin aldehyde and endrin ketone, and DDT, which breaks down into DDE and DDD. The source of breakdown can be aged samples and standards, the GC column or, most commonly, active sites in the GC injection port. Routine maintenance of the injection port, prevention of sample flashback, and thorough deactivation of the inlet liner and GC columns are essential to minimize compound breakdown.

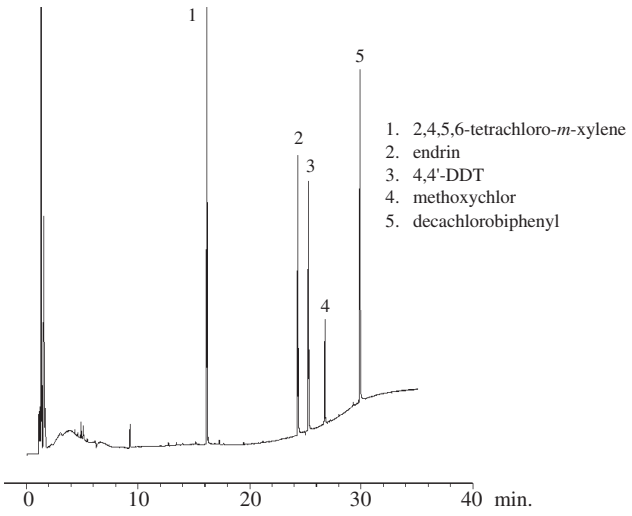
We chose a pesticide analysis to compare Siltek™-deactivated products against undeactivated products. To evaluate inlet liner effects on endrin and DDT breakdown, an HP 5890 GC equipped with an Rtx®-CLPesticides2 column and an electron capture detector (ECD) was used. A 50pg/µL standard of tetrachloro-*m*-xylene, endrin, 4,4'-DDT, methoxychlor, and decachlorobiphenyl was injected directly onto the column and onto several different liners. The area of the breakdown products was then measured. Helium was used as the carrier gas and the following oven program was used: 120°C (hold 1 min.), to 300°C at 9°C/min. (hold 10 min.). The injector was set at 250°C and the ECD at 300°C.

To measure the column (i.e., "system") breakdown, an on-column injection was made. This eliminated the effects of the injection port. Using this technique, endrin breakdown was 1% and DDT breakdown was below detection limits (Figure 1).

A sample was then introduced by direct injection into an untreated glass Uniliner® sleeve. The advantage of using a Uniliner® sleeve for a direct injection is that the GC column forms a press-tight seal with the liner, allowing the vaporized sample to deposit onto the column with minimal injection port contact. Deactivation is critical for this technique because a direct injection maximizes contact of the sample vapor with the liner. The injection resulted in 62% endrin breakdown with no DDT breakdown (Figure 2).

Figure 1

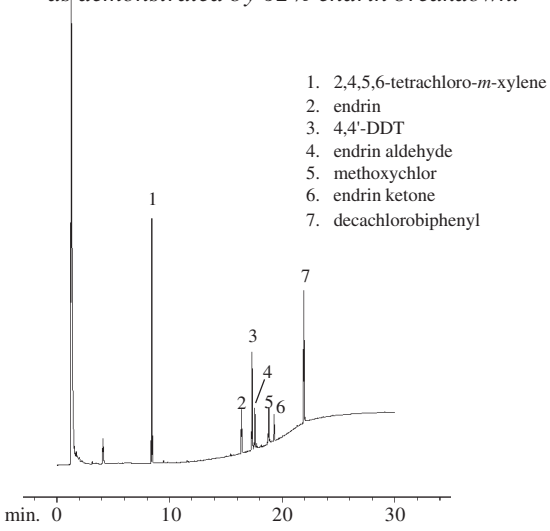
An on-column injection demonstrates system breakdown of less than 1% for endrin and DDT using a Siltek™-deactivated inlet liner.



30m, 0.53mm ID, 0.42µm Rtx®-CLPesticides2 (cat.# 11340). **Inj.:** 1µL of 50pg/µL standard. **Oven temp.:** 40°C (hold 1 min.) to 300°C @ 9°C/min. (hold 10 min.); **Inj. temp.:** 250°C; **Det.:** ECD, 300°C; **Carrier gas:** helium.

Figure 2

A chromatographic system that is inadequately deactivated will cause poor linearity, poor reproducibility, as demonstrated by 62% endrin breakdown.

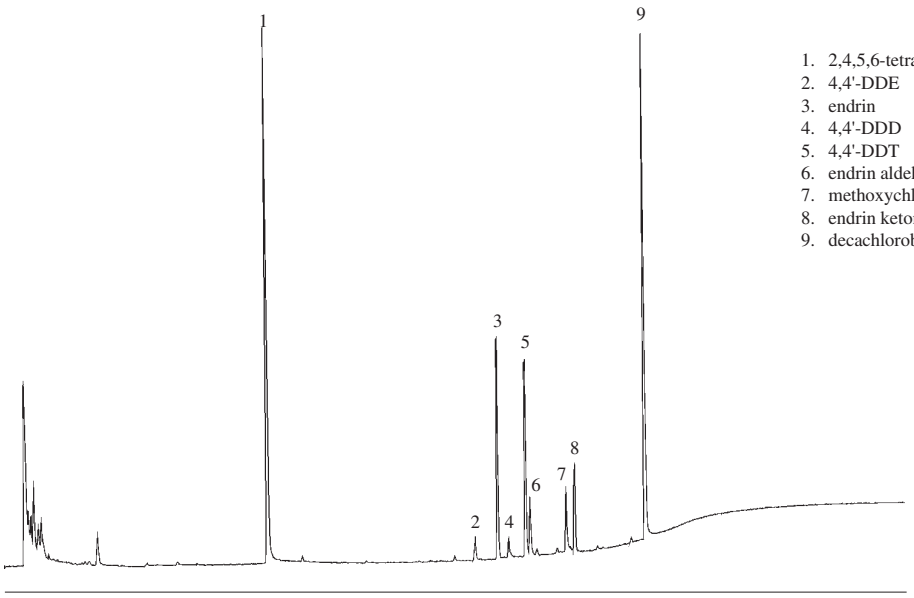


30m, 0.53mm ID, 0.42µm Rtx®-CLPesticides2 (cat.# 11340). **Inj.:** 1µL of 50pg/µL standard. **Oven temp.:** 120°C (hold 1 min.) to 300°C @ 9°C/min. (hold 10 min.); **Inj. temp.:** 250°C; **Det.:** ECD, 300°C; **Carrier gas:** helium.

environmental

Figure 3

Untreated metal liners and injection port surfaces will cause high levels of endrin and DDT breakdown.



1. 2,4,5,6-tetrachloro-*m*-xylene
2. 4,4'-DDE
3. endrin
4. 4,4'-DDD
5. 4,4'-DDT
6. endrin aldehyde
7. methoxychlor
8. endrin ketone
9. decachlorobiphenyl

30m, 0.53mm ID, 0.42µm Rtx®-CLPesticides2 (cat.# 11340). **On-column conc.:** 50ppb; **Oven temp.:** 120°C (hold 1 min.) to 300°C @ 9°C/min. (hold 10 min.); **Inj./det. temp.:** 250°C/300°C; **Purge:** on 0.75 min.; **Carrier gas:** helium, 33cm/sec.

To compare the effects caused by hot metal surfaces, a splitless injection was made into an untreated stainless steel sleeve. Because the splitless sleeve does not form a press-tight seal with the analytical column, the sample vapor is less restricted and is free to contact the metal disk located at the bottom of the HP injection port, as well as the injection port body. Endrin breakdown (40%) was less than in the untreated glass liner, but DDT breakdown (20%) was significant (Figure 3).

As a final comparison, the standard mixture was injected via direct injection into a Uniliner® sleeve that was processed with Siltek™ deactivation (Figure 4). Endrin breakdown measured less than 1% on the Uniliner® sleeve that had been deactivated using the Siltek™ deactivation process. DDT breakdown again was below detection limits and, therefore, insignificant. A completely deactivated system is most effective for analyzing US EPA Method 8081A samples on the same HP system used for the previous injections (Figure 5).

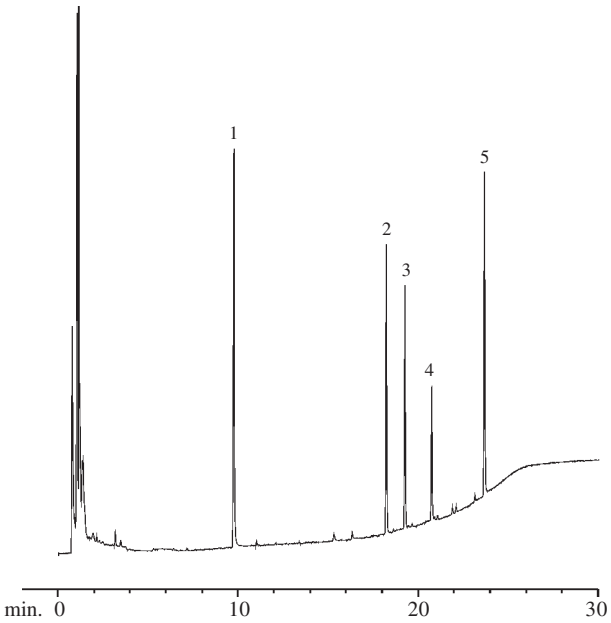
Several conclusions can be drawn from the results of this series of injections. It appears that endrin is more prone to break down on glass surfaces than metal, and DDT is more prone to break down on metal surfaces. In addition, direct injection reduces contact between the sample and the metal injection port. And, most importantly, careful deactivation of glass sleeves is crucial for minimizing endrin breakdown. Restek's Siltek™ deactivation yields a minimal endrin breakdown of 1%!

Our research demonstrates that a direct injection into a Siltek™-deactivated Uniliner® sleeve provides the best protection against problematic breakdown that occurs in the injection port when analyzing chlorinated pesticides. For a complete, highly inert pathway to analyze these compounds, Restek also offers analytical columns for chlorinated pesticide analysis (Rtx®-CLPesticides and Rtx®-CLPesticides2 columns) and Siltek™-deactivated guard columns.

For more information on Siltek™—the next generation of deactivation, please request our Siltek™ Benefits brochure (cat.# 59803).

Figure 4

Siltek™-deactivated Uniliner® inlet liners result in less than 1% endrin breakdown and undetectable DDT breakdown.

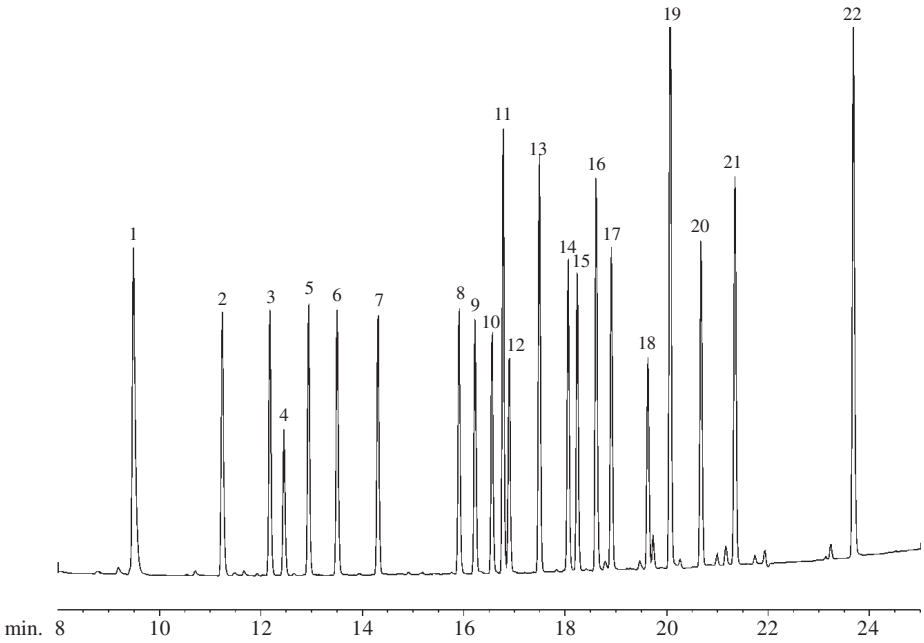


1. 2,4,5,6-tetrachloro-*m*-xylene
2. endrin
3. 4,4'-DDT
4. methoxychlor
5. decachlorobiphenyl

30m, 0.53mm ID, 0.42µm Rtx®-CLPesticides2 (cat.# 11340). **Inj.:** 1µL of 50pg/µL standard. **Oven temp.:** 120°C (hold 1 min.) to 300°C @ 9°C/min. (hold 10 min.); **Inj. temp.:** 250°C; **ECD temp.:** 300°C; **Carrier gas:** helium.

Figure 5

A completely deactivated GC system shows excellent resolution of the complete list of the chlorinated pesticides in US EPA Method 8081A.



1. 2,4,5,6-tetrachloro-*m*-xylene
2. α-BHC
3. γ-BHC
4. β-BHC
5. δ-BHC
6. heptachlor
7. aldrin
8. heptachlor epoxide
9. γ-chlordane
10. α-chlordane
11. 4,4'-DDE
12. endosulfan I
13. dieldrin
14. endrin
15. 4,4'-DDD
16. endosulfan II
17. 4,4'-DDT
18. endrin aldehyde
19. methoxychlor
20. endosulfan sulfate
21. endrin ketone
22. decachlorobiphenyl

30m, 0.32mm ID, 0.5µm Rtx®-CLPesticides (cat.# 11139) with a 5m, 0.32mm ID Siltek™-deactivated guard column (cat.# 10027) and a Siltek™-deactivated gooseneck liner (cat.# 20798-214.1). **On-column conc.:** 16-160pg; **Oven temp.:** 120°C (hold 1 min.) to 300°C @ 9°C/min. (hold 10 min.); **Inj. temp.:** 250°C, splitless (hold for 0.75 min.); **ECD temp.:** 300°C with anode purge; **Carrier gas:** helium, 31cm/sec.

Product Listing

Rtx®-CLPesticides Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.25	340°C	11120	11123
0.32mm	0.50	340°C	11136	11139
0.53mm	0.50	340°C	11137	11140

ID	df (µm)	Stable to	10m	20m
0.18mm	0.18	340°C	42101	42102

Rtx®-CLPesticides Column Kits

These kits include both a CLPesticides and CLPesticides2 column, a Universal Angled 'Y' Press-Tight® Connector, and a 5m guard column. (Note: Columns are not preconnected in these kits.)

Description	cat.#
0.53mm ID Rtx®-CLPesticides Kit	11197
0.32mm ID Rtx®-CLPesticides Kit	11198
0.25mm ID Rtx®-CLPesticides Kit	11199

Organochlorine Pesticide Mix AB #2

aldrin	8µg/mL	dieldrin	16µg/mL
α-BHC	8	endosulfan I	8
β-BHC	8	endosulfan II	16
δ-BHC	8	endosulfan sulfate	16
γ-BHC (lindane)	8	endrin	16
α-chlordane	8	endrin aldehyde	16
γ-chlordane	8	endrin ketone	16
4,4'-DDD	16	heptachlor	8
4,4'-DDE	16	heptachlor epoxide (B)	8
4,4'-DDT	16	methoxychlor	80

in hexane/toluene (1:1), 1mL/ampul.

	each	5-pack	10-pack
	32292	32292-510	
w/data pack	32292-500	32292-520	32392

Organochlorine Pesticide Mix C #2

chlorobenzilate	32µg/mL
diallate (cis & trans)	80
1,2-dibromo-3-chloropropane	8
hexachlorobenzene	8
hexachlorocyclopentadiene	8
isodrin	8

in hexane/toluene (1:1), 1mL/ampul.

	each	5-pack	10-pack
	32295	32295-510	
w/data pack	32295-500	32295-520	32395

Rtx®-CLPesticides2 Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.20	340°C	11320	11323
0.32mm	0.25	340°C	11321	11324
0.53mm	0.42	340°C	11337	11340

ID	df (µm)	Stable to	10m	20m
0.18mm	0.14	340°C	42301	42302

Siltek™-Deactivated Guard Columns

nominal ID	nominal OD	5-meter	10-meter
0.25mm	0.37 ± 0.04mm	10026	10036
0.32mm	0.45 ± 0.04mm	10027	10037
0.53mm	0.69 ± 0.04mm	10028	10038

Siltek™-Deactivated Press-Tight® Connectors

type	qty.	cat.#
straight	25-pk.	20449
angled 'Y'	3-pk.	20469

For other Siltek™-deactivated Press-Tight® connectors, add suffix "-266" to the catalog number.

Siltek™-Deactivated Inlet Liners

Siltek™	Siltek™ with Siltek™-deact. wool	Siltek™ with CarboFrit™	qty.
-214.1	-213.1	-216.1	each
-214.5	-213.5	-216.5	5-pk.
-214.25	-213.25	-216.25	25-pk.

For Siltek™-deactivated liners, add the corresponding suffix number to the liner's catalog number.

Siltek™-Deactivated Borosilicate Wool

qty.	cat.#
10 grams	21100

For more information on Siltek™— the next generation of deactivation, please request our Siltek™ Benefits brochure (cat.# 59803).

Restek Trademarks: Press-Tight, Rtx, Siltek, Uniliner.

RESTEK ISO 9001 REGISTERED

RESTEK environmental

Applications note

Siltek™ Deactivation Delivers Inertness to Analyte Breakdown and Reactivity, and Durability to Physical and Chemical Challenges

A common concern in gas chromatographic (GC) analyses is the interaction of analytes with active surfaces in the GC pathway. The injection port is the first source of active sites, often leading to adsorption and breakdown of analytes. However, not all analyses are affected by reactivity within the injection port. Hydrocarbons, typically, are not susceptible to adsorption or breakdown. In contrast, active compounds such as pesticides, drugs, phenols, amines, and alcohols, which are often injected via splitless mode, are more prone to these problems. With a splitless injection, carrier gas flow rate through the liner is very slow, increasing the sample residence time in the injector and the chance for reactivity. Complete and effective liner deactivation is crucial to minimize available active sites and ensure repeatable results.

Restek has designed Siltek® deactivation to deliver both enhanced inertness and durability. Gas chromatography accessories coated with Siltek® deactivation provide durability for matrices of extreme pH or high-temperature applications.

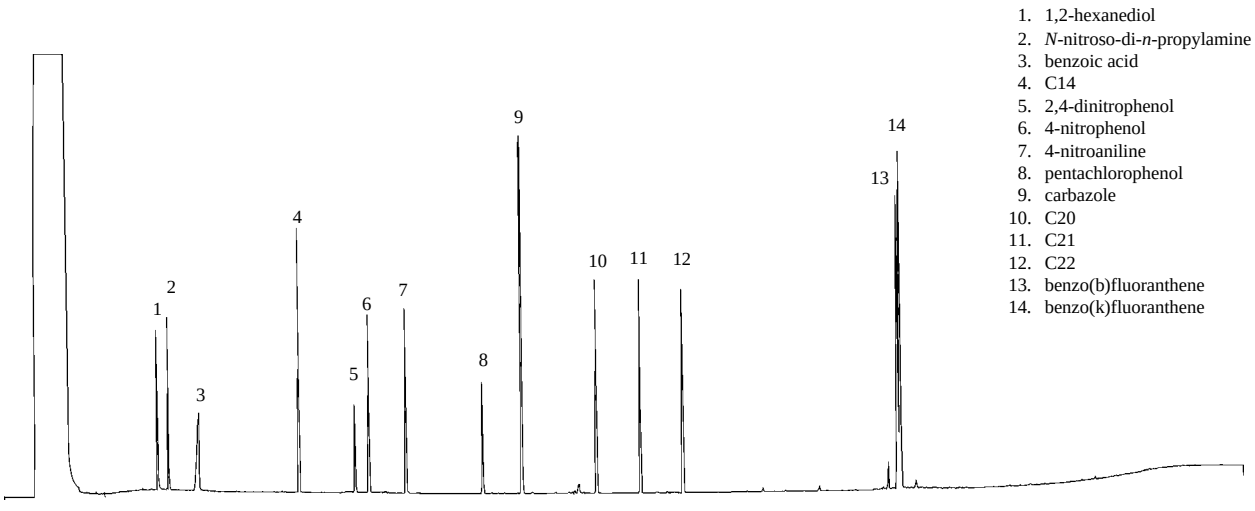
Inertness

Semivolatile analysis places extreme demands on the GC system. One key to successfully analyzing semivolatiles is having the capability to handle basic and acidic compounds in the GC system. The analytical column must provide selectivity for both classes without resulting in poor peak shape. Additionally, liner deactivation is critical to analytical success because the vaporized sample comes in contact with the inlet liner first.

The Restek XTI test mix was chosen to evaluate the inertness of a Siltek™-deactivated liner. This mix contains both acidic and basic probes, some of which are pollutants monitored in US Environmental Protection Agency (EPA) Method 8270 (4-nitroaniline, *N*-nitroso-di-*n*-propylamine, 2,4-dinitrophenol, pentachlorophenol, benzoic acid, benzo(b)- and benzo(k)fluoranthene). A splitless injection of the XTI mix with an on-column concentration of 4-10ng shows an excellent response for all of the probes, including the active compounds dinitrophenol, 1,2-hexanediol, and benzoic acid (Figure 1).

Figure 1

Siltek™-deactivated liner shows excellent inertness for acidic and basic probes.



30m, 0.25mm ID, 0.25µm XTI®-5 (cat.# 12223) with a Siltek™-deactivated 4mm splitless single gooseneck sleeve (cat.# 20798-214.1). **Oven temp.:** 40°C (hold 2 min.) to 100°C @ 30°C/min., to 180°C @ 9°C/min., to 330°C @ 30°C/min. (hold 10 min.); **Inj. temp.:** 250°C; **Det.:** 330°C; **Carrier gas:** He.

environmental

Thermal Stability

To test the durability of the Siltek® liner deactivation, two sources of stress were investigated—high inlet temperature over a period of 10 days and repeated exposure to aqueous injections of low, then high pH. High inlet temperatures can promote degradation of the deactivation layer by causing it to bake or bleed off of the liner. In the first study, a baseline splitless XTI injection was performed, and response factors (relative to C14) were calculated. The injection port was then set at 330°C overnight and another XTI injection was made. This process was continued for 10 days. After 10 days at 330°C, the Siltek® deactivation retained its integrity, achieving essentially unchanged response factors, even for the critical probes (Figure 2).

Resistance to Chemical Attack

For the next durability study, a Siltek®-deactivated liner was repeatedly exposed to aqueous HCl injections, pH 1.4. The ability to withstand low pH aqueous samples is important with environmental applications that require acidification of the matrix. Very low or very high pH samples can cause pinpoint holes in the deactivation layer that will eventually undercut the layer and strip it away. For this study, a baseline XTI injection was made via direct injection and relative response factors were calculated. In the direct injection mode, a leak-free connection is formed in the liner, minimizing sample exposure within the injection port. Ten microliters of the pH 1.4 sample were injected, followed by a direct injection of the XTI test mixture.

This cycle continued until a total of 180µL were injected (Figure 3). Key probes, such as 2,4-dinitrophenol (DNP), pentachlorophenol (PCP), *N*-nitroso-di-*n*-propylamine (*n*-propylamine), and 1,2-hexanediol (diol) retained their responses up to at least 120µL injected.

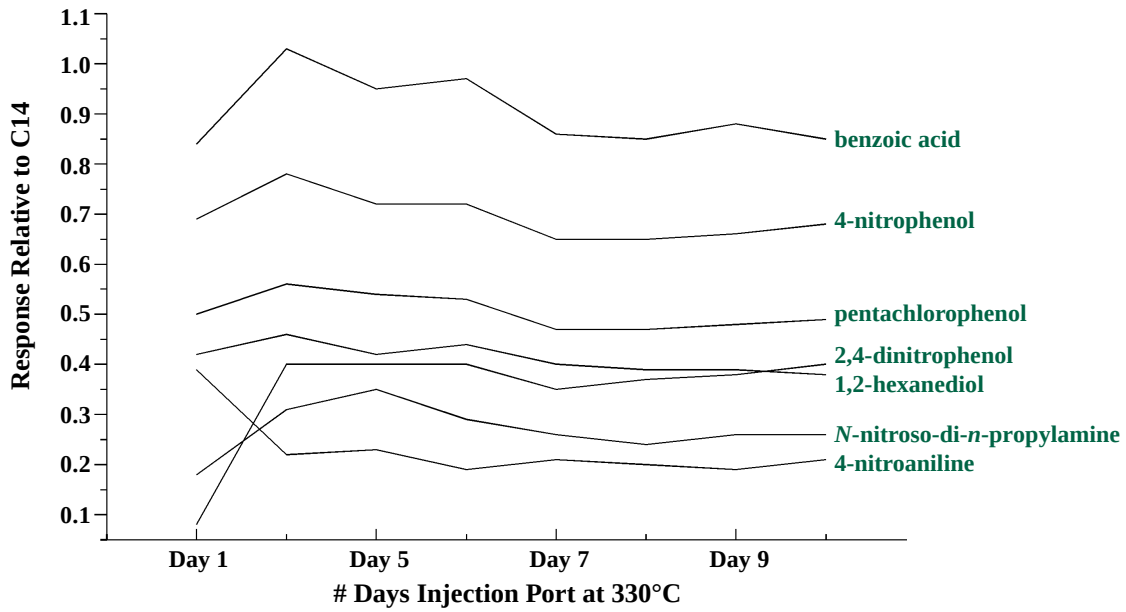
The experiment was repeated with an identical set-up using aqueous NH_4OH injections, pH 10.1 (Figure 4). Under these demanding conditions, the response for the XTI compounds was consistent for 70 injections. As expected, the response of the acidic compounds began to decrease with repeated injections but many compounds continued to have excellent response for more than 120µL injections.

Siltek® deactivation offers both inertness and resistance to temperature and pH extremes within a GC system. It is available as a deactivation for fused silica guard columns and inlet liners.

For more information on Siltek™—the next generation of deactivation, please request our Siltek™ Benefits brochure (cat.# 59803).

Figure 2

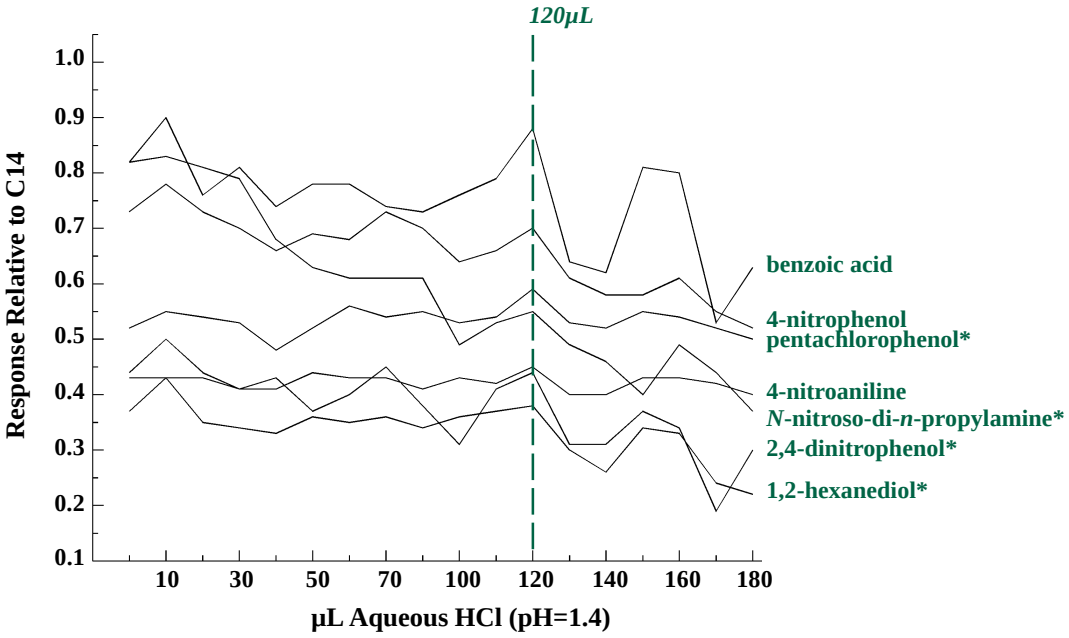
Siltek™ deactivation remains effective at an injection port temperature of 330°C for ten days.



30m, 0.25mm ID, 0.25µm XTI®-5 (cat.# 12223) with a Siltek™-deactivated splitless sleeve (cat.# 20798-214.1). **Oven temp.:** 40°C (hold 2 min.) to 190°C @ 6°C/min., to 330°C @ 30°C/min. (hold 10 min.); **Inj. temp.:** 330°C; **Det.:** 330°C; **Carrier gas:** He.

Figure 3

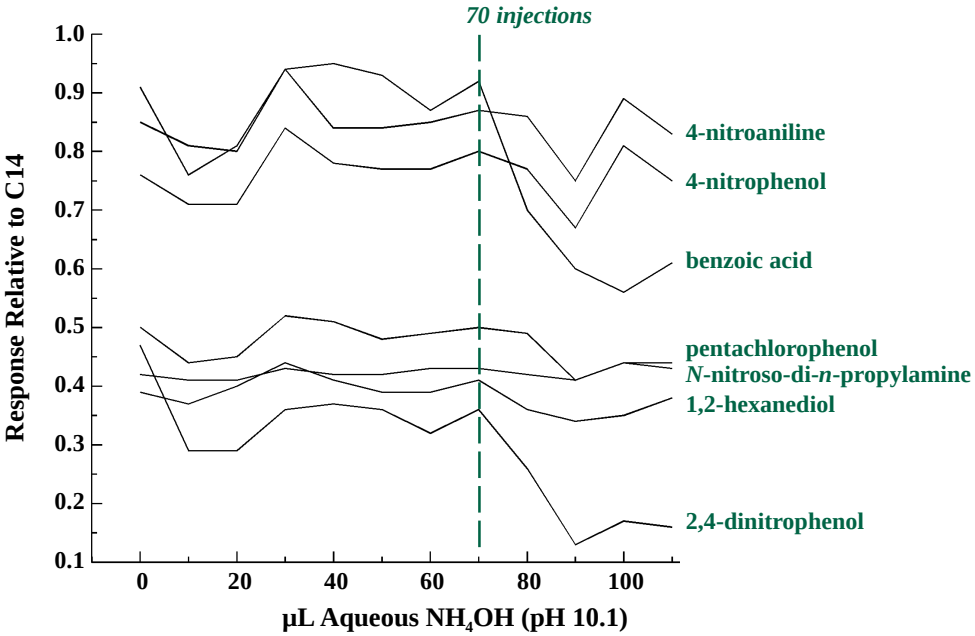
Difficult probes retain their response on Siltek™ deactivation up to 120µL of an aqueous solution of pH 1.4.*



30m, 0.25mm ID, 0.25µm XTI®-5 (cat.# 12223) with a Siltek™-deactivated 4mm open-top Uniliner® w/o wool (cat.# 20843-214.1).
Oven temp.: 40°C (hold 2 min.) to 190°C @ 6°C/min., to 330°C @ 30°C/min. (hold 10 min.); **Inj. temp.:** 250°C; **Det.:** 330°C; **Carrier gas:** He.

Figure 4

Response for XTI compounds on Siltek™ deactivation was consistent for 70 injections of an aqueous solution at pH 10.1.



30m, 0.25mm ID, 0.25µm XTI®-5 (cat.# 12223) with a Siltek™-deactivated 4mm open-top Uniliner® w/o wool (cat.# 20843.214.1).
Oven temp.: 40°C (hold 2 min.) to 190°C @ 6°C/min., to 330°C @ 30°C/min. (hold 10 min.); **Inj. temp.:** 250°C; **Det.:** 330°C; **Carrier gas:** He.

Product Listing

XTI®-5 Columns

ID	df (µm)	Temp. limits	15m	30m
0.25mm	0.25	-60 to 360°C	12220	12223
	0.50	-60 to 330/350°C	12235	12238
	1.00	-60 to 325/350°C	12250	12253
0.32mm	0.25	-60 to 360°C	12221	12224
	0.50	-60 to 330/350°C	12236	12239
	1.00	-60 to 325/350°C	12251	12254
0.53mm	0.50	-60 to 330/360°C	12237	12240
	1.00	-60 to 325/350°C	12252	12255
	1.50	-60 to 310/330°C	12267	12270

Inlet Liners for HP GCs

Liner type	ID/OD/Length (mm)	ea.	5-pk.	25-pk.
4mm split w/wool	4.0/6.3/78.5	20781	20782	20783
2mm splitless	2.0/6.5/78.5	20712	20713	20714
4mm splitless	4.0/6.5/78.5	20772	20773	20774
4mm gooseneck	4.0/6.5/78.5	20798	20799	20800
4mm double gooseneck	4.0/6.5/78.5	20784	20785	20786
Cyclosplitter®	4.0/6.3/78.5	20706	20707	20708

Inlet Liners for Varian GCs

Liner type	ID/OD/Length (mm)	ea.	5-pk.	25-pk.
2mm splitless	2.0/6.3/74	20721	20722	20723
4mm splitless	4.0/6.3/74	20904	20905	20906
0.5mm SPI	0.53/4.6/54	20775	20776	20777
0.8mm SPI	0.80/4.6/54	20778	20779	20780
SPI with buffer	2.4/4.6/54	20850	20851	20852

Siltek™-Deactivated Guard Columns

Nominal ID	Nominal OD	5m	10m
0.25mm	0.37 ± 0.04mm	10026	10036
0.32mm	0.45 ± 0.04mm	10027	10037
0.53mm	0.69 ± 0.04mm	10028	10038

Siltek™-Deactivated Press-Tight® Connectors

Type	Qty.	cat.#
straight	25-pk.	20449
angled 'Y'	3-pk.	20469

For other Siltek™-deactivated Press-Tight® connectors, add suffix "-266" to the catalog number.

Siltek™-Deactivated Inlet Liners

Siltek™	Siltek™ with Siltek™-deact. wool	Siltek™ with CarboFrit™	Qty.
-214.1	-213.1	-216.1	each
-214.5	-213.5	-216.5	5-pk.
-214.25	-213.25	-216.25	25-pk.

For Siltek™-deactivated liners, add the corresponding suffix number to the liner's catalog number.

Siltek™-Deactivated Glass Wool

Qty.	cat.#
10 grams	21100

For more information on Siltek™— the next generation of deactivation, please request our Siltek™ Benefits brochure (cat.# 59803).

Restek Trademarks: Cyclosplitter, Press-Tight, Rtx, Siltek, Uniliner.

RESTEK ISO 9001 REGISTERED

RESTEK pharmaceutical

Applications note

Allure™ PFP Propyl and Ultra PFP HPLC Columns Provide Improved Analyses of Basic Compounds

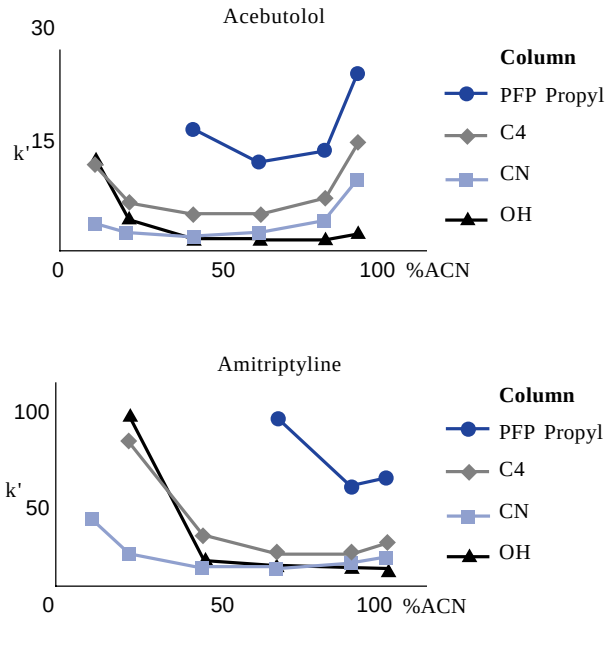
High performance liquid chromatography (HPLC) methods must be optimized to provide the greatest selectivity and sensitivity, and the best peak shape. Unfortunately, many analysts consider switching the stationary phase—the heart of the HPLC system—only as a last resort. Too often analysts coax the stationary phase to perform a non-native separation by using modifiers and ion pairing agents, which leads to reduced sensitivity and equilibration problems (the C18 phase is the most often misused). Selection of the proper stationary phase for your separation can improve LC sensitivity, analyte retention, and peak shape without the use of modifiers or ion pairing agents. For example, Restek's Allure™ PFP Propyl and Ultra PFP HPLC columns easily perform separations of many basic analytes.

Research shows the Allure™ PFP Propyl stationary phase not only provides the greatest retention and capacity factor (k') (Figure 1) for basic analytes such as beta blockers and tricyclic antidepressants, but also the best peak shape. "…The results indicate that both the fluorine groups and the propyl chain are important on the phenyl ring to obtain the best peak shape and retention of the basic solutes when ammonium formate:acetonitrile (10:90) is used as the mobile phase."[1] As peak asymmetry is improved, sensitivity is increased.

Figure 1

Allure™ PFP Propyl demonstrates increased retention of basic analytes

5mM NH_4OAc, pH 4.5:ACN; 30x2.1mm; 0.4mL/min.



Data Courtesy of Shane Needham, Pfizer Inc PLC-0001

Basic analytes are difficult to retain on C18 phases if the analytes have pKa's greater than 8. They can be retained on C8 or C18 columns by using modifiers, but at the expense of sensitivity.[2] Sensitivity can be reduced further on an LC/mass spectrometer (MS) ESI interfaces when the buffer concentrations exceed 50mM. The Allure™ PFP Propyl and Ultra PFP columns eliminate the need for modifiers, and analytes such as cocaine (COC) and its metabolite, ecgonine methyl ester (EME), can be separated and retained using 90% acetonitrile in under 4.5 minutes (Figure 2). As the concentration of the organic solvent in the mobile phase increases, the desolvation process becomes more effective and the LC/MS ESI signal increases.[3] Because of this interaction, using the Allure™ PFP Propyl column with a high organic concentration increases the response of COC and other basic solutes by as much as twelve times over that from a C18 column.[4]

Proper retention also is needed to separate basic analytes from naturally occurring substances in the blood, urine, or body tissues. If the analytes elute too closely to the void volume, ionization suppression can occur.[5] To be cost effective, however, the analytes should be separated in less than 6 minutes. The high selectivity of the Allure™ PFP Propyl column more than adequately separates EME from COC and the column void volume, respectively. (Figure 2).[6]

The Ultra PFP column is similar to the Allure PFP Propyl. The PFP column features a 100 angstrom pore size and is available in 3 and 5 micron particle sizes. Like the PFP Propyl, the PFP has high retention for basic and multi-halogenated analytes. Table I displays the retention of many basic beta-blockers on the PFP phase versus many other phases using 90% acetonitrile as the mobile phase. The HPLC/MS ESI analysis of beta-blockers using the Ultra PFP column shows good intensity (Figure 6). The PFP phase is also able to retain multi-halogenated analytes such as the thyroid hormone, levothyroxine, and purines and pyrimidines (Figures 4 & 5). The PFP phase has the greatest retention, which will improve the separation of one analyte from another.

The attraction mechanism of pi-acid HPLC phases, such as PFPP and PFP, allow stronger retention of bases than alkyl phases like C18. Alkyl phases like C18 cannot adequately retain strongly basic analytes unless they have significant non-polar functional groups to allow hydrophobic retention.

pharmaceutical

Therefore, Restek's Allure™ PFP Propyl and Ultra PFP columns are the best choice for the analysis of basic analytes. COC, EME, quinine, morphine, beta-blockers, and tricyclic antidepressants have been analyzed successfully in urine, blood, and tissue samples. In addition, pyridines, pyrimidines, and multi-halogenated compounds have been successfully separated on the Ultra PFP column.

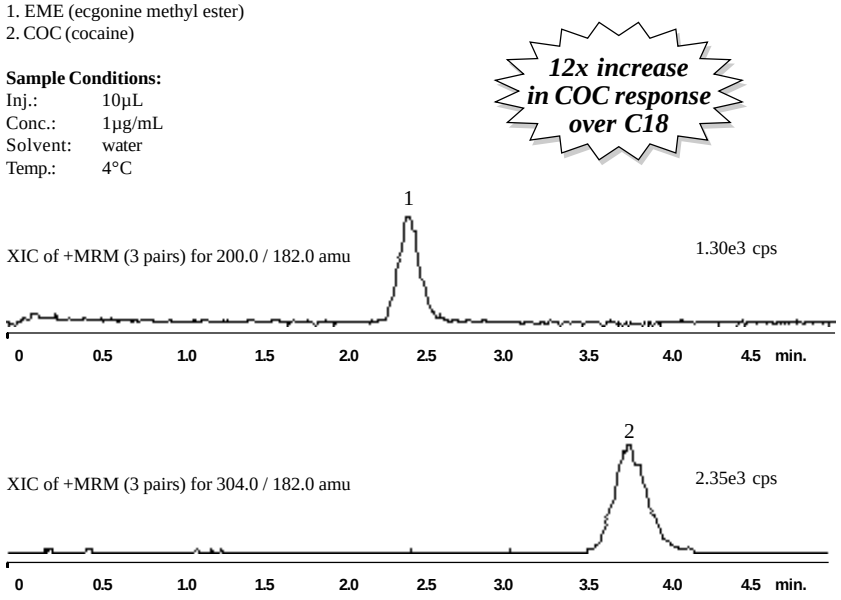
The Allure™ PFP Propyl and Ultra PFP columns provide superior retention and peak shape for analytes having a pKa >8 without the need for modifers, and they can provide increased sensitivity for LC/MS ESI due to the high level of organic solvent used in the mobile phase.

Figure 2

Allure™ PFP Propyl column baseline separates cocaine (COC) and its metabolite, ecgonine methyl ester (EME), with excellent peak shape in less than five minutes.

Peak List:
1. EME (ecgonine methyl ester)
2. COC (cocaine)

Sample Conditions:
Inj.: 10µL
Conc.: 1µg/mL
Solvent: water
Temp.: 4°C



Column:	**Allure™ PFP Propyl**
Catalog#:	9169532
Dimensions:	30mm x 2.1mm
Particle size:	5µm
Pore size:	60Å
Conditions:	
Mobile phase:	5mM pH 3.0 ammonium formate: acetonitrile (10:90)
Flow:	0.6mL/min.
Column temp.:	ambient
Det.:	PE/Sciex API 3000
Interface:	Turbo Ion Spray, ESI
Interface temp.:	150°C
Ion mode:	positive
ESI probe voltage:	5000V
Orifice:	+/- 71V
Ring:	+/- 265V
Collision gas:	Nitrogen
Collision gas pressure:	2.2 mTorr
Collision gas energy(COC):	28 eV
Collision gas energy(EME):	26 eV
Electron multiplier:	2100 volts
Auxillary gas flow:	7000cc/min.
Nebulizer gas setting:	15 lb/in.^{2}
Curtain gas setting:	12 lb/in.^{2}

Data Courtesy of Shane Needham, Pfizer Inc

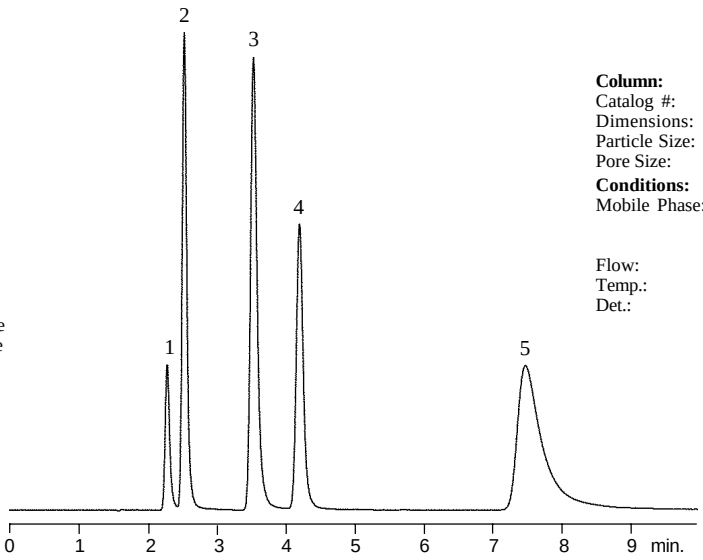
LC_0126

Figure 3

Nucleic bases as guanine, cytosine, uradine, thymine, and adenine are all easily retained upon the Allure™ PFP Propyl.

Peak List:
1. cytosine
2. uracil
3. guanine
4. thymine
5. adenine

Sample:
Inj.: 1.0 mL
Conc. 100µg/mL
Solvent: 20 mM Ammonium Acetate, pH 5.0. Ammonium hydroxide added to keep guanine in solution



Column:	**Allure™ PFP Propyl**
Catalog #:	9169565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	60Å
Conditions:	
Mobile Phase:	20mM Ammonium Acetate, pH 5.0: Methanol (85:15, v/v)
Flow:	1.0mL/min
Temp.:	ambient
Det.:	UV @ 260nm

LC_0137

Figure 4

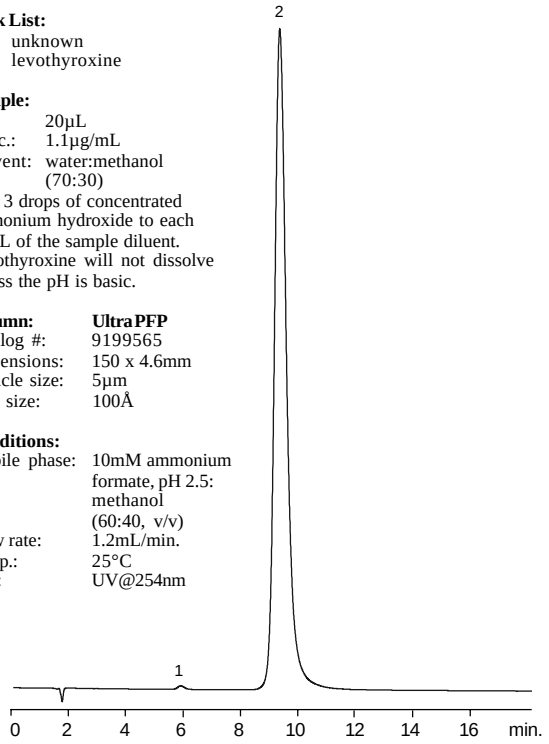
Multi-halogenated analytes as levothyroxine can also take advantage of the Ultra PFP's strong retention

Peak List:
1. unknown
2. levothyroxine

Sample:
Inj.: 20µL
Conc.: 1.1µg/mL
Solvent: water:methanol (70:30)
Add 3 drops of concentrated ammonium hydroxide to each 10mL of the sample diluent. Levothyroxine will not dissolve unless the pH is basic.

Column: **Ultra PFP**
Catalog #: 9199565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 10mM ammonium formate, pH 2.5: methanol (60:40, v/v)
Flow rate: 1.2mL/min.
Temp.: 25°C
Det.: UV@254nm



LC_0093

Figure 5

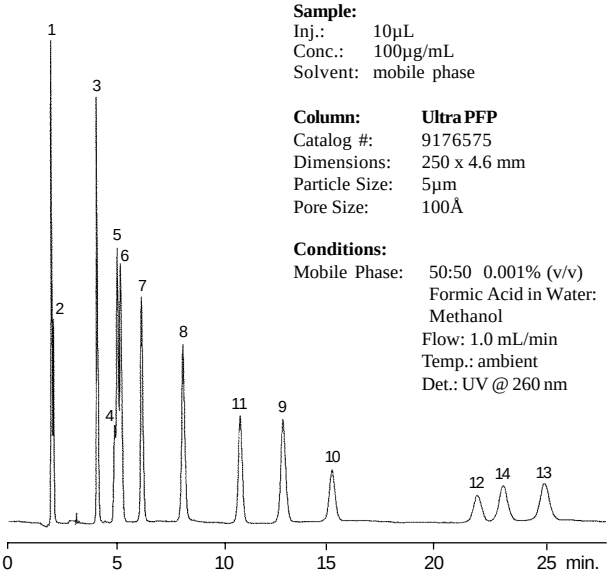
The strong retention of the PFP makes it an excellent phase for the analysis of purines and pyrimidines.

Peak List:
1. cytosine
2. 5-methylcytosine
3. uracil
4. uric acid
5. hypoxanthine
6. xanthine
7. thymine
8. purine
9. 6-methylpurine
10. theobromine
11. ethyluracil
12. 1,7-dimethylxanthine
13. theophylline
14. propyluracil

Sample:
Inj.: 10µL
Conc.: 100µg/mL
Solvent: mobile phase

Column: **Ultra PFP**
Catalog #: 9176575
Dimensions: 250 x 4.6 mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 50:50 0.001% (v/v) Formic Acid in Water: Methanol
Flow: 1.0 mL/min
Temp.: ambient
Det.: UV @ 260 nm

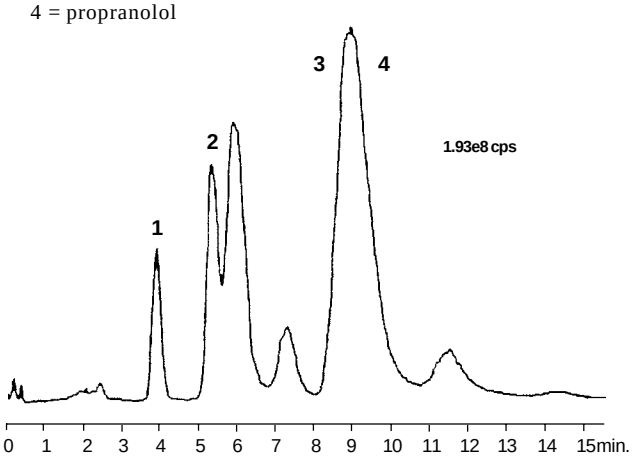


LC_0136

Figure 6

HPLC/MS ESI analysis of beta-blockers on the Ultra PFP column shows good intensity

Peak list:
1 = atenolol
2 = acetbutolol
3 = alprenolol
4 = propranolol



Data courtesy of Shane Needham, Pfizer Inc

Table I

Retention Data of Basic Solutes with 90% Acetonitrile

	Retention Time (min.)			
β-blockers	**CN**	**OH**	**PFP**	**C4***
Acebutolol	3.22	2.02	6.32	<0.5
Alprenolol	3.79	1.74	9.99	<0.5
Atenolol	3.04	2.15	4.63	<0.5
Labetolol	3.56	1.32	4.93	<0.5
Metoprolol	3.42	1.83	7.70	<0.5
Nadolol	3.37	2.15	5.58	<0.5
Oxprenolol	3.81	1.78	9.20	<0.5
Pindolol	3.64	1.81	6.60	<0.5
Propranolol	3.27	1.78	10.3	<0.5
Sotalol	3.12	1.69	5.09	<0.5
Timolol	3.37	1.74	7.53	<0.5

*<20% acetonitrile needed for adequate retention

Data courtesy of Shane Needham, Pfizer Inc

References

1. S. R. Needham, P.R. Brown, and K. Duff, *Rapid Commun. Mass Spectrom.* 13, 2231-2236 (1999).
2. J. Svensson., *J. Anal. Toxicol.*, 10 (1986) 122-124.
3. P. Sjoberg and K. Markides., *J. Chromatogr. A.*, 855 (1999) 317-327.
4. S. R. Needham, *op. cit.*
5. B. Matuszewski, M. Constanzer, and C. Chavez-Eng. *Anal. Chem* 70 (1998) 882-889.
6. S. R. Needham, *op. cit.*

References not available from Restek.

Allure™ PFP Propyl, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9169531	9169532	9169533	9169535
50mm	9169551	9169552	9169553	9169555
100mm	9169511	9169512	9169513	9169515
150mm	9169561	9169562	9169563	9169565
200mm	9169521	9169522	9169523	9169525
250mm	9169571	9169572	9169573	9169575

Allure™ PFP Propyl, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9169532-700	9169533-700	9169535-700
50mm	—	9169552-700	9169553-700	9169555-700
100mm	—	9169512-700	9169513-700	9169515-700
150mm	—	9169562-700	9169563-700	9169565-700
200mm	—	9169522-700	9169523-700	9169525-700
250mm	—	9169572-700	9169573-700	9169575-700

Ultra PFP, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9176531	9176532	9176533	9176535
50mm	9176551	9176552	9176553	9176555
100mm	9176511	9176512	9176513	9176515
150mm	9176561	9176562	9176563	9176565
200mm	9176521	9176522	9176523	9176525
250mm	9176571	9176572	9176573	9176575

Ultra PFP, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9176532-700	9176533-700	9176535-700
50mm	—	9176552-700	9176553-700	9176555-700
100mm	—	9176512-700	9176513-700	9176515-700
150mm	—	9176562-700	9176563-700	9176565-700
200mm	—	9176522-700	9176523-700	9176525-700
250mm	—	9176572-700	9176573-700	9176575-700

RESTEK environmental

Applications note

Rtx®-CLPesticides and Rtx®-CLPesticides2 Columns: The Ideal Confirmational Pair for Analyzing Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are a group of industrial organochlorine chemicals that have become a major environmental concern. Since the 1950's, over one million metric tons of PCBs have been produced. They are very persistent in the environment and bioaccumulate in living systems. All PCBs are practically insoluble in water, but they are soluble in hydrophobic media like fats or oily substances. They were used commercially because they are chemically inert liquids and are difficult to burn; they have low vapor pressures, are inexpensive to make, and are excellent electrical insulators. As a result, they were used extensively as coolant fluids in transformers and capacitors; and later as plasticizers, de-inking solvents, heat transfer fluids in machinery, and water-proofing agents, among other uses.

Because of their persistence and their solubility in fatty tissue, PCBs in food chains undergo biomagnification. Strong heating of PCBs in the presence of oxygen can lead to the formation of polychlorodibenzofurans (PCDF), which are structurally and toxicologically similar to dioxins. Commercial PCB mixtures (e.g., Aroclor® mixtures) contain small amounts of PCDF as a result of the synthesis. PCB mixtures are not highly toxic, but toxicity due to the PCDF concentration has caused concern.

Certain PCB congeners can be highly toxic; toxicity depends on where the chlorine substitution resides on the biphenyl molecule. The congeners without chlorine substitution on the ortho positions are the most toxic. These are termed "coplanar PCBs" because the phenyl rings can maintain a planar geometry to each other. This makes these compounds "dioxin-like," and the most toxic of these PCB congeners is one-tenth the toxicity of the 2,3,7,8-tetrachloro dibenzo dioxin. The coplanar PCBs also have been implicated as endocrine disrupters.[1]

It is important, therefore, when designing a PCB analysis method to determine if the separation will be by specific congener (for toxicity) or by commercial Aroclor® mixture. The commercial synthesis of PCBs results in chlorination of the biphenyl molecule, and this reaction produces a mixture of many of the 209 congeners of the PCB family.

Naming of the specific congeners follows the positional numbering shown in Figure 1. Because the IUPAC names for these compounds are long, the congeners are normally referred to by their IUPAC number or BZ number as defined by Ballschmitter and Zell.[2] The exact proportions of congeners in the Aroclor® mixtures depends on the ratio of chlorine to biphenyl, the reaction time, and the temperature. Although many of the PCB compounds are solids, the mixtures usually are liquids or low-melting-point solids. Commercially, the PCB compounds were not isolated. Instead, they were sold as partially separated mixtures, with the average chlorine content in different products ranging from 21% to 68%. These Aroclor® mixtures are, therefore, composed of a number of individual PCB congeners and have a characteristic profile depending on the percent of chlorine substitution. The Aroclor® mixtures are named by the number of carbons (12), followed by the weight % of chlorine (42). Thus Aroclor® 1242 represents a mixture of PCB compounds with an average weight percent of 42.

There are 9 common Aroclor® mixtures: 1221, 1232, 1242, 1248, 1254, 1260, 1262, 1268, and 1016. Aroclor® 1016 does not follow the same naming sequence, and appears chromatographically similar to 1242. Samples from contamination sites often are quantitated and reported as concentration of PCBs, i.e., as Aroclor® mixtures. This analysis requires the individual PCB Aroclor® mixtures to be analyzed as standards, then the sample extract chromatograms are compared to the standards to qualitatively identify the Aroclor® mixtures. Once this identification has been made, the quantitation can be performed by selecting five of the largest peaks and treating them as individual compounds, then reporting the average concentration.

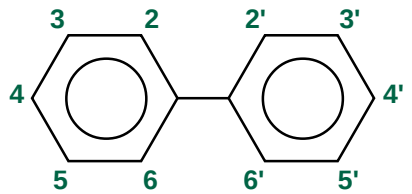
Due to the unreactive nature of the PCBs, instrument conditions and column choice is less critical than when analyzing chlorinated pesticides. When choosing columns, it is important to select stationary phases that have low bleed and high thermal stability; allowing the columns to be baked out at the end of the run to prevent carryover from one injection to the next. Because many instruments used for the analysis of PCBs also may be used for pesticide and herbicide analyses, the column pair of choice is the Rtx®-CLPesticides and Rtx®-CLPesticides2 columns. This column pair provides excellent separation of the pesticide and herbicide compounds, low bleed, high thermal stability, and they are designed to compliment each other for primary column analysis and secondary column confirmation.

Figure 2 shows the chromatograms obtained for seven commercial Aroclor® mixtures injected on the Rtx®-CLPesticides column. Figure 3 shows the chromatograms for the same mixtures injected on the Rtx®-CLPesticides2 column.

Table 1 (on back) lists the retention times for the individual 209 PCB congeners on these same stationary phases. The analysis of PCBs by congener requires each peak to be treated like an individual component; making a standard curve for each of the congeners of interest. While many laboratories are interested in the analysis of PCBs by congener, most do not need, or desire, to analyze all 209. For this reason, the retention table is listed, and conditions may be modified to better suit the particular separation in your laboratory. If you have questions regarding the analysis of PCBs by congener, contact Restek's technical service team at 800-356-1688 or 814-353-1300, ext. 4, or contact your local Restek representative.

Figure 1

Structure of polychlorinated biphenyls.

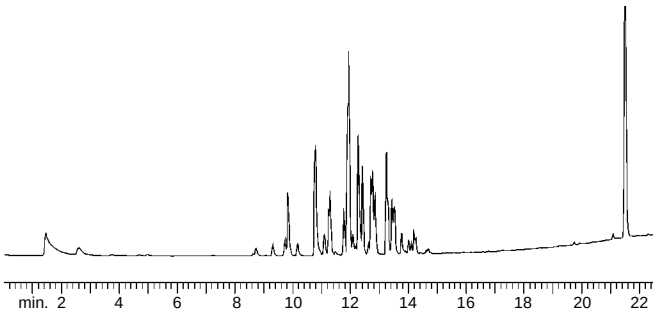


environmental

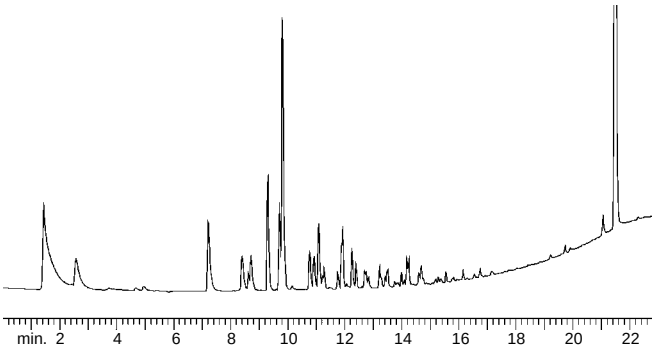
Figure 2

Aroclor® standards run on the Rtx®-CLPesticides column at 320ppb.

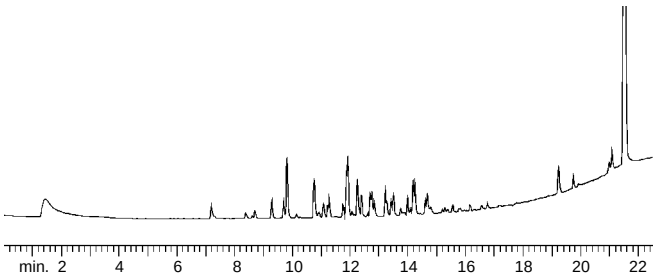
Aroclor® 1016



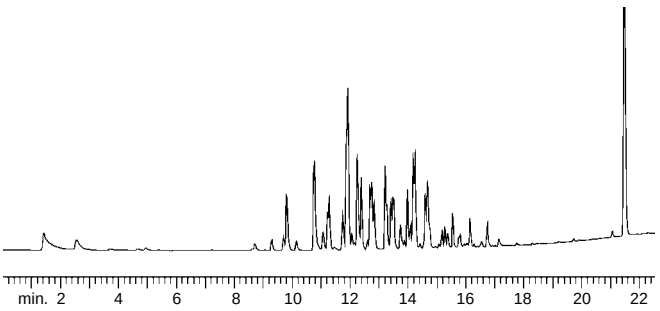
Aroclor® 1221



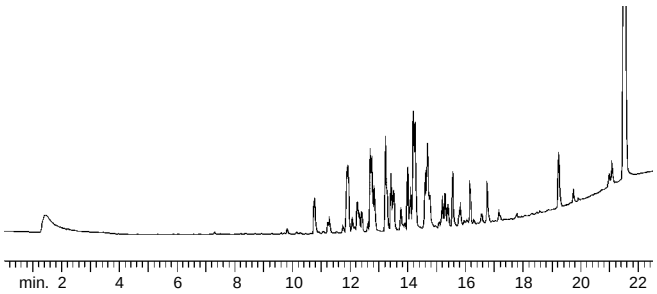
Aroclor® 1232



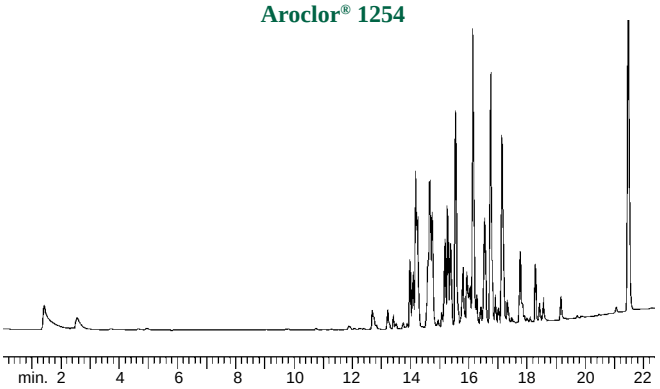
Aroclor® 1242



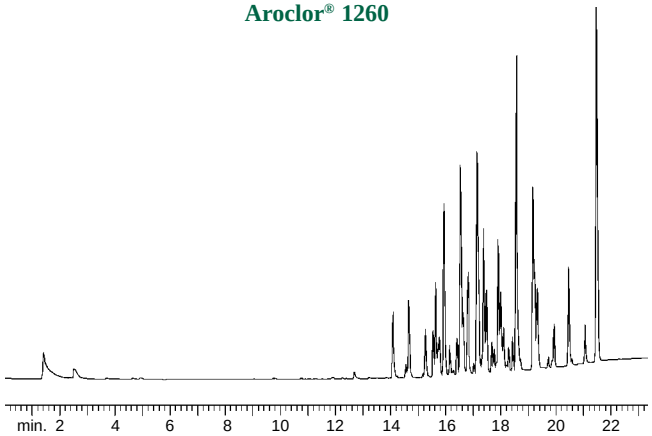
Aroclor® 1248



Aroclor® 1254



Aroclor® 1260

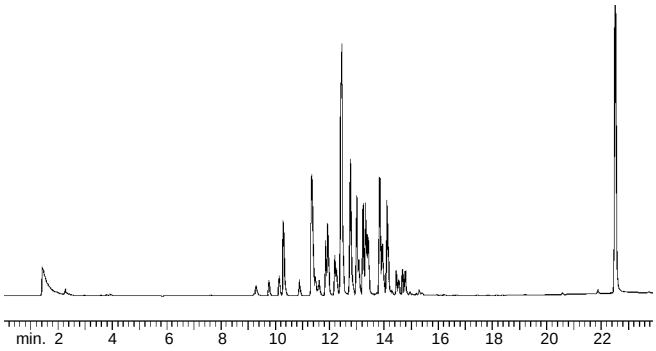


30m, 0.32mm ID, 0.50µm Rtx®-CLPesticides (cat.# 11139). 0.5µL direct injection of commercial Aroclor® mixtures, 160pg on-column concentration. **Oven temp.:** 120°C (hold 1 min.) to 300°C @ 9°C/min. (hold 10 min.); **Inj. temp.:** 210°C; **Det.:** ECD with anode purge, 310°C; **Carrier gas:** He.

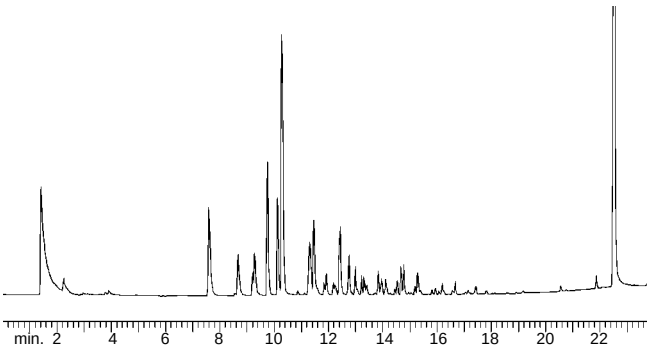
Figure 3

Aroclor® standards run on the Rtx®-CLPesticides2 column at 320ppb.

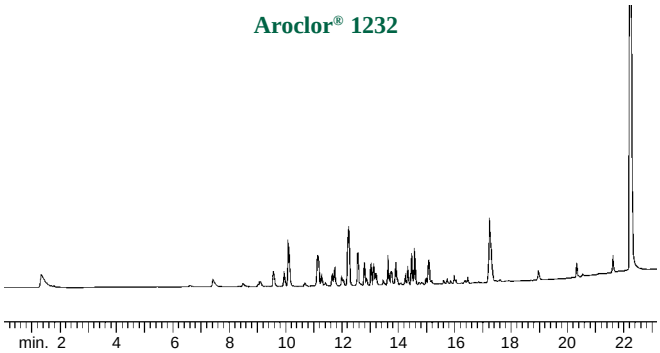
Aroclor® 1016



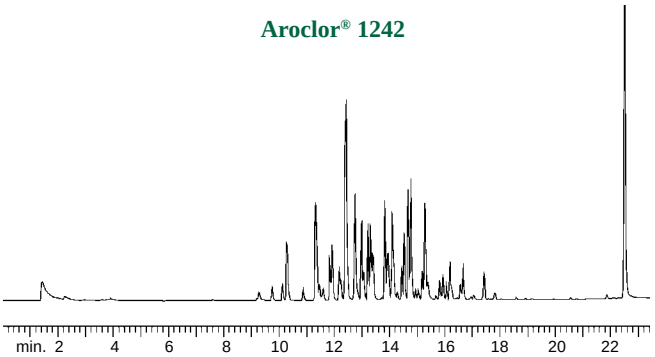
Aroclor® 1221



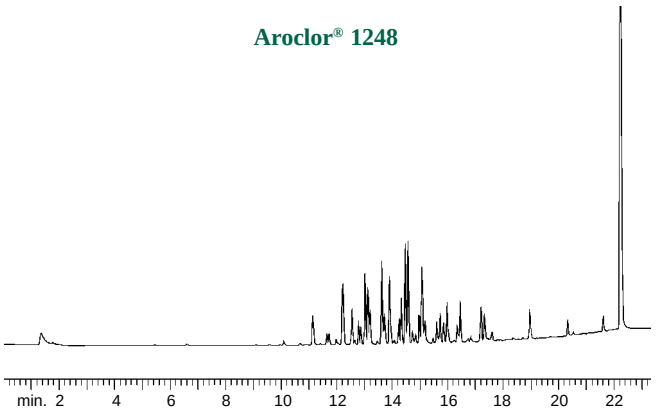
Aroclor® 1232



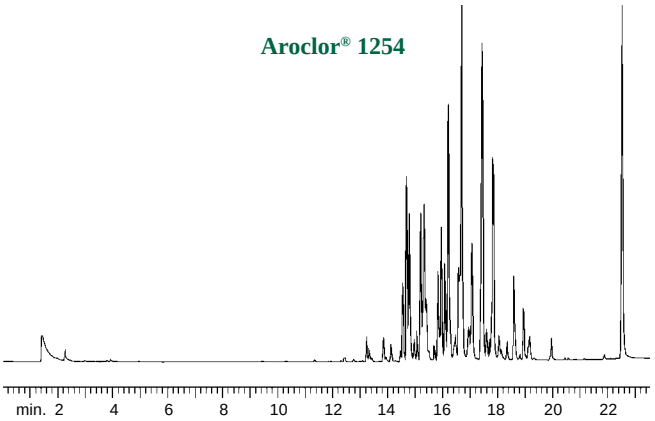
Aroclor® 1242



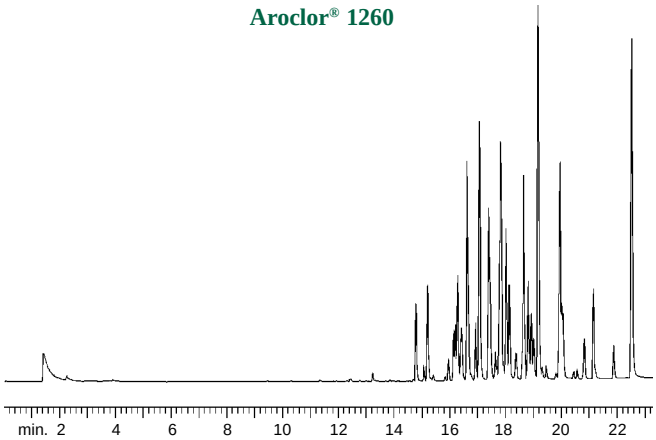
Aroclor® 1248



Aroclor® 1254



Aroclor® 1260



30m, 0.32mm ID, 0.25µm Rtx®-CLPesticides2 (cat.# 11324). 0.5µL direct injection of commercial Aroclor® mixtures, 160pg on-column concentration. **Oven temp.:** 120°C (hold 1 min.) to 300°C @ 9°C/min. (hold 10 min.); **Inj. temp.:** 210°C; **Det.:** ECD with anode purge, 310°C; **Carrier gas:** He.

Table I—PCB Congener Retention Time Data

Rtx®-CLP PCB IUPAC#	Rtx®-CLP RT (min)	Rtx®-CLP2 PCB IUPAC#	Rtx®-CLP2 RT (min)	Rtx®-CLP PCB IUPAC#	Rtx®-CLP RT (min)	Rtx®-CLP2 PCB IUPAC#	Rtx®-CLP2 RT (min)	Rtx®-CLP PCB IUPAC#	Rtx®-CLP RT (min)	Rtx®-CLP2 PCB IUPAC#	Rtx®-CLP2 RT (min)	Rtx®-CLP PCB IUPAC#	Rtx®-CLP RT (min)	Rtx®-CLP2 PCB IUPAC#	Rtx®-CLP2 RT (min)	Rtx®-CLP PCB IUPAC#	Rtx®-CLP RT (min)	Rtx®-CLP2 PCB IUPAC#	Rtx®-CLP2 RT (min)
1	13.36	1	14.33	52	25.24	73	26.47	155	28.50	88	29.89	135	31.90	147	33.60	182	35.37	129	36.95
2	ND	2	ND	65	25.27	52	26.56	66	28.54	155	30.07	139	32.10	82	33.69	187	35.38	159	36.95
3	15.86	3	16.61	43	25.29	46	26.70	80	28.77	91	30.22	149	32.22	108	33.75	183	35.60	126	37.00
4	16.57	10	17.79	62	25.30	43	26.71	55	28.90	55	30.39	140	32.35	107	33.79	126	35.67	166	37.06
10	16.38	4	17.94	73	25.33	49	26.73	60	29.28	92	30.54	124	32.37	139	33.80	159	35.76	183	37.16
7	17.84	7	18.98	49	25.34	75	26.79	92	29.28	101	30.81	108	32.37	149	33.86	185	35.98	162	37.34
9	17.86	9	18.99	39	25.34	38	26.86	84	29.34	90	30.85	107	32.43	123	33.89	162	36.12	167	37.61
6	18.73	6	19.80	75	25.40	65	26.87	89	29.42	60	30.91	123	32.49	106	33.96	128	36.14	185	37.65
5	18.91	8	20.13	47	25.48	47	26.88	90	29.44	56	30.96	106	32.51	118	34.01	181	36.32	128	38.08
8	18.96	5	20.21	48	25.52	62	26.92	56	29.48	113	30.96	118	32.65	140	34.06	167	36.37	181	38.20
19	19.64	14	20.56	104	25.53	48	26.93	101	29.51	99	31.07	142	32.67	133	34.25	202	36.50	174	38.24
14	19.80	30	21.17	38	25.78	104	27.31	150	29.60	84	31.16	188	32.68	165	34.31	174	36.50	202	38.27
30	19.82	19	21.36	44	26.32	35	27.61	99	29.68	89	31.26	143	32.68	143	34.36	177	36.69	177	38.56
18	21.00	11	21.93	59	26.34	59	27.80	113	29.76	79	31.28	134	32.69	188	34.39	201	36.85	201	38.70
17	21.06	12	22.24	42	26.44	44	27.82	152	30.01	119	31.42	131	32.88	134	34.46	204	36.89	204	38.74
11	21.10	13	22.34	35	26.56	72	27.85	112	30.02	150	31.43	114	32.93	161	34.49	171	36.92	171	38.81
24	21.27	18	22.40	64	26.71	42	28.00	119	30.08	112	31.46	184	33.00	142	34.54	173	37.09	192	38.86
12	21.31	17	22.46	96	26.71	37	28.07	109	30.10	109	31.58	133	33.03	146	34.57	197	37.18	156	38.88
13	21.34	15	22.66	41	26.86	68	28.11	116	30.18	78	31.83	165	33.19	114	34.60	156	37.27	173	39.04
27	21.67	24	22.88	37	26.89	103	28.38	79	30.27	83	31.84	146	33.30	131	34.68	192	37.57	172	39.08
15	21.68	27	23.00	72	26.90	71	28.40	145	30.29	111	31.87	161	33.30	184	34.73	157	37.61	197	39.09
32	22.01	32	23.49	103	26.97	64	28.41	83	30.33	152	31.89	122	33.40	153	34.86	172	37.65	157	39.26
16	22.11	23	23.63	71	26.98	41	28.50	86	30.46	116	32.00	132	33.55	122	34.88	200	37.87	180	39.42
54	22.36	16	23.65	68	27.00	57	28.61	117	30.49	148	32.04	153	33.56	168	34.98	180	37.95	193	39.51
23	22.37	34	23.69	100	27.22	100	28.70	97	30.56	86	32.07	179	33.74	127	35.11	193	38.11	191	39.71
29	22.52	29	23.78	57	27.44	96	28.72	115	30.57	97	32.13	168	33.75	132	35.46	191	38.25	200	39.93
34	22.69	26	24.27	40	27.44	67	28.83	148	30.69	120	32.14	105	33.95	141	35.60	198	39.17	169	40.54
50	23.01	54	24.37	67	27.61	40	29.10	136	30.72	117	32.19	176	34.07	105	35.61	170	39.19	198	40.76
26	23.15	25	24.39	61	27.73	58	29.13	78	30.73	125	32.19	141	34.15	179	35.63	190	39.19	190	40.95
25	23.20	50	24.55	63	27.76	63	29.18	87	30.79	145	32.20	127	34.16	137	35.96	169	39.30	199	41.04
31	23.46	31	24.74	94	27.78	61	29.29	125	30.84	115	32.26	137	34.34	176	36.01	199	39.38	170	41.07
28	23.56	28	24.81	58	27.92	94	29.30	111	30.91	87	32.40	186	34.39	130	36.20	203	39.56	203	41.23
21	23.79	21	25.32	93	28.00	74	29.37	85	30.98	81	32.43	130	34.58	160	36.27	196	39.62	196	41.33
53	23.85	53	25.44	74	28.00	80	29.42	154	31.07	154	32.45	160	34.66	163	36.37	208	40.44	189	42.17
51	24.04	33	25.48	98	28.07	121	29.47	81	31.10	85	32.65	163	34.80	164	36.39	189	40.71	208	42.38
33	24.22	20	25.53	102	28.13	98	29.68	120	31.13	136	32.74	138	34.81	186	36.41	207	40.82	207	42.82
20	24.23	51	25.68	88	28.16	102	29.68	110	31.38	110	32.95	158	34.90	178	36.44	195	40.86	195	42.92
45	24.33	36	25.85	95	28.22	70	29.70	151	31.60	77	33.06	164	34.93	138	36.48	194	42.05	194	43.68
22	24.53	22	25.97	70	28.39	93	29.72	144	31.79	151	33.14	178	34.99	158	36.50	205	42.28	205	43.83
46	24.93	45	26.12	76	28.44	76	29.74	77	31.79	144	33.35	166	35.15	175	36.73	206	43.34	206	45.15
69	24.96	69	26.21	121	28.46	95	29.86	82	31.87	135	33.44	175	35.22	182	36.90	209	44.19	209	46.32
36	25.05	39	26.39	91	28.47	66	29.88	147	31.89	124	33.56	129	35.23	187	36.91				

30m, 0.32mm ID Rtx®-CLPesticides (cat.# 11139) and Rtx®-CLPesticides2 (cat.# 11324) columns.
Oven temp.: 100°C (hold 1 min.) to 290°C @ 4°C/min.; **Inj. temp.:** 210°C; **Det. temp.:** 310°C; **Carrier gas:** He @ 36cm/sec.

References (not available from Restek):

1 Environmental Chemistry, Colin Baird, W.H. Freeman and Co., 1998, pp. 337-353.

2 Ballschmiter, K, and Zell, M., Fresenius Z. *Anal. Chem.*, 302, 20, (1980)

Product Listing

Rtx®-CLPesticides Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.25	340°C	11120	11123
0.32mm	0.50	340°C	11136	11139
0.53mm	0.50	340°C	11137	11140
ID	**df (µm)**	**Stable to**	**10m**	**20m**
0.18mm	0.18	340°C	42101	42102

Rtx®-CLPesticides2 Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.20	340°C	11320	11323
0.32mm	0.25	340°C	11321	11324
0.53mm	0.42	340°C	11337	11340
ID	**df (µm)**	**Stable to**	**10m**	**20m**
0.18mm	0.14	340°C	42301	42302

HPLC

Applications note

Ultra C4 HPLC Column Provides High Stability At Low pH

The use of a low pH (< 2.0) mobile phase can cause acid hydrolysis of the HPLC column stationary phase, resulting in reduced retention and poor reproducibility. During hydrolysis the phase is stripped from the silica backbone. Generally, acid hydrolysis of alkyl bonded phases increases with a decrease in the chain length of the stationary phase ligand. Typically, stationary phase ligands as short as C4 will be much less stable than C18 ligands. To overcome the problem, some HPLC column manufacturers offer C4 phases made with relatively high ligand density, which are more stable than lower density C4 phases. However, these high ligand density phases still exhibit gradual loss of retention when exposed to low pH mobile phases.

The research chemists at Restek have designed the new Ultra C4 phase to further increase stability by using both high ligand density and a unique bonding chemistry to reduce acid hydrolysis. The improved stability of the Ultra C4 phase was confirmed using a controlled study comparing the Ultra C4 to another leading C4 phase. This leading C4 phase contains a relatively high ligand density, making it more stable than other C4 columns (Table 1). The results of the study are shown in Figure 1.

Experimental Conditions

Both columns were simultaneously exposed to repeated acetonitrile/water mobile phase gradients in the presence of 0.1% (v/v) trifluoroacetic acid (TFA) and a temperature of 50°C. The pH of 0.1% TFA in water is 1.9. As the mobile phase pH is decreased towards 2, all bonded phase silicas become less stable because the siloxane (Si-O-Si) linkage to the stationary phase is susceptible to acid hydrolysis, resulting in loss of stationary phase[1]. Elevated column temperature accelerates acid hydrolysis and the resulting loss of the stationary phase.

The hydrophobic retention of each column was measured before exposure to hydrolysis conditions, and periodically during the acid hydrolysis experiment. The capacity factor (k') of phenylheptane was used to measure hydrophobic retention, and was calculated using uracil as the void marker (t_o). After equilibration in a mobile phase of H_2O:ACN (25:75, v/v) at a flow rate of 1.25mL/min, each column was injected with 5µL of a mixture of uracil (0.1mg/mL) and phenylheptane (10mg/mL). UV detection was performed at 254nm.

In addition to the Ultra C4 and competitor C4 columns that were exposed to hydrolytic conditions, an additional Ultra C4 column was used as a control. The control column was periodically analyzed for hydrophobic retention along with the test columns. While the test columns were exposed to the TFA gradients, the control column was only exposed to H_2O:ACN (25:75). The hydrophobic retention of the control column varied less than 2% throughout this experiment.

Figure 1

Ultra C4 shows greater retention and stability due to its unique bonding chemistry

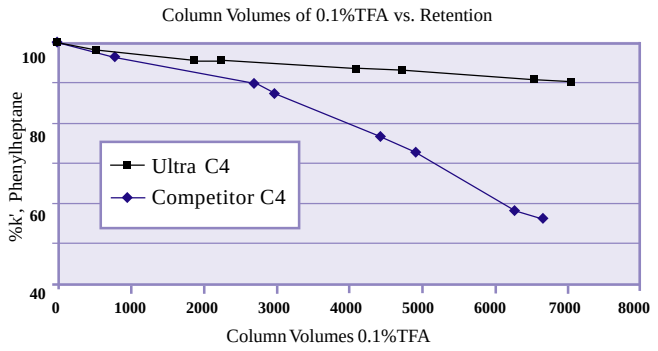


Table 1

C4 Columns Evaluated

Column	Dimensions	Particle Size	Pore Size	Carbon %
Competitor C4	50x4.6mm	5µm	100Å	8
Ultra C4	50x4.6mm	5µm	100Å	9

Table 2

Acid Hydrolysis Conditions

Mobile phase A: 0.1% TFA in deionized water
Mobile phase B: 0.1% TFA in acetonitrile
Temperature: 50°C

Gradient:	Time (min.)	%A	%B
	0	100	0
	20	0	100
	40	0	100
	40.1	100	0
	60	100	0

The acid hydrolysis conditions of the experiment (Table 2) were modeled after those of Kirkland, Glajch, and Farlee.[2] Both test columns were exposed simultaneously to repeated cycles of the mobile phase gradient listed in Table 2. A flow rate of 2.0mL/min was split between the two test columns using a tee. The eluent from each column was collected and monitored. A restrictor was added to the outlet of one of the columns to make the flow rate as similar as possible. The total flow through each column at the conclusion of the experiment was comparable—3784mL for the Ultra C4

column versus 3671mL for the competitor C4 column. Mobile phase volumes were converted to column volumes using the initial elution volumes for uracil (0.553 mL for competitor C4, 0.538mL for Ultra C4). Following the 50th gradient cycle, the columns were exposed to 8.5 hours of continuous flow of 100% mobile phase A. The test columns were exposed to a total of 54 gradient cycles.

Results

Figure 1 shows the percent change in capacity factor (k') of phenylheptane plotted against the column volumes of the TFA mobile phase. After approximately 3000 column volumes of TFA, the competitor C4 column showed twice the loss in retention as compared to the Ultra C4 column. Even after 7000 column volumes, the Ultra C4 column lost less than 10% of its original retention, while the competitor C4 column showed a 44% loss in retention.

Conclusions

Restek's Ultra C4 column combines high ligand density and a unique bonding chemistry to attain excellent resistance to acid hydrolysis. This resistance to stationary phase loss ensures highly reproducible and consistent retention even when using a very acidic mobile phase.

References

1. J.J. Kirkland, J.W. Henderson, J.J. Destefano, M.A. Van Straten, H.A. Claessens, J. Chromatogr., A, 762 (1-2), 97-112 (1997).
2. J.J. Kirkland, J.L. Glajch, and R.D. Farlee, *Anal. Chem*. **61**, 2-11 (1989).

References not available from Restek.

Ultra C4, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9102331	9102332	9102333	9102335
50mm length	9102351	9102352	9102353	9102355
100mm length	9102311	9102312	9102313	9102315

Ultra C4, 3µm Columns with Trident™ Inlet

Particle Size: 3µm		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	—	9102332-700	9102333-700	9102335-700
50mm length	—	9102352-700	9102353-700	9102355-700
100mm length	—	9102312-700	9102313-700	9102315-700

Ultra C4, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9102531	9102532	9102533	9102535
50mm length	9102551	9102552	9102553	9102555
100mm length	9102511	9102512	9102513	9102515
150mm length	9102561	9102562	9102563	9102565
200mm length	9102521	9102522	9102523	9102525
250mm length	9102571	9102572	9102573	9102575

Ultra C4, 5µm Columns with Trident™ Inlet

Particle Size: 5µm		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	—	9102532-700	9102533-700	9102535-700
50mm length	—	9102552-700	9102553-700	9102555-700
100mm length	—	9102512-700	9102513-700	9102515-700
150mm length	—	9102562-700	9102563-700	9102565-700
200mm length	—	9102522-700	9102523-700	9102525-700
250mm length	—	9102572-700	9102573-700	9102575-700

RESTEK environmental

Applicationsnote

Improving throughput of semivolatile GC/MS analysis using a performance-based measurement system, Rtx[fi]-5Sil MS column, and Uniliner[fi] inlet liner.

Restek has developed a GC/MS method for analyzing semivolatile compounds (e.g., US Environmental Protection Agency [EPA] Method 8270) that will help increase productivity in the lab. The changes include modifying the final extract volume, using a drilled Uniliner[fi] liner, optimizing GC analysis conditions, and modifying the calibration curve to offset the increased extract volume. Following is an explanation of each modification.

1) Increase the final extract volume from 1mL to 5mL.

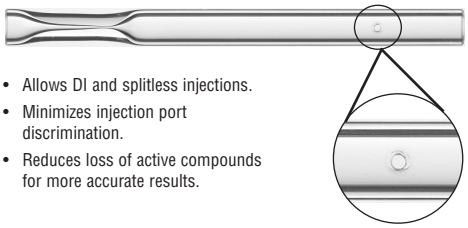
Increasing the final extract volume will dilute the nonvolatile material extracted from the sample, resulting in less contamination to the injection system and analytical column. This has the potential to allow the GC/MS to stay in calibration longer, and to reduce maintenance time and expense. Also, the increased extract volume will result in better recoveries of low-boiling compounds that may be lost when the extract is concentrated to 1mL.

2) Replace the splitless inlet liner with a drilled Uniliner[fi] liner.

When the sample is injected into the injection port, it expands and comes into contact with the bottom of the injection port. This may cause adsorption or breakdown of the active compounds in the sample. A drilled Uniliner[fi] liner (Figure 1) can be used in place of a standard splitless liner to significantly reduce sample exposure to the injection port. This unique inlet liner can be used for both direct and splitless injections. Because the column is sealed to the liner with a press-tight connection, there is no chance for any sample contact with metal surfaces below the liner. The small hole located on the side of the Unliner[fi] liner allows carrier gas to be routed through the split vent line during the splitless purge operation of the injection system. Because the drilled Uniliner[fi] liner directs more of the sample onto the column, less discrimination of high molecular weight compounds occurs.

Figure 1

The drilled Uniliner[fi] liner provides more accurate results.

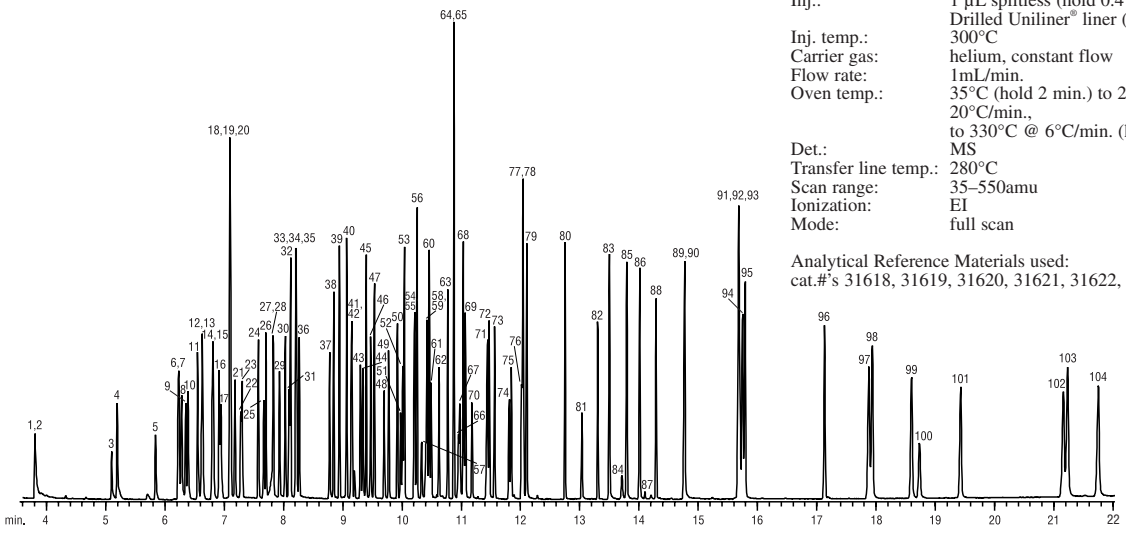


- Allows DI and splitless injections.
- Minimizes injection port discrimination.
- Reduces loss of active compounds for more accurate results.

Figure 2

The Rtx[fi]-5Sil MS column, combined with a Siltek -treated drilled Uniliner[fi] liner, exhibits excellent peak shape and response for semivolatile compounds.

See page 3 for peak list.



Rtx®-5Sil MS, 30m, 0.25mm ID, 0.25µm (cat.# 12723)
Sample: 16µg/mL in methylene chloride, IS @ 8µg/mL
Inj.: 1 µL splitless (hold 0.4 min.), Drilled Uniliner® liner (cat.# 21054)
Inj. temp.: 300°C
Carrier gas: helium, constant flow
Flow rate: 1mL/min.
Oven temp.: 35°C (hold 2 min.) to 260°C @ 20°C/min., to 330°C @ 6°C/min. (hold 1 min.)
Det.: MS
Transfer line temp.: 280°C
Scan range: 35–550amu
Ionization: EI
Mode: full scan

Analytical Reference Materials used:
cat.#'s 31618, 31619, 31620, 31621, 31622, 31206, 31062, 31063

environmental

3) Use a thin-film column by reducing the concentration of calibration standards.

A thin-film Rtx®-5Sil MS column can reduce the analysis time to less than 22 minutes for the compounds listed in EPA Method 8270 (see compound list for Figure 2, p.3). The Rtx®-5Sil MS column features a silarylene stationary phase that exhibits lower bleed and optimized separation of semivolatile compounds. Usually, a thinner film column has less sample capacity than a thicker film column, which can lead to column overload. To prevent column overload, the concentration of the calibration standards should be reduced by 1/5, so that the on-column concentration ranges from 4ng to 32ng. Table I shows the response factors and linearity of active and late-eluting semivolatile compounds. Also, reducing the standard concentration by a factor of 5 off-sets the increased extract volume, resulting in the same reporting limits.

4) Optimize oven temperature programming.

A multi-ramp GC temperature program can optimize the separation of critical compound pairs. Increasing the initial hold time helps resolve early-eluting compounds; then a fast ramp rate can be used through non-critical areas, and a lower ramp rate used to elute later compounds. Extracted ion chromatograms of the closely eluting compounds show resolution between them (Figure 3).

5) Calibration Curve

We used 1/5 the recommended concentration level of Method 8270 1 L injection of 4, 10, 16, 24, and 32ppm standard. The internal standards were also reduced to 1/5 the concentration and are at 8ppm. As seen in Figure 2, the 16ng on-column injection shows excellent signal-to-noise ratio, and low column bleed and injection port discrimination.

Conclusion

A number of techniques can be used to increase sample throughput for the analysis of semivolatile compounds. Increasing extract volume will reduce preparation time and injection port contamination. Using a drilled Uniliner® injection port liner results in a more inert sample pathway and eliminates injection port discrimination. In addition, the use of a thin-film column reduces analysis time helping laboratories increase sample output.

Table I

Response factors and linearity of active and late-eluting semivolatile compounds.

Compound	Ret. time (min.)	Int. Stnd. for quant.	m/z	4ppm RRF	10ppm RRF	16ppm RRF	24ppm RRF	32ppm RRF	Ave. RRF	5-point %RSD	4-point %RSD (w/o 4ppm)
N-nitrosodimethylamine	3.79	1	74	0.724	0.736	0.775	0.742	0.748	0.745	3	2
pyridine	3.80	1	79	1.055	0.951	1.058	0.967	1.004	1.007	5	5
aniline	6.28	1	93	1.777	1.773	1.962	1.933	1.946	1.878	5	5
N-nitroso-di-n-propylamine	7.12	1	169	0.776	0.746	0.801	0.740	0.770	0.767	3	4
benzoic acid	7.84	2	122	0.148	0.193	0.201	0.203	0.228	0.195	15	7
2,4-dichlorophenol	7.94	2	162	0.215	0.248	0.240	0.249	0.259	0.242	7	3
hexachlorocyclopentadiene	9.14	3	237	0.283	0.310	0.323	0.333	0.357	0.321	9	6
3-nitroaniline	10.21	3	138	0.323	0.318	0.343	0.339	0.348	0.334	4	4
2,4-dinitrophneol	10.34	3	184	0.110	0.139	0.156	0.155	0.169	0.146	16	8
4-nitrophenol	10.41	3	109	0.162	0.168	0.185	0.187	0.202	0.181	9	7
azobenzene	11.07	3	77	1.387	1.446	1.436	1.369	1.414	1.410	2	2
nitrosodiphenylamine	11.04	4	169	0.718	0.698	0.723	0.771	0.738	0.729	4	4
pentachlorophenol	11.81	4	266	0.094	0.122	0.132	0.132	0.146	0.125	15	7
benzidine	13.72	5	184	0.213	0.178	0.188	0.206	0.269	0.211	17	19
benzo(b)fluoranthene	17.88	6	252	1.344	1.448	1.504	1.506	1.628	1.486	7	5
benzo(ghi)perylene	21.76	6	276	1.341	1.428	1.492	1.488	1.593	1.468	6	5
ISTD											
1,4-dichlorobenzene-d14	6.62	1	152								
naphthalene-d8	8.10	2	136								
acenaphthene-d10	10.22	3	164								
phenanthrene-d10	12.02	4	188								
chrysene-d12	15.70	5	240								
perylene-d12	18.73	6	264								

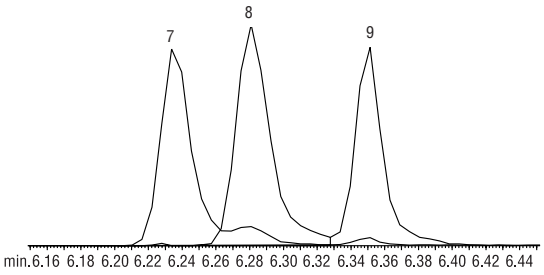
Peak List for Figure 2

1. N-nitrosodimethylamine
2. pyridine
3. methyl methanesulfonate
4. 2-fluorophenol
5. ethyl methanesulfonate
6. phenol-d6
7. phenol
8. aniline
9. bis(2-chloroethyl)ether
10. 2-chlorophenol
11. 1,3-dichlorobenzene
12. 1,4-dichlorobenzene-d4
13. 1,4-dichlorobenzene
14. 1,2-dichlorobenzene
15. benzyl alcohol
16. 2-methylphenol
17. bis(2-chloroisopropyl)ether
18. acetophenone
19. 4-methylphenol/3-methylphenol
20. N-nitroso-di-*n*-propylamine
21. hexachloroethane
22. nitrobenzene-d5
23. nitrobenzene
24. isophorone
25. 2-nitrophenol
26. 2,4-dimethylphenol
27. bis(2-chloroethoxy)methane
28. benzoic acid
29. 2,4-dichlorophenol
30. 1,2,4-trichlorobenzene
31. naphthalene-d8
32. naphthalene
33. 2,6-dichlorophenol
34. 4-chloroaniline
35. hexachloropropene
36. hexachlorobutadiene
37. 4-chloro-3-methylphenol
38. isosafrole
39. 2-methylnaphthalene
40. 1-methylnaphthalene
41. hexachlorocyclopentadiene
42. 1,2,4,5-tetrachlorobenzene
43. 2,4,6-trichlorophenol
44. 2,4,5-trichlorophenol
45. 2-fluorobiphenyl
46. safrole
47. 2-chloronaphthalene
48. 2-nitroaniline
49. 1,4-naphthoquinone
50. dimethylphthalate
51. 1,3-dinitrobenzene
52. 2,6-dinitrotoluene
53. acenaphthylene
54. acenaphthene-d10
55. 3-nitroaniline
56. acenaphthene
57. 2,4-dinitrophenol
58. pentachlorobenzene
59. 4-nitrophenol
60. dibenzofuran
61. 2,4-dinitrotoluene
62. 2,3,4,6-tetrachlorophenol
63. diethyl phthalate
64. fluorene
65. 4-chlorophenyl phenyl ether
66. 4-nitroaniline
67. 4,6-dinitro-2-methylphenol
68. diphenylamine
69. azobenzene
70. 2,4,6-tribromophenol
71. phenacetin
72. 4-bromophenyl phenyl ether
73. hexachlorobenzene
74. pentachlorophenol
75. pentachloronitrobenzene
76. phenanthrene-d10
77. dinoseb
78. phenanthrene
79. anthracene
80. di-*n*-butylphthalate
81. 4-nitroquinoline-1-oxide
82. isodrin
83. fluoranthene
84. benzidine
85. pyrene
86. *p*-terphenyl-d14
87. aramite
88. chlorbenzilate
89. kepone
90. butyl benzyl phthalate
91. benzo(a)anthracene
92. 3,3'-dichlorobenzidine
93. chrysene-d12
94. chrysene
95. bis(2-ethylhexyl)phthalate
96. di-*n*-octyl phthalate
97. benzo(b)fluoranthene
98. benzo(k)fluoranthene
99. benzo(a)pyrene
100. perylene-d12
101. 3-methylcholanthrene
102. indeno(1,2,3-cd)pyrene
103. dibenzo(a,h)anthracene
104. benzo(ghi)perylene

Figure 3

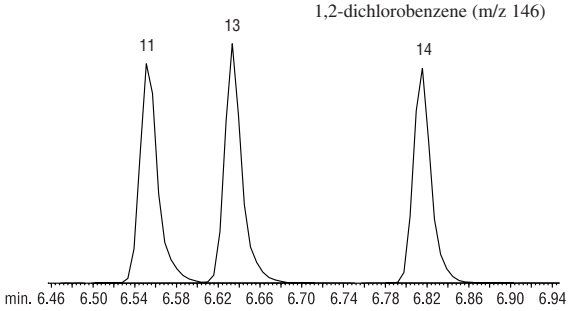
Extracted ion chromatograms show resolution of closely-eluting compounds.

phenol (m/z 94)
aniline (m/z 93)
bis(2-chloroethyl)ether (m/z 93)



GC_EV00562

1,3-dichlorobenzene (m/z 146)
1,4-dichlorobenzene (m/z 146)
1,2-dichlorobenzene (m/z 146)

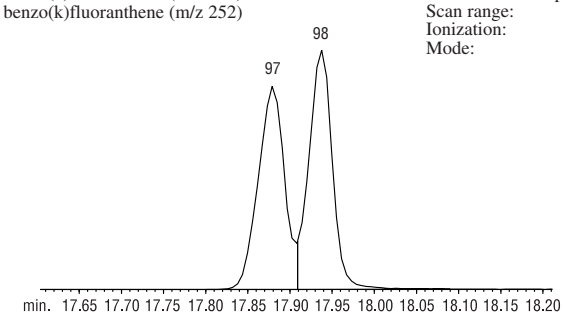


GC_EV00563

Rtx®-5Sil MS, 30m, 0.25mm ID, 0.25µm (cat.# 12723)

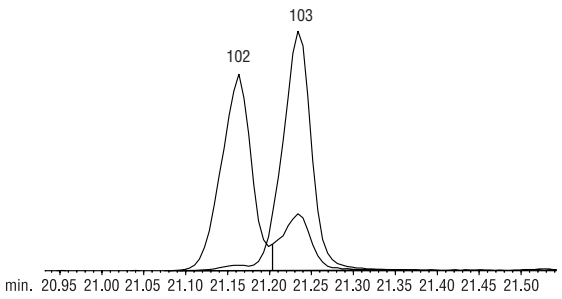
Sample:	16µg/mL in methylene chloride
Inj.:	1µL splitless (hold 0.4 min.), Drilled Uniliner® liner (cat.# 21054)
Inj. temp.:	300°C
Carrier gas:	1mL/min., constant flow
Linear velocity:	34cm/sec.
Oven temp.:	35°C (hold 2 min.) to 260°C @ 20°C/min., to 330°C @ 6°C/min. (hold 1 min.)
Det.:	MS
Transfer line temp.:	280°C
Scan range:	35–550amu
Ionization:	EI
Mode:	full scan

benzo(b)fluoranthene (m/z 252)
benzo(k)fluoranthene (m/z 252)



GC_EV00564

indeno(1,2,3-cd)pyrene (m/z 276)
dibenzo(a,h)antracene (m/z 278)



GC_EV00565

Product Listing

Rtx®-5Sil MS (Fused Silica)

(Equivalent selectivity of Crossbond® 5% diphenyl/95% dimethyl polysiloxane) Stable to 360°C

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.10	-60 to 330/350°C	12705	12708
	0.25	-60 to 330/350°C	12720	12723
	0.50	-60 to 330/350°C	12735	12738
	1.00	-60 to 325/350°C	12750	12753
0.28mm	0.25	-60 to 330/350°C	12790	12793
	0.50	-60 to 330/350°C	12791	12794
	1.00	-60 to 325/350°C	12792	12795
0.32mm	0.10	-60 to 330/350°C	12706	12709
	0.25	-60 to 330/350°C	12721	12724
	0.50	-60 to 330/350°C	12736	12739
	1.00	-60 to 325/350°C	12751	12754
0.53mm	0.50	-60 to 320/340°C	12737	12740
	1.00	-60 to 320/340°C	12752	12755
	1.50	-60 to 310/330°C	12767	12770

Rtx®-5MS (Fused Silica)

(Crossbond® 5% diphenyl - 95% dimethyl polysiloxane) Stable to 360°C

ID	df (µm)	temp. limits	15-Meter	30-Meter	60-Meter
0.25mm	0.10	-60 to 330/350°C	12605	12608	12611
	0.25	-60 to 330/350°C	12620	12623	12626
	0.50	-60 to 330/350°C	12635	12638	12641
	1.00	-60 to 325/350°C	12650	12653	
0.32mm	0.10	-60 to 330/350°C	12606	12609	12612
	0.25	-60 to 330/350°C	12621	12624	12627
	0.50	-60 to 330/350°C	12636	12639	12642
	1.00	-60 to 325/350°C	12651	12654	
0.53mm	0.50	-60 to 320/340°C	12637	12640	
	1.00	-60 to 320/340°C	12652	12655	
	1.50	-60 to 310/330°C	12667	12670	

Innovative Integra-Guard™ Columns

ID	Length	Suffix #	ID	Length	Suffix #
0.25mm	5m	-124	0.32mm	5m	-125
	10m	-127		10m	-128
0.28mm	5m	-243	0.53mm	5m	-126
	10m	-244		10m	-129

Drilled Uniliner® Liners for Agilent 6890 GCs (For 0.32/0.53mm ID Columns)

	Benefits/Uses:	ID**/OD & Length (mm)	cat.# ea.	cat.# 5-pk.
 Drilled Uniliner®	allows direct injection when using an EPC-equipped GC	4.0 ID 6.3 OD x 78.5	21054	21055

*** Nominal ID at syringe needle expulsion point.*

Merlin Microseal™ Septa

Microseal™ High-Pressure Septa 400 Series	Merlin#	Similar to Agilent#	cat.#
Nut kit (1 nut, fits 300 & 400 series septa)	403	5182-3445	22809
Standard kit (nut, 2 high-pressure septa)	404	Not offered	22810
Starter kit (nut, 1 high-pressure septum)	405	5182-3442	22811
High-pressure replacement septum (1 septum)	410	5182-3444	22812

Microseal™ Septa, 300 Series	Merlin#	Similar to Agilent#	cat.#
Standard kit (nut, 2 septa)	304	5181-8833	22813
Starter kit (nut, 1 septum)	305	5181-8816	22814
Microseal replacement septum (1 septum)	310	5181-8815	22815
Replacement PTFE washers (2-pk.)	311	5181-0853	22808

Determination of Omega-3 (n-3) and Omega-6 (n-6) Fatty Acid Composition in Evening Primrose Oil, Flax Seed Oil, Black Currant Oil, and Borage Oil

Essential fatty acids (EFAs) are polyunsaturated fatty acids (PUFAs) that the human body requires, yet cannot produce, and therefore must be obtained through dietary sources or nutritional supplements. α-Linolenic acid (LNA) and γ-Linolenic acid (GLA) are important Omega-3 (n-3) and Omega-6 (n-6) fatty acids. Accurate determination and quantitation of these EFAs, especially the separation of LNA and GLA, can be performed by capillary gas chromatography (GC). The FAMEWAX™ column is ideal to provide the composition of the EFAs found in evening primrose oil, flax seed oil, black currant seed oil, and borage oil.

Why are these fatty acids essential?

The two families of EFAs are the Omega-3 (n-3) series and the Omega-6 (n-6) series. The Omega-3 (n-3) series includes LNA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The Omega-6 (n-6) series includes linoleic acid (LA), GLA, dihommogamma-linolenic acid (DGLA), and arachidonic acid (AA).

These EFAs are nutrients that perform key functions in our bodies. For example, they determine membrane fluidity and reactivity, oxidation rate, metabolic rate, and energy production. In addition, they are a factor in maintaining body temperature, insulating nerves, and cushioning body tissue. EFAs also are precursors to prostaglandins, hormone-like substances that are critical to the body's overall health maintenance. Prostaglandins regulate blood pressure, blood clotting, stimulation of the immune system, and general regulation of heart, kidneys, liver, lungs, and brain. They are short-lived molecules and constantly need to be replenished. Without EFAs this can be impossible.

Background

EFAs are similar to vitamins in their importance to one's overall health. However, vitamins are required in small dietary quantities (µg/day), whereas EFAs are a macronutrient (i.e., necessary in g/day). A joint study released by the Food and Agriculture Organization and the World Health Organization recommends that at least 3% of our daily calorie intake be in the form of EFA.[1]

Polyunsaturated oils, such as safflower, sunflower, and corn oil are good sources of LA. Once ingested, LA can be converted to the other Omega-6 acids: GLA, DGLA, and AA.

Green, leafy vegetables and flax oil are good sources of LNA. From the LNA provided in our diets, our bodies can produce the other Omega-3 (n-3) acids: EPA and DHA.

Unfortunately, one's diet may not be well-fortified with these food sources. Also, physiological conditions can inhibit the conversion process of LA and LNA to the other essential Omega-3 and Omega-6 fatty acids.[1] Therefore, nutritional supplements can be used to help people attain the suggested daily intake. Evening primrose oil, flax seed oil, black currant seed oil, and borage oil are rich sources of these EFAs and are available in capsules as nutritional supplements.

A number of clinical conditions have been treated with oils rich in GLA. Oral dosages of evening primrose oil have been used to treat premenstrual tension, rheumatoid arthritis, breast disorders, and atopic eczema.[2]

Analysis

The oils were obtained from soft-gel capsules of evening primrose oil, flax seed oil, black currant oil, and borage oil. The fats were initially in the form of triglycerides. They were saponified into their free acids and esterified for better volatility and inertness by GC. To do this, 5mL of hexane and 250µL of 2N KOH were added to 0.24g oil. The mixture was shaken for 2 minutes in a closed 20mL vial. After settling, the supernatant was injected.

In the 1980s, packed and capillary GC, as well as liquid chromatography (LC), were evaluated for the analysis of EFAs in evening primrose oil and soybean oil.[2] According to one reference, "gas chromatography using a capillary column (25m, Carbowax® 20 M) was the best tool for the separation of GLA (C18:3n6) and LNA (C18:3n3)."[3]

Based on this finding, we used a Restek column—the 30m, 0.25mm ID, 0.25 µm FAMEWAX™ column (cat.# 12497)—to analyze these oils. The FAMEWAX™ column contains a polyethylene glycol stationary phase, which is slightly more polar than the Stabilwax® column. The FAMEWAX™ column offers excellent selectivity and efficiency, not only to separate saturated (C16:0 and C18:0) and monounsaturated (C18:1n9) fatty acids from the Omega-3 and -6 fatty acids of interest, but also to resolve the isomers of linolenic acid (C18:3n3 and C18:3n6).

We used an HP 5890 GC with a flame ionization detector (FID) and a split/splitless injection port, used in the split mode, with a split vent flow of 40mL/min. The inlet liner was a deactivated 4mm ID split sleeve (cat.# 20781). The injector and detector ports were set at 225°C and 230°C, respectively. The oven temperature program was initially set at 165°C for the first 30 minutes, and then increased at a rate of 1.5°C/min to 220°C, where it remained for the last 15 minutes. The carrier gas was helium and the linear velocity of 40cm/sec. was measured at the initial temperature.

Results

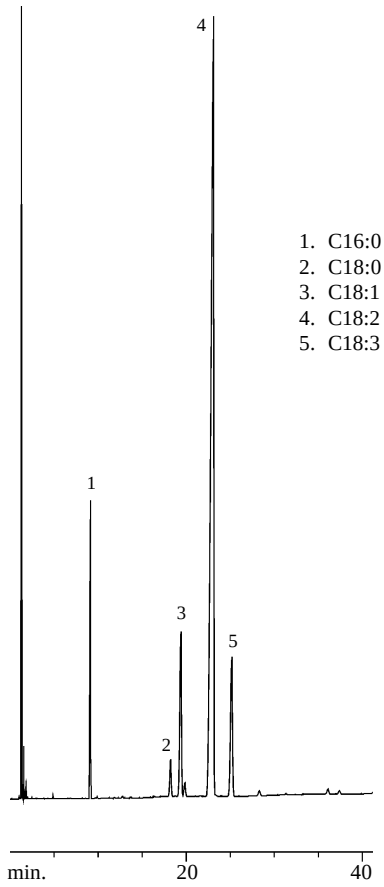
Regarding the content of the Omega-3 and Omega-6 essential fatty acids in the four oils studied, all contain LA (18:2n6) (***Figures 1-4 and Table I***). Evening primrose oil (***Figure 1***) contains the largest amount of LA and would be the best source. Flax seed oil (***Figure 2***) reveals a significant amount of LNA (C18:3n3), and would be the best source of this EFA. Only black currant seed oil (***Figure 3)*** contains an approximately equal amount of both isomeric linolenic acids. All chromatograms, except that of flax seed oil, illustrate the presence of GLA (C18:3n6). In fact, borage oil (***Figure 4***) and black currant seed oil are significant sources of GLA.

Conclusion

Omega-3 (n-3) and Omega-6 (n-6) EFAs perform key functions in our bodies. Quantifying these compounds in nutritional supplements such as evening primrose oil, flax seed oil, black currant seed oil, and borage oil is successfully achieved using the FAMEWAX™ column. This column offers excellent efficiency and selectivity towards these polyunsaturated methyl esters, providing an accurate determination of the fatty acid profiles. Thus the FAMEWAX™ column is an excellent column choice for this and similar applications.

Figure 1

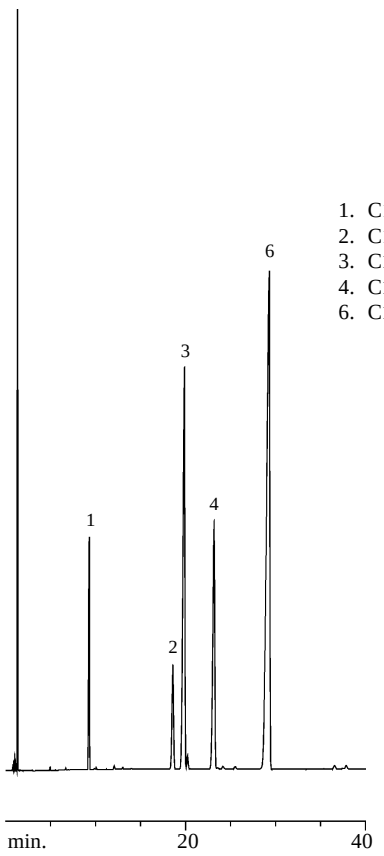
Evening primrose oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min. (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

Figure 2

Flax seed oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min. (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

Achieve baseline resolution of complex polyunsaturated FAMEs in significantly less time using the FAMEWAX™ column as compared to other Carbowax® columns!

Call 800-356-1688 or 814-353-1300, ext. 3, or your local Restek representative to order your FAMEWAX™ column today.

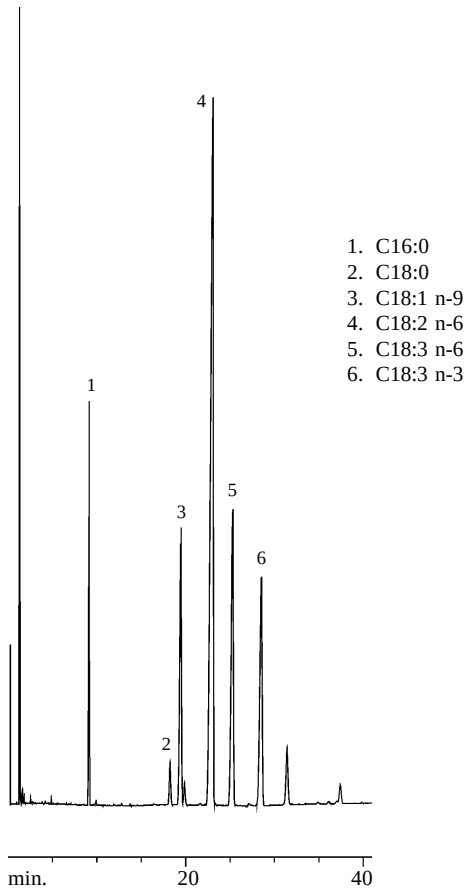
Table I

Composition of Omega-3 and Omega-6 EFAs in the four oils (% area).

	C16:0	*C18:0*	*C18:1n9*	*C18:2n6*	*C18:3n6*	*C18:3n3*
Evening Primrose Oil	6.5	1.8	8.6	**73.5**	8.7	n/a
Flax Seed Oil	4.9	5.2	23.7	15.2	n/a	**50.1**
Black Currant Seed Oil	6.7	1.6	11.3	**47.1**	15.3	13.1
Borage Oil	11.5	4.9	19.5	**40.3**	22.1	n/a

Figure 3

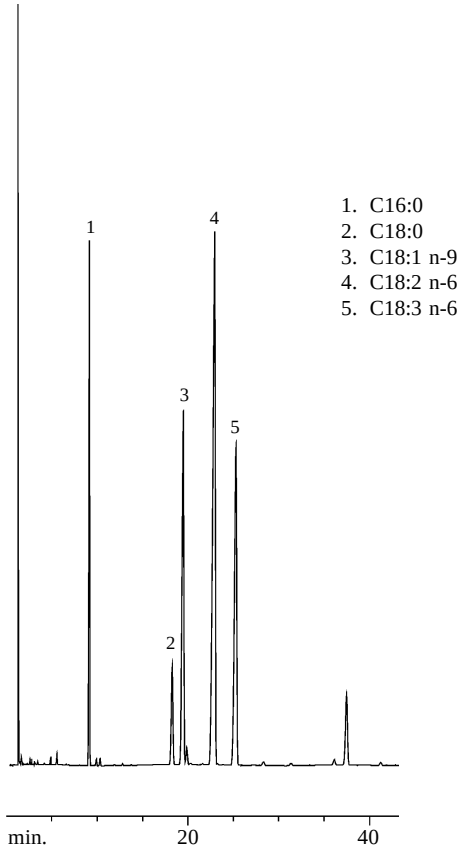
Black currant seed oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min. (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

Figure 4

Borage seed oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min. (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

References

1. Health and Healing News, "Evening Primrose Oil—Superfood for the '90s." http://www.hhnews.com/epo.htm
2. Robert A. Gibson, David R. Lines and Mark Neumann, "Gamma Linolenic Acid (GLA) Content of Encapsulated Evening Primrose Oil Products," *Lipids*, Vol. 27, no. 1 (1992).
3. M.S. Manku, "A Comparison of GLC and HPLC Methods for Determining Fatty Acid Composition of Evening Primrose and Soybean Oil," Journal of Chromatographic Science, Vol. 21, August (1983).

Product Listing

FAMEWAX™ Columns

- Ideal for FAME analysis.
- Similar to Omegawax™ columns.

ID	df (µm)	Stable to	30m
0.25mm	0.25	250°C	12497
0.32mm	0.25	250°C	12498
0.53mm	0.50	250°C	12499

Thermolite® Septa (green)

- Lowest bleed on FIDs, ECDs, and MSDs.
- Excellent puncturability.
- Preconditioned/ready to use.
- Does not adhere to hot metal surfaces.
- Usable to 340°C inlet temperatures.
- Packaged in non-contaminating tins.

Septum Diameter	25-pk.	50-pk.	100-pk.
9.5mm (3/8")	20359	20360	20361
10mm	20378	20379	20380
11mm (7/16")	20363	20364	20365
Shimadzu Plug	20372	20373	20374

High-Capacity Split Vent Trap

- Reduces the release of hazardous materials into the lab when using a split injection mode.
- Lasts one month or 1,500 injections.
- Connecting lines and mounting kit included.

Each	5-pk.
20698	20699

Fatty Acid Methyl Ester Mixtures

Neat fatty acid methyl esters can be used to prepare specific mixtures not commercially available. These products are of the highest purity available. Each compound is packaged under a nitrogen blanket to ensure product stability. A Certificate of Analysis is provided with each ampul. *Packaged 100mg/ampul.*

Carbon No.	Compound	CAS#	cat.#
C14:0	methyl myristate	124-10-7	35045
C14:1Δ9 cis	methyl myristoleate	56219-06-8	35046
C15:0	methyl pentadecanoate	7162-64-1	35047
C16:0	methyl palmitate	112-39-0	35048
C16:1Δ9 cis	methyl palmitoleate	1120-25-8	35049
C17:0	methyl heptadecanoate	1731-92-6	35050
C18:0	methyl stearate	112-61-8	35051
C18:1Δ9 cis	methyl oleate	112-62-9	35052
C18:2Δ9, 12 cis	methyl linoleate	112-63-0	35053
C18:3Δ9,12,15 cis	methyl linolenate	301-00-8	35054
C19:0	methyl nonadecanoate	1731-94-8	35055
C20:0	methyl arachidate	1120-28-1	35056
C20:1Δ11 cis	methyl eicosenoate	2390-09-2	35057
C20:2Δ11,14 cis	methyl eicosadienoate	2463-02-7	35058
C20:3Δ11,14,17 cis	methyl eicosatrienoate	55682-88-7	35059
C20:4Δ5,8,11,14 cis	methyl arachidonate	2566-89-4	35060

Autosampler Syringe 6-Packs for HP 7673 GCs

- Hamilton and SGE syringes are designed and tested to meet critical autosampler specifications.
- Needle point styles are developed to withstand multiple, fast septum injections.

Volume (µL)	Needle Term.	Gauge	Hamilton Restek cat.#	SGE Restek cat.#
5	ASN/F	23s	20170	24783
5	ASN/F	26s	21230	24782
5	ASN/F	23s-26s	24594	21214
10	ASN/F	23s	20169	24787
10	ASN/F	26s	24599	24786
10	ASN/F	23s-26s	24600	21215

RESTEK environmental

Applications note

The Rtx®-5Sil MS Column Provides the Best Resolution for Gasoline Range Organic (GRO) Compounds Listed in Alaska Method AK101AA

The Alaska Department of Environmental Conservation (ADEC) developed a new technique for the gas chromatographic (GC) analysis of gasoline range organic (GRO) compounds in soil, water, and waste water—Method AK101AA. This method quantitates aromatic and aliphatic compounds from C6 (hexane) to C10 (decane), and is capable of a higher level of accuracy over existing GRO methods. Restek's Rtx®-5Sil MS column is ideal for the analysis of GRO compounds, and specifically meets the requirements of Method AK101AA.

The key difference between AK101AA and other GRO methods that use photoionization detection/flame ionization detection (PID/FID) for differentiation of aliphatic and aromatic compounds, is that AK101AA uses the C9 alkyl benzenes (e.g., methyl ethylbenzenes) as target compounds in the calibration mixture. Using specific compound identification reduces error over other GRO methods that rely only on PID ranges for the determination of aromatic compounds. This can help determine the difference between highly degraded gasoline and light components of diesel fuels, such as kerosene and arctic fuel.

Method AK101AA prevents a high bias in reporting of aromatic compounds when they are in the presence of alkenes and alkynes. These are straight-chain unsaturated compounds that can give a false positive on the PID. Because all gasoline compounds respond on the FID, the total quantitation of gasoline is achieved with this detector, and the identification of single compounds are performed with the more selective PID detector. Method AK101AA also disregards analytes eluting before C6 because these pentanes and oxygenates have similar retention and are poorly resolved.

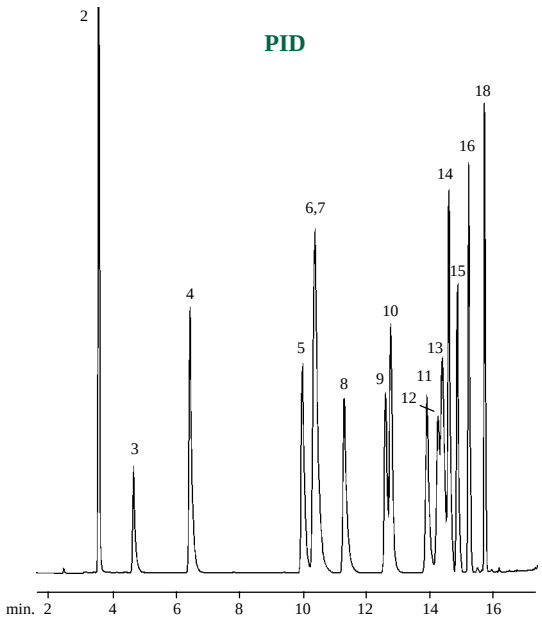
Peak List and Conditions for Figure 1

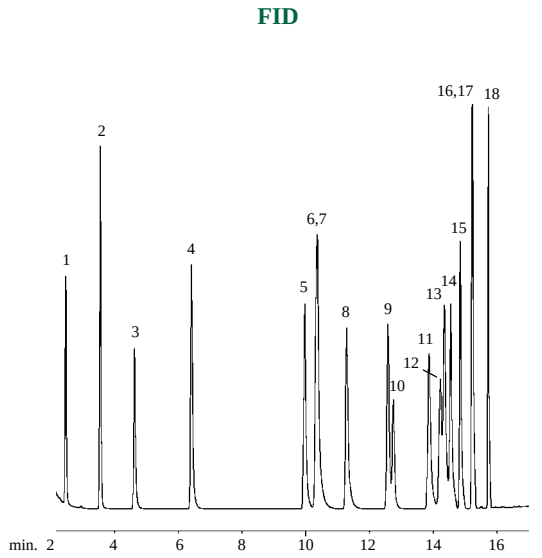
1. hexane
2. benzene
3. α,α,α-trifluorotoluene (ss)
4. toluene
5. ethylbenzene
6. *m*-xylene
7. *p*-xylene
8. *o*-xylene
9. isopropylbenzene
10. 4-bromo-fluorobenzene (ss)
11. *n*-propylbenzene
12. 1-ethyl-3-methylbenzene
13. 1-ethyl-4-methylbenzene
14. 1,3,5-trimethylbenzene
15. 1-ethyl-2-methylbenzene
16. 1,2,4-trimethylbenzene
17. decane
18. 1,2,3-trimethylbenzene

40m, 0.45mm ID, 1.50µm Rtx®-5Sil MS (cat.# 12798). Injection of 4-bromofluorobenzene (cat.# 30026); α,α,α-trifluorotoluene (cat.# 30048), decane, hexane, and Alaska aliphatic/aromatic GRO mix (cat.# 30461). **GC:** Finnigan 9001; **Column flow:** 9mL/min.; **Concentrator:** Tekmar LSC-3000 Purge & Trap, BTEX trap; **Interface:** direct with Siltek™ transfer line; **Oven temp.:** 40°C (hold 2 min.) to 85°C @ 4°C/min. (hold 1 min.) to 225°C @ 40°C/min. (hold 2 min.); **Det.:** FID (280°C)/PID (200°C); **Make-up flow rate:** 15mL/min.

Figure 1

The Rtx®-5Sil MS column resolves the alkyl benzenes and all the branched aromatic compounds listed in the Alaska GRO Method AK101AA providing more accurate identification and quantitation.



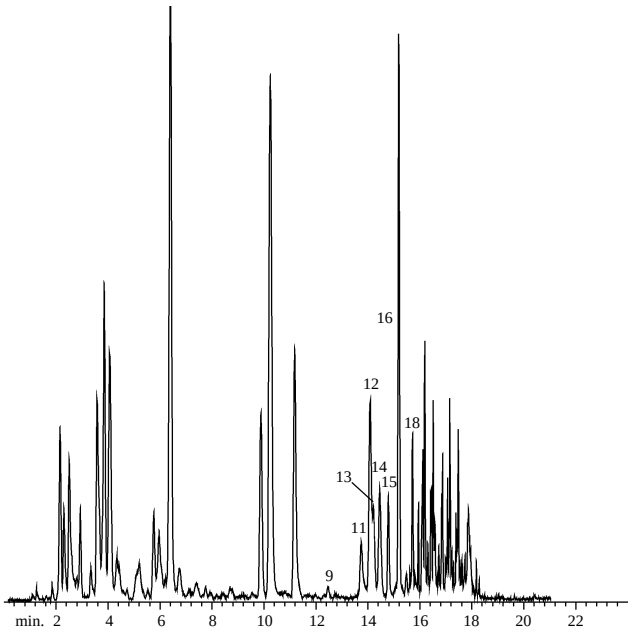


environmental

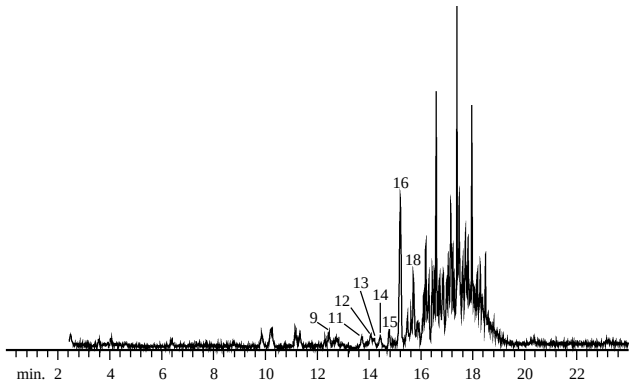
Figure 2

Comparing patterns and elution can help determine unweathered gasoline, kerosene, and diesel fuel.

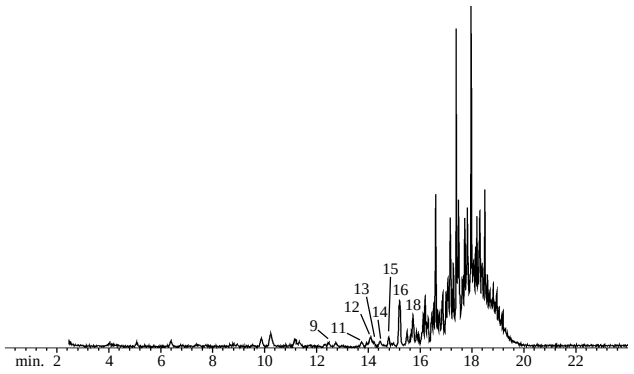
Unweathered gasoline



Unweathered kerosene



Unweathered diesel fuel



The Rtx®-5Sil MS column is capable of resolving all 13 aromatic compounds listed in the method. Columns commonly used for GRO analysis, such as the Rtx®-5 and Rtx®-502.2 columns, cannot adequately resolve the C9 alkyl benzenes. The Rtx®-5Sil MS phase design, column dimensions, and suggested GC conditions are optimized to provide the best possible resolution of the alkyl benzenes and all the branched aromatics listed in the Alaska method (Figure 1). Additionally, the 40m length and a 0.45mm internal diameter (ID) results in faster GC run-times, reduced cost, and lower column bleed. Bleed levels are exceptionally low even at temperatures up to 300°C.

The FID chromatogram in Figure 1 shows the elution of the AK101AA target compounds with the addition of two surrogates and two window markers—α,α,α-trifluorotoluene and 4-bromofluorobenzene, and C6 and C10 , respectively. Two of the eight aromatic C9 compounds—1,2,4-trimethylbenzene and 1,2,3-trimethylbenzene—have longer retention times on the Rtx®-5Sil MS column than the C10 marker, thus these two analytes elute after decane. All the C9 aromatic compounds are included for analyte quantitation on the PID, whereas the FID uses the total gasoline range quantitation, which ends with C10.

Unweathered gasoline, kerosene, and diesel fuel were analyzed under the same conditions to illustrate the differences in their patterns and elution (Figure 2). These three different fractions of petroleum were analyzed using GC/mass spectrometry (MS), with the C9 aromatic compounds labeled. The distribution and concentration of the C9 aromatics is different depending on the following: degree of weathering, type of fuel, and source of the petroleum. It is important to run both weathered and unweathered fuels using PID/FID and your conditions to assist in determining the types of petroleum and degrees of weathering.

Figure 3 shows examples of the C9 aromatic compounds found in gasoline and kerosene, analyzed using GC/MS in selected ion monitoring (SIM) mode and scanning for M/Z 120. These alkylbenzenes are the only compounds that share ions 105 and 120 in this region of the chromatogram. Concentrations of C9 compounds found in kerosene and gasoline differ by an order of magnitude; therefore, the standards were made in two different concentrations to produce a similar signal intensity on the MS system. The area of each C9 aromatic hydrocarbon was compared relative to the total area of all eight of these compounds in

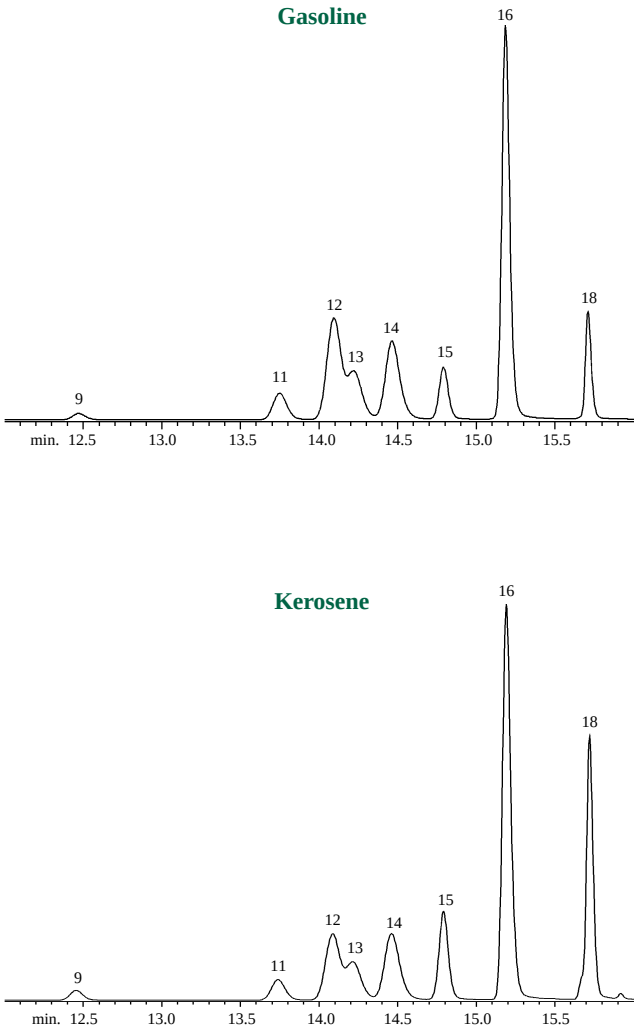
Peak List and Conditions for Figure 2

1. hexane
2. benzene
3. α,α,α-trifluorotoluene (ss)
4. toluene
5. ethylbenzene
6. *m*-xylene
7. *p*-xylene
8. *o*-xylene
9. isopropylbenzene
10. 4-bromo-fluorobenzene (ss)
11. *n*-propylbenzene
12. 1-ethyl-3-methylbenzene
13. 1-ethyl-4-methylbenzene
14. 1,3,5-trimethylbenzene
15. 1-ethyl-2-methylbenzene
16. 1,2,4-trimethylbenzene
17. decane
18. 1,2,3-trimethylbenzene

40m, 0.45mm ID, 1.50µm Rtx®-5Sil MS (cat.# 12798).
Injections of gasoline, kerosene, and diesel.
Column flow: 9mL/min.; **Concentrator:** Tekmar LSC-3000 Purge & Trap, BTEX trap; **Interface:** direct with Siltek™ transfer line; **Oven temp.:** 40°C (hold 2 min.) to 85°C @ 4°C/min. (hold 1 min.) to 225°C @ 40°C/min. (hold 2 min.); **Det.:** GC/MS TIC.

Figure 3

Distribution of C9 for varying types of petroleum are different, which can aid in fuel identification and quantitation.



Peak List and Conditions for Figure 3

1. hexane
2. benzene
3. α,α,α-trifluorotoluene (ss)
4. toluene
5. ethylbenzene
6. *m*-xylene
7. *p*-xylene
8. *o*-xylene
9. isopropylbenzene
10. 4-bromo-fluorobenzene (ss)
11. *n*-propylbenzene
12. 1-ethyl-3-methylbenzene
13. 1-ethyl-4-methylbenzene
14. 1,3,5-trimethylbenzene
15. 1-ethyl-2-methylbenzene
16. 1,2,4-trimethylbenzene
17. decane
18. 1,2,3-trimethylbenzene

40m, 0.45mm ID, 1.50µm Rtx®-5Sil MS (cat.# 12798). Injections of gasoline and kerosene. **Column flow:** 9mL/min.; **Concentrator:** Tekmar LSC-3000 Purge & Trap, BTEX trap; **Interface:** direct with Siltek™ transfer line; **Oven temp.:** 40°C (hold 2 min.) to 85°C @ 4°C/min. (hold 1 min.) to 225°C @ 40°C/min. (hold 2 min.); **Det.:** GC/MS SIM mode for ion 120 only.

each chromatogram. 1-ethyl-2-methylbenzene and 1,2,3-trimethylbenzene distributions are different for gasoline and kerosene using our standards. Other slight differences were noted between 1-ethyl-3-methylbenzene and 1,3,5-trimethylbenzene requiring separation of the ethyl-methylbenzene isomers. It is important to stress that results may vary due to different fuel sources and the degree of weathering; the point is that the distribution of C9 for varying types of petroleum are different, and these differences can aid in fuel identification and quantitation.

The standards for Method AK101AA consist of a mixture of 13 aromatic compounds. Restek offers this mixture complete with quality assurance (QA) documentation and a Certificate of Analysis, which can be used for audits as well as for internal QA needs. Our Alaska GRO standards are made in the correct concentrations, ensuring accurate identification and quantitation of environmental samples.

Use of the Rtx®-5Sil MS column and the analytical method outlined in Method AK101AA will achieve the best possible resolution of the alkyl benzenes and all the listed branched aromatics.

Product Listing

Rtx®-5Sil MS Columns

ID	df (µm)	Stable to	30m	40m	60m
0.45mm	1.50	300/320°C	—	12798	—

Siltek™ Guard Columns

nominal ID	nominal OD	5-meter	10-meter
0.25mm	0.37 ± 0.04mm	10026	10036
0.32mm	0.45 ± 0.04mm	10027	10037
0.53mm	0.69 ± 0.04mm	10028	10038

Alaska UST Method AK101AA

benzene
ethylbenzene
1-ethyl-2-methylbenzene
1-ethyl-3-methylbenzene
1-ethyl-4-methylbenzene
isopropylbenzene
n-propylbenzene
toluene
1,2,3-trimethylbenzene
1,2,4-trimethylbenzene
1,3,5-trimethylbenzene
o-xylene
m-xylene
p-xylene

1,000µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30461	30461-510	
w/data pack	30461-500	30461-520	30561

4-bromofluorobenzene

2,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30026	30026-510	
w/data pack	30026-500	30026-520	30126

10,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30082	30082-510	
w/data pack	30082-500	30082-520	30182

α,α,α-trifluorotoluene

2,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30048	30048-510	
w/data pack	30048-500	30048-520	30148

10,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30083	30083-510	
w/data pack	30083-500	30083-520	30183

Unleaded Gasoline Composite

From samples of regular- and premium-grade unleaded gasoline from three sources, blended to form a composite sample.

2,500µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30081	30081-510	
w/data pack	30081-500	30081-520	30181

50,000µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30205	30205-510	
w/data pack	30205-500	30205-520	30305

50,000µg/mL ea. in P&T methanol, 5mL/ampul.

	each	5-pack	10-pack
	30206	30206-510	
w/data pack	30206-500	30206-520	30306

Suitable for Matrix Spikes and Laboratory Control Samples

WA VPH Marker Standard

decane (C10)
dodecane (C12)
hexane (C6)
1-methylnaphthalene
naphthalene
octane (C8)
pentane (C5)
toluene
1,2,3-trimethylbenzene

1,000µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30450	30450-510	
w/data pack	30450-500	30450-520	30550

WA VPH Standard

benzene
decane (C10)
dodecane (C12)
ethylbenzene
hexane (C6)
1-methylnaphthalene
methyl-*tert*-butyl ether
naphthalene
octane (C8)
pentane (C5)
toluene
1,2,3-trimethylbenzene
m-xylene
o-xylene
p-xylene

1,000µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30451	30451-510	
w/data pack	30451-500	30451-520	30551

Restek Trademarks: Rtx, Siltek.

RESTEK pharmaceutical

Applications note

Excellent LC/MS Separation of Penicillins and Cephalosporins Using Ultra IBD Columns

Antibiotics are the most widely used medications in the world. Whether by prescription, addition to animal feed stocks, or use of cleaning agents, everyone in the civilized world is either directly or indirectly exposed to antibiotics in daily life. The overuse of antibiotics, however, has allowed resistant bacteria to thrive. The death of 12,500 people in Guatemala from an episode of Shingella fever can be traced to a simple mutation of the bacterial strain. Research indicated that the bacterium incorporated a single plasmid into its RNA sequence and resultantly became resistant to four different antibiotics. This illustrates the danger of resistance caused by adaptation. To combat resistant bacteria, new antibiotic derivatives must be created to overcome the bacteria's new defense mechanisms. Typically, HPLC columns can be used to analyze penicillins and their structurally related cephalosporins. However, the similarity of many derivatives may require additional interactions to effectively separate related compounds. Restek's Ultra IBD column is better able to resolve these compounds using polar and hydrophobic interactions.

Background

Penicillins and cephalosporins represent nearly sixty percent of antibiotics worldwide. These antibiotics possess a sulfur atom within a five- or six-membered ring, attached to a four-member ß-lactam ring. They are produced by fermentation processes using either selected fungi or species of *Streptomyces* bacteria. Derivatives are produced in two fashions:

1. Biosynthetic process—The fungus or bacteria are genetically engineered to produce a new derivative, or the starting materials are altered to produce biosynthetic variants during fermentation.

2. Semi-synthetic processes—The materials from a biosynthetic process are converted to chemical derivatives. Penicillin derivatives are created from penicillin G or V, while cephalosporin derivatives are created from cephalosporin C or cephamycin C.

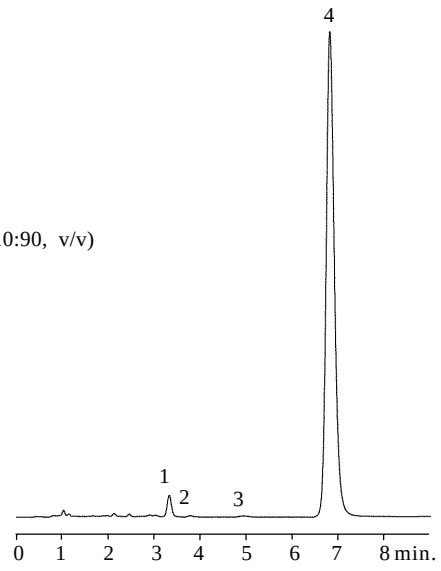
Unfortunately, biosynthetic fermentation does not produce a "pure" antibiotic. Even after cleanup of the fermentation mash, some side reaction products will remain. Many of these side products are closely related to the primary analyte (Figure 1). Desired products, however, are created in the semi-synthetic process. Penicillin V is converted to amoxicillin through chemical intermediates and varies only slightly in structure (Figure 2). Similar reactions also occur during production of cephalosporin derivatives. The loss of

Figure 1

Ultra IBD separates penicillin V from other fermentation impurities.

Peak List:
1. unknown
2. unknown
3. unknown
4. penicillin V

Sample:
Inj.: 2.5µL
Conc.: 1.2mg/mL
Solvent: acetonitrile:water (10:90, v/v)



Column:	**Ultra IBD**
Catalog #:	9175565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å

Conditions:

Mobile Phase:	10mM ammonium formate, pH 2.5: acetonitrile (60:40, v/v)
Flow:	1.2mL/min.
Temp.:	30°C
Det.:	UV @ 270nm

LC_0096

pharmaceutical

a hydride ion to create a phenyl ring is the only structural difference between cephradine and its side product cephalexin (Figure 3). Semi-synthetic processes are used to create derivatives like cephaloridine.

Unfortunately, many penicillins and cephalosporins are acid labile so that liquid chromatographic (LC) analysis of these molecules only should be performed if the sample is dissolved in a neutral media. Furthermore, if analysis time on the column is prolonged, breakdown of the analytes may occur *in situ* with a mobile phase that is not at a neutral pH. When measuring trace quantities of the analytes, especially by LC/mass spectrometry (MS), maintaining physiological pH near 7.4 may become important for stability and accurate quantitation.

Discussion of Analysis

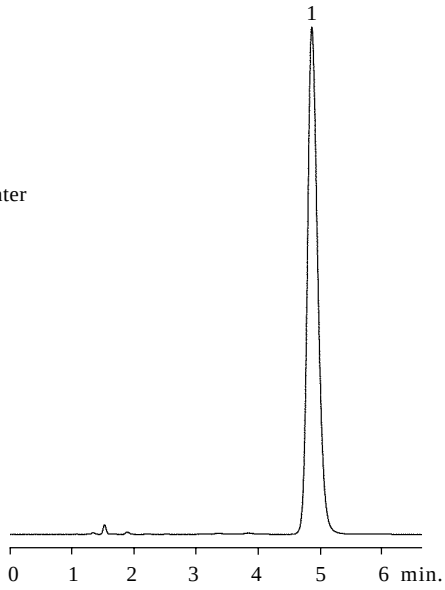
The Restek Ultra IBD phase provides greater versatility for the LC/MS analysis of penicillins and cephalosporins compared to a C18 column. The Ultra IBD column is capable of providing retention for cephaloridine in reverse phase mode with up to 45% organic solvent in the mobile phase. A conventional C18 column loses all retention near 35% organic solvent. Unlike a C18 column, the IBD is capable of polar interactions in a normal phase mode with analytes that possess charged functional groups. The ability

Figure 2

Ultra IBD shows excellent peak shape for amoxicillin.

Peak List:
1. amoxicillin

Sample:
Inj.: 5µL
Conc.: 1.5mg/mL
Solvent: acetonitrile:water (10:90, v/v)



Column: **Ultra IBD**
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 10mM ammonium formate, pH 2.5:acetonitrile (95:5, v/v)
Flow: 1.2mL/min.
Temp.: 30°C
Det.: UV @ 270nm

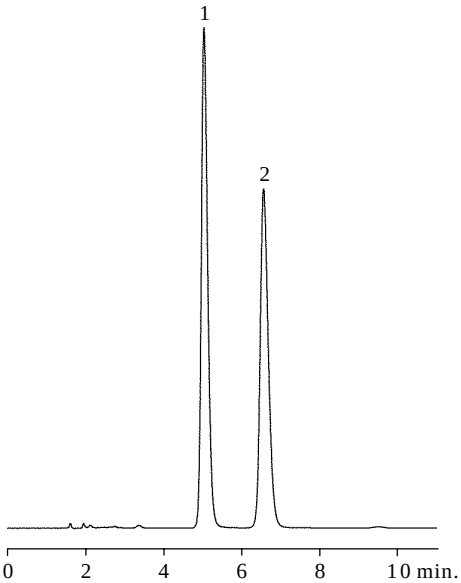
LC_0095

Figure 3

Ultra IBD shows great separation between cephalexin and cephradine, which differ only by a hydride ion.

Peak List:
1. cephalexin
2. cephradine

Sample:
Inj.: 10µL
Conc.: 500µg/mL
Solvent: acetonitrile:water (10:90, v/v)



Column: **Ultra IBD**
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 10mM ammonium formate, pH 2.5: acetonitrile (90:10, v/v)
Flow: 1.2mL/min.
Temp.: 30°C
Det.: UV @ 270nm

LC_0094

to retain a compound such as cephaloridine in normal phase mode using levels of organic solvents above 50% in the mobile phase, will allow increased sensitivity by LC/MS (Figure 4).

The IBD column also provides other chromatographic benefits. The excellent peak shape for cephaloridine in both the reverse and normal phase modes (Figure 5) increases sensitivity and improves quantitation. Furthermore, the retention of cephalosporin and cephaloridine is essentially unaffected by the pH. This allows full control in the pH range of 2 to 8 for optimum stabilization of the cephalosporins and penicillins during analysis, provided hydrolysis is not an issue. The IBD column has a unique blend of hydrophobic and polar character for better resolution of closely related compounds.

Conclusion

Closely related compounds such as penicillins and cephalosporins may require more than one type of interaction for optimum resolution of closely related components. The Restek IBD phase provides those interactions using only simple mobile phases. The excellent peak shape, resolution enhancement, and wide pH make it the ideal choice for the analysis of penicillin- and cephalosporin-based antibiotics by HPLC or LC/MS.

Figure 4

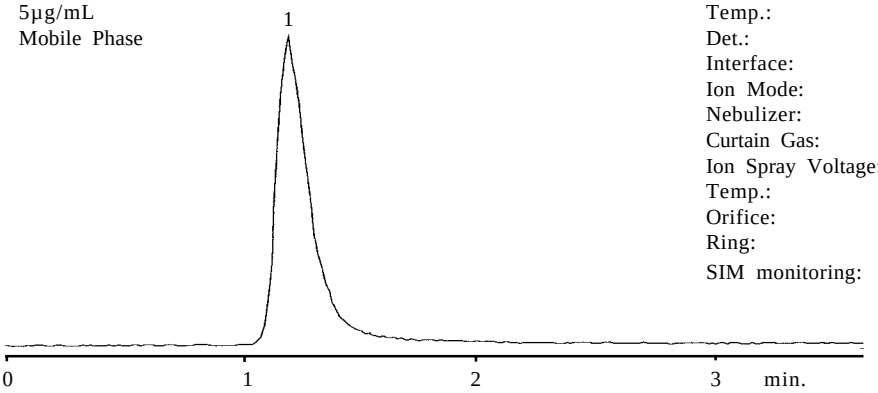
Ultra IBD allows increased LC/MS sensitivity of cephaloridine in normal phase mode.

Peak List:
1. cephaloridine

Sample:
Inj.: 5µL
Conc.: 5µg/mL
Solvent: Mobile Phase

Column: **Ultra IBD**
Catalog #: 9175552
Dimensions: 50 x 2.1mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 5mM ammonium acetate pH 7.4: acetonitrile (20:80)
Flow: 0.2mL/min.
Temp.: ambient
Det.: PE/Sciex API 150 EX
Interface: Turbo Ion Spray
Ion Mode: Positive
Nebulizer: 8L/hour
Curtain Gas: 12L/Hour
Ion Spray Voltage: 4700.0v
Temp.: 350°C
Orifice: + 10.0v
Ring: + 25.0v
SIM monitoring: 416 ± 3dal.



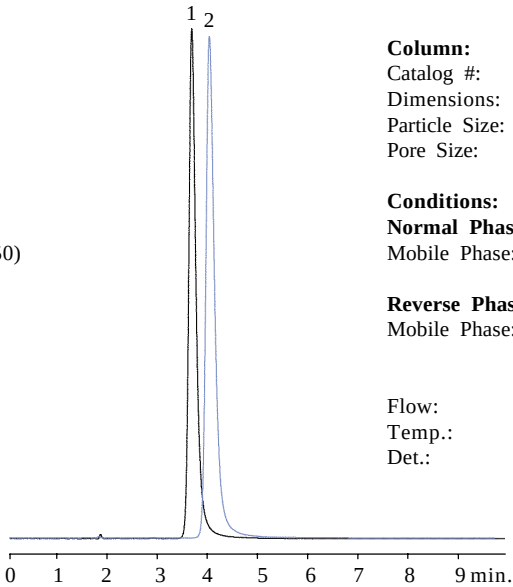
LC_0124

Figure 5

Ultra IBD shows excellent peak shape for cephaloridine in both normal and reverse phase modes.

Peak List:
1. cephaloridine reverse phase
2. cephaloridine normal phase

Sample:
Inj.: 5µL
Conc.: 1mg/mL
Solvent: acetonitrile:water (50:50)



Column: **Ultra IBD**
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Normal Phase:
Mobile Phase: acetonitrile: pH 4.0 20mM ammonium phosphate (80:20, v/v)
Reverse Phase:
Mobile Phase: acetonitrile: pH 4.0 20mM ammonium phosphate (20:80, v/v)

Flow: 1.2mL/min.
Temp.: 27°C
Det.: UV @ 254nm

LC_0101&LC_0102

Ultra IBD, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365

Ultra IBD, 3µm Columns with Trident™ Inlet

Particle Size: 3µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175332-700	9175333-700	9175335-700
50mm length	9175352-700	9175353-700	9175355-700
100mm length	9175312-700	9175313-700	9175315-700
150mm length	9175362-700	9175363-700	9175365-700

Ultra IBD, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

Ultra IBD, 5µm Columns with Trident™ Inlet

Particle Size: 5µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175532-700	9175533-700	9175535-700
50mm length	9175552-700	9175553-700	9175555-700
100mm length	9175512-700	9175513-700	9175515-700
150mm length	9175562-700	9175563-700	9175565-700
200mm length	9175522-700	9175523-700	9175525-700
250mm length	9175572-700	9175573-700	9175575-700

Ultra IBD Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	917550212	3
10 x 4.0mm	917550210	3
20 x 4.0mm	917550220	2

RESTEK nutraceutical

Applications note

The Institute for Nutraceutical Advancement (INA) Validates GC Methods for Saw Palmetto Using Rtx®-5 and Stabilwax® Columns

History of the INA Methods Validation Program

Consumer demand for natural products and dietary supplements has grown exponentially, with increasing amounts of botanical materials being used in the manufacture of a large variety of products. As the supplies and number of suppliers multiply, the consistency of raw materials has become an issue for virtually every major player in the natural products industry. Even companies with conscientious and responsible quality control procedures have found it difficult to ensure consistency in their products due to the lack of any published standards for analysis.

These issues, along with other science and market-based factors, led 29 companies to come together in an international effort to validate and make available analytical methods that will meet the demand for global consistency in the testing of botanicals.

The effort is called the Methods Validation Program, or MVP, and it is the first project for the newly formed Institute for Nutraceutical Advancement (INA). INA is a non-corporate division of Denver-based Industrial Laboratories, an independent laboratory that provides analytical and consulting services to the natural products industry.

The INA MVP is being developed under the direction of a broad range of representatives from within the natural products industry, including suppliers, manufacturers, retailers, marketing companies, a grower and an independent laboratory. Companies from both the United States and Europe are represented. In addition, ten major natural products organizations, including the Food and Drug Administration (FDA), have accepted seats on the INA MVP Advisory Committee as a way of ensuring that the process is inclusive. (Additional information is available at http://www.nutraceuticalinstitute.com/whoweare.)

All currently validated methods from the INA can be viewed at their website: http://www.nutraceuticalinstitute.com/methods. Although many use high performance liquid chromatography (HPLC), two methods involve gas chromatography (GC) for the analysis of fatty acids and sterols in saw palmetto. This fruit contains several principles thought to have physiological activity, including fatty acids such as caproic, capric, lauric, myristic, oleic, palmitic, stearic, and 1 to 2% essential oils. Furthermore, saw palmetto contains phytosterols and high molecular weight polysaccharides such as ß-sitosterol, ß-sitosterol 3-O-ß-D-glucoside, campesterol, stigmasterol. Purified ethanolic or CO_2 extracts of saw palmetto usually contain 70 to 80% free fatty acids. The fatty acids present are in several forms: free fatty acids, fatty acid esters of the fatty alcohols, and fatty acid esters of the phytosterols. This oil is commonly blended with excipients to form a dry powder at 30% free fatty acids concentration.

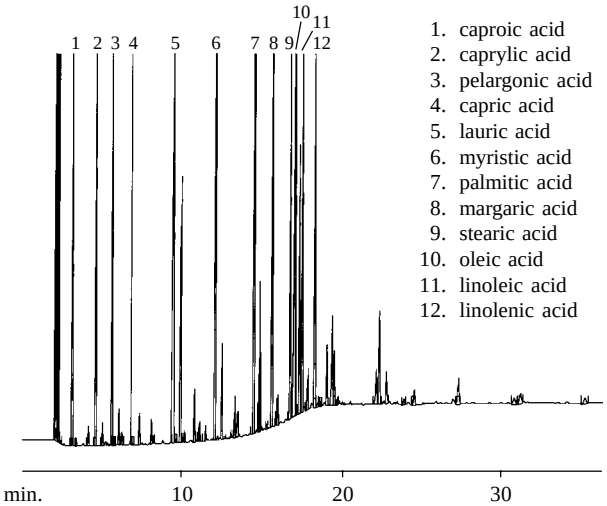
Determination of Fatty Acids in Serenoa Repens (Saw Palmetto or Sabel) by GC

This assay can be used to determine fatty acid distribution in saw palmetto fruit, oil extract, and blended powders. Determination is performed using GC, after transesterification of the triglycerides into the methyl esters occurs. For more specific information on the method itself and all procedures involved, please refer to http://www.nsfina.org/methods/sterolsset.html

The fatty acids from saw palmetto are separated in Figure 1, which was obtained using a Restek Stabilwax® column and a Shimadzu GC-14A GC, with split injection and a flame ionization detector (FID). The Stabilwax® column offers the necessary efficiency and selectivity to provide accurate identification of the fatty acids (as methyl esters).

Figure 1

The Stabilwax® column offers the necessary efficiency and selectivity to provide accurate identification of the fatty acids (as methyl esters).



50m, 0.25mm ID, 0.25µm Stabilwax® (cat.# 10626-105). 1µL split injection. **Oven temp.:** 110°C (hold 1 min.) to 240°C @ 8°C/min. (hold 25 min.); **Inj./FID temp.:** 230°C/250°C; **Carrier gas:** Hydrogen @ 2.5mL/min.; **Split flow:** 37.5mL/min.; **Septum purge:** 3mL/min.

nutraceutical

Determination of Sterols in Serenoa Repens (Saw Palmetto or Sabel) by GC

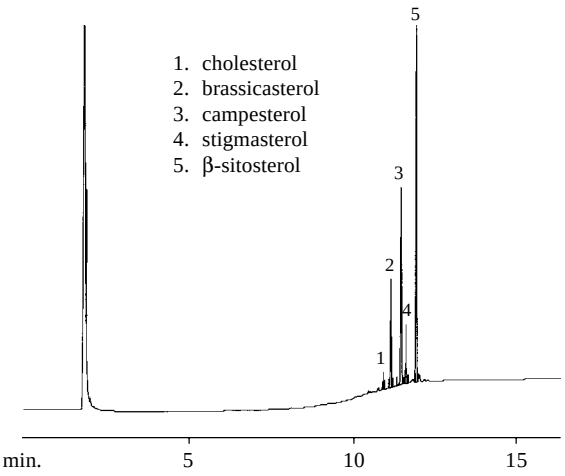
This assay can be used to determine stigmasterol, campesterol, brassicasterol, and ß-sitosterol in saw palmetto fruit, oil extract, and blended powders. Determination is performed using GC after hydrolysis, saponification, and derivatization. For more specific information on the method itself and all procedures involved, please refer to http://www.nutraceuticalinstitute.com/methods/sterols.html

The sterols from saw palmetto are shown in Figure 2, which was obtained by using a Restek Rtx®-5 column and a Hewlett Packard 5890 Series II GC equipped with an FID and an autosampler. The Restek Rtx®-5 column contains a 5% diphenyl/95% dimethyl polysiloxane phase, and has the thermal stability to provide elution and accurate quantitation of the phytosterols, even up to 340°C.

Special thanks to Dr. Mark Lange, Director, and to Kathryn Bass, Marketing Director, of MVP for allowing us to print this material. Much of this text has been directly downloaded from the INA website.

Figure 2

The Rtx®-5 column has the thermal stability to provide elution and accurate quantitation of the phytosterols, even up to 340°C.



60m, 0.25mm ID, 0.25µm Rtx®-5 (cat.# 10226). 1µL splitless injection*.
Oven temp.: 200°C (hold 1 min.) to 340°C @ 15°C/min. (hold 10 min.);
Inj./FID temp.: 345°C/355°C.
**Split injection may be used. A split flow of 112mL/min. is suggested. However, split injection may result in higher variability of results.*

Product Listing

Stabilwax® Columns

ID	df (µm)	Stable to	50m
0.25mm	0.25	250°C	10626-105

Rtx®-5 Columns

ID	df (µm)	Stable to	60m
0.25mm	0.25	360°C	10226

Inlet Sleeves for HP GCs

Description	ID/OD/Length	ea.	5-pk.	25-pk.
2mm Splitless	2.0/6.5/78.5mm	20712	20713	20714
4mm Splitless	4.0/6.5/78.5mm	20772	20773	20774

Inlet Sleeves for Shimadzu GCs

Description	ID/OD/Length	ea.	5-pk.	25-pk.
99mm Split	3.5/5.0/99mm	20860	20861	20862
Cyclosplitter®	3.5/5.0/99mm	20870	20871	—

For more information on Restek's Stabilwax® and Rtx®-5 GC columns, please request our informative* Fast Facts Flyers *(Lit. cat.# 59316 for Stabilwax® and Lit. cat.# 59310 for Rtx®-5).

RESTEK pharmaceutical

Applications note

Analyze Nucleotides, Nucleosides, Purine, and Pyrimidine Bases Simultaneously with the Ultra IBD Column

Mixtures of nucleotides, nucleosides, and their respective purine or pyrimidine bases are difficult to analyze by reversed phase/high performance liquid chromatography (RP/HPLC). These compounds cover a wide range of polarities and functionalities, from the acidic nucleotides to the basic purines and pyrimidines, making it very difficult to retain and resolve all of them with conventional alkyl stationary phases. Traditional HPLC analysis of these compounds often uses a combination of reversed phase-ion pairing (RP-IP) and/or ion exchange (IEX) mode. Nucleotides often are analyzed by anion exchange, while nucleosides sometimes are analyzed by cation exchange. These methods are not compatible with all the solutes in the mixtures and they lack ruggedness.

This Applications Note demonstrates that all three classes of compounds (nucleotides, nucleosides, and bases) can be analyzed by RP/HPLC using one column and the same, simple isocratic mobile phase. This provides greater convenience, reproducibility, and ruggedness in developing methods for these mixtures. By using a unique, intrinsically base-deactivated stationary phase (i.e., the Ultra IBD column), simple RP/HPLC conditions were identified that can resolve any common purine or pyrimidine base from its related ribonucleoside and mono-, di-, and triphosphate nucleotides.

Nucleotides and nucleosides are derived from the nitrogenous bases shown in Figure 1. These nitrogenous bases are either purines (adenine and guanine) or pyrimidines (cytosine, uracil, and thymine). A nucleoside consists of a purine or pyrimidine base linked to a five-carbon sugar (pentose). A nucleotide is composed of a nucleoside plus one or more phosphate groups. Figure 2 shows the structures of a nucleoside and three nucleotides derived from adenine.

Figure 1

Nitrogenous purine and pyrimidine bases form nucleotides and nucleosides

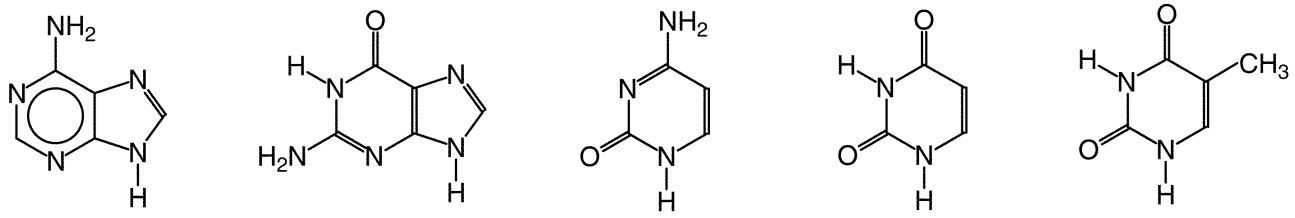
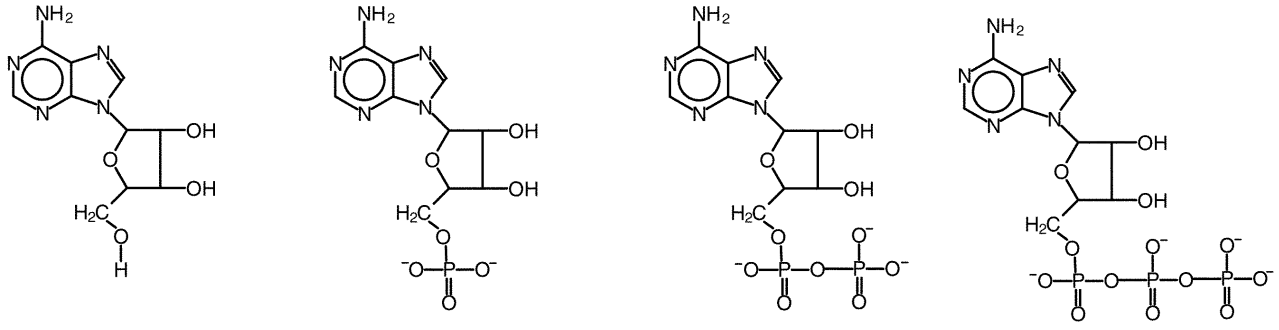


Figure 2

A nucleoside and three nucleotides derived from adenine



pharmaceutical

Adenosine is a ribonucleoside (adenine + ribose). ATP is a particularly important nucleotide, serving as a universal source of energy for biological processes.

The Ultra IBD column is particularly effective for retaining and resolving complex mixtures of nucleotides, nucleosides, and purine and pyrimidine bases. The unique Ultra IBD stationary phase is composed of a polar group within, or intrinsic to, an alkyl chain. The polar group gives extra retention for many polar analytes as well as unique selectivity, a very high level of base deactivation, and compatibility with highly aqueous mobile phases. The Ultra IBD column is ideal for LC/MS because it often can resolve acidic, basic, zwitterionic and/or neutral compounds in a single analysis using simple mobile phases.

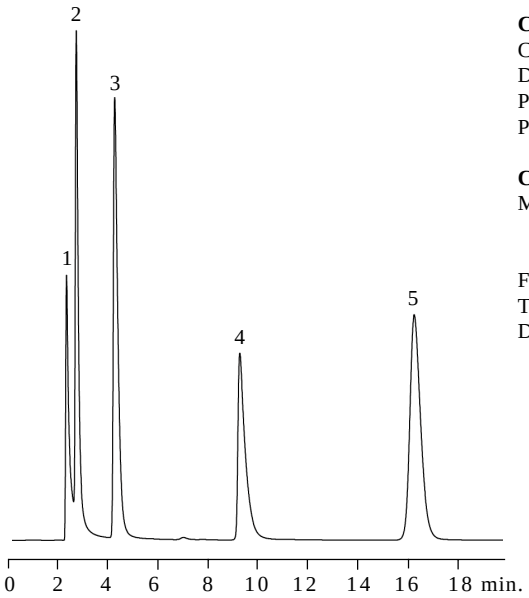
Figures 3 through 7 each show separations of one of the major purines or pyrimidines from its respective ribonucleo-

Figure 3

Ultra IBD separates adenine, adenosine, ATP, ADP, and AMP with simple isocratic reverse phase conditions.

Peak List:	Conc. (µg/mL):
1. ATP	258
2. ADP	320
3. AMP	274
4. adenine	84
5. adenosine	254

Sample:
Inj.: 20µL
Solvent: 20mM ammonium acetate, pH 5.8



Column:	**Ultra IBD**
Catalog #:	9175565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å

Conditions:
Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
Flow: 1.0mL/min
Temp.: 35°C
Det.: UV @ 260nm

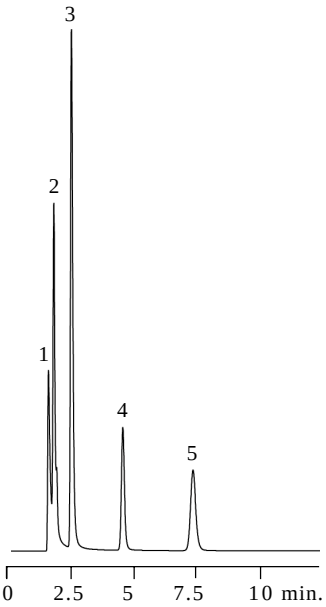
LC_0129

Figure 4

Ultra IBD separates guanine, guanosine, GTP, GDP, and GMP with simple isocratic reverse phase conditions.

Peak List:	Conc. (µg/mL):
1. GTP	212
2. GDP	260
3. GMP	362
4. guanine	80
5. guanosine	226

Sample:
Inj.: 20µL
Solvent: 20mM ammonium acetate, pH 5.8



Column:	**Ultra IBD**
Catalog #:	9175565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å

Conditions:
Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
Flow: 1.0mL/min
Temp.: 35°C
Det.: UV @ 260nm

LC_0131

side and mono-, di-, and triphosphate nucleotide. Guanosine, uridine, cytidine, and thymidine are ribonucleosides derived from guanine, uracil, cytosine, and thymine, respectively. Note that each of these separations was achieved using the same conditions and that, in each case, the order of elution is the same: the triphosphate, the diphosphate, then the monophosphate nucleotide, followed by the base, and lastly the nucleoside. There are slight "shoulders" on the peaks for GDP (Figure 4), TTP (Figure 6), and UMP (Figure 7). These compounds were present in the standards and were presumed to be impurities or degradation products.

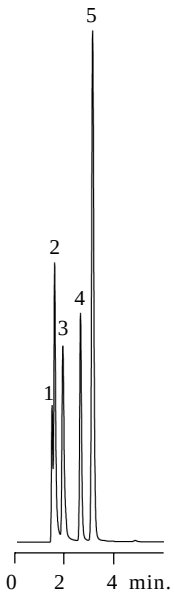
Table 1 lists the typical retention times obtained for all 25 of the compounds separated in Figures 3–7. While not all 25 compounds can be resolved in a single HPLC analysis, it is possible to analyze all of them using these chromatographic conditions and MS or MS/MS detection. Note that the mobile phase is compatible with MS detection, as all of its

Figure 5

Ultra IBD separates cytosine, cytidine, CTP, CDP, and CMP with simple isocratic reverse phase conditions.

Peak List: Conc. (µg/mL):
1. CTP 248
2. CDP 322
3. CMP 248
4. cytosine 100
5. cytidine 340

Sample:
Inj.: 20µL
Solvent: 20mM ammonium acetate, pH 5.8



Column: **Ultra IBD**
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
Flow: 1.0mL/min
Temp.: 35°C
Det.: UV @ 260nm

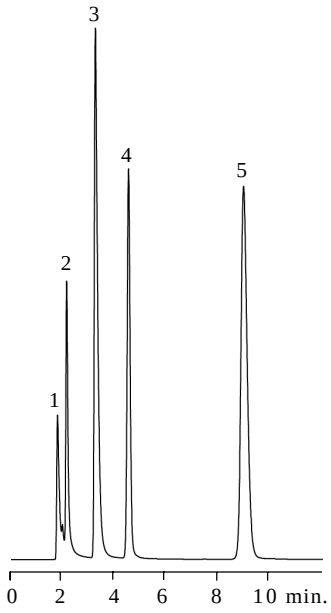
LC_0130

Figure 6

Ultra IBD separates thymine, thymidine, TTP, TDP, TMP with simple isocratic reverse phase conditions.

Peak List: Conc. (µg/mL):
1. TTP 224
2. TDP 152
3. TMP 346
4. thymine 88
5. thymidine 318

Sample:
Inj.: 20µL
Solvent: 20mM ammonium acetate, pH 5.8



Column: **Ultra IBD**
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
Flow: 1.0mL/min
Temp.: 35°C
Det.: UV @ 260nm

LC_0132

components are volatile. Figure 8 shows that the Ultra IBD column can resolve a mixture of 11 various nucleotides, nucleosides, and bases using ultraviolet (UV) detection.

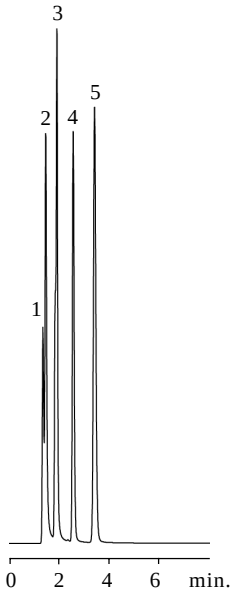
The unique stationary phase of the Ultra IBD column can retain and resolve mixtures of nucleotides, nucleosides, and purine and pyrimidine bases by RP/HPLC, using isocratic elution with a simple, volatile mobile phase. A single set of chromatographic conditions can resolve any of the common purine or pyrimidine bases from its respective ribonucleoside and mono-, di-, and triphosphate nucleotides.

Figure 7

Ultra IBD separates uracil, uridine, UTP, UDP, UMP with simple isocratic reverse phase conditions.

Peak List: Conc. (µg/mL):
1. UTP 360
2. UDP 360
3. UMP 284
4. uracil 90
5. uridine 230

Sample:
Inj.: 20µL
Solvent: 20mM ammonium acetate, pH 5.8



Column: **Ultra IBD**
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
Flow: 1.0mL/min
Temp.: 35°C
Det.: UV @ 260nm

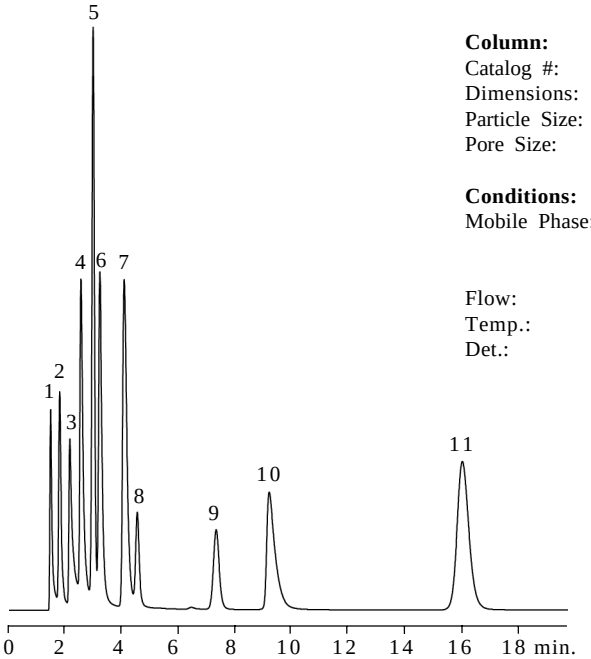
LC_0133

Figure 8

Ultra IBD separates an 11-component mixture of nucleotides, nucleosides, and nitrogenous bases.

Peak List: Conc. (µg/mL):
1. CDP 146
2. CMP 113
3. ATP 117
4. ADP 145
5. cytidine 155
6. TMP 157
7. AMP 125
8. guanine 36
9. guanosine 103
10. adenine 38
11. adenosine 115

Sample:
Inj.: 20µL
Solvent: 20mM ammonium acetate, pH 5.8



Column: **Ultra IBD**
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
Flow: 1.0mL/min
Temp.: 35°C
Det.: UV @ 260nm

LC_0128

Table 1

Typical retention times for common nucleotides, nucleosides, and purine and pyrimidine bases.

Compound	Retention Time[1] (min.)
CTP	1.5
UTP	1.5
CDP	1.6
UDP	1.6
GTP	1.7
TTP	1.8
CMP	1.9
GDP	1.9
UMP	2.0
TDP	2.2
ATP	2.3
GMP	2.6
Cytosine	2.6
ADP	2.7
Uracil	2.7
Cytidine	3.1
TMP	3.3
Uridine	3.6
AMP	4.2
Guanine	4.6
Thymine	4.6
Guanosine	7.4
Thymidine	9.0
Adenine	9.3
Adenosine	16.2

1. Retention times are for 150x4.6mm column; Flow rate: 1.0mL/min; Mobile phase: 20mM ammonium acetate, pH 5.8: Methanol. (97.5:2.5, v/v).

Ultra IBD, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365

Ultra IBD, 3µm Columns with Trident™ Inlet

Particle Size: 3µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175332-700	9175333-700	9175335-700
50mm length	9175352-700	9175353-700	9175355-700
100mm length	9175312-700	9175313-700	9175315-700
150mm length	9175362-700	9175363-700	9175365-700

Ultra IBD, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

Ultra IBD, 5µm Columns with Trident™ Inlet

Particle Size: 5µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175532-700	9175533-700	9175535-700
50mm length	9175552-700	9175553-700	9175555-700
100mm length	9175512-700	9175513-700	9175515-700
150mm length	9175562-700	9175563-700	9175565-700
200mm length	9175522-700	9175523-700	9175525-700
250mm length	9175572-700	9175573-700	9175575-700

Ultra IBD Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	917550212	3
10 x 4.0mm	917550210	3
20 x 4.0mm	917550220	2

RESTEK environmental

Applications note

CarboPrep™ SPE Cleanup of Method 8141A Organophosphorous Pesticides and Herbicides

Organophosphorus pesticides (OPP) are widely applied in agriculture and home use. With the decline of organochlorine pesticides, OPPs have become the most widely used class of insecticides in the US. [1] Analysis of OPPs requires protection of prepared samples from thermal- and photodegradation and special detectors. In addition, the US EPA does not recommend GPC or Florisil® cleanup techniques for solvent-extracted samples. This makes gas chromatography (GC) analysis of low-level, complex matrices even more difficult. This Applications Note shows how CarboPrep™ 90 solid phase extraction (SPE) cartridges may be useful as an alternative in the cleanup of sample extracts containing OPP. Using Rtx®-CLPesticides columns also will help to improve GC analysis of these insecticides.

CarboPrep™ SPE cartridges contain a nonporous, chromatographic grade- graphitized carbon that is optimized for cleanup of environmental sample extracts. Graphitized carbon packings also have been effective in the concentration and extraction of a variety of pesticides in drinking water samples.[2] The high flow rates that can be used with this material allow rapid extraction of 1 to 4 liters of aqueous samples. The cartridges have a low background level, especially suitable for pesticides. The carbon surface provides maximum capacity with a minimum bed weight, reducing the volume of solvent used during extraction.

Studies using spiked solvent samples show that CarboPrep™ 90 SPE cartridges may be useful as an alternative in the cleanup of OPP insecticides before nitrogen phosphorus detection (NPD) or flame photometric detection (FPD) analysis. Previous studies at Restek have shown excellent performance in the recovery of organochlorine pesticides in sample extracts.[3] The same SPE cartridge and preparation method were used to extract the OPPs as in the previous studies (see procedure outlined in Figure 1).

Repetitive extracts show that 80% of the compounds tested exhibited greater than 80% recovery (Table 1). The average recovery range (n=5) was 42% for monocrotophos to 109% for merphos. CarboPrep™ 90 SPE cartridges also were shown to remove sample matrix interferences such as hydrocarbons and humic substances that cause chromatographic interference peaks and leave nonvolatile organic residue in the injection port. For fast, quantitative recovery of OPP compounds, CarboPrep™ 90 SPE cartridges offer a better alternative than GPC or Florisil® SPE.

References:

1. "Recognition and Management of Pesticides Poisonings". http://www.epa.gov/oppfead1/safety/healthcare/handbook/handbook.htm, p.34
2. "Development of a Multiresidue Method for Analyzing Pesticide Traces in Water," C. Crescenzi, A. DiCorcia , E. Guerriero, R. Samperi, ES&T, 1997, 31 479-488.
3. "CarboPrep™ SPE Cleanup of Method 8081A Chlorinated Pesticides" Restek Application Note #59110.

Table I

Recovery of OPP from Solvent Extracts Using CarboPrep™ 90 SPE Cartridges

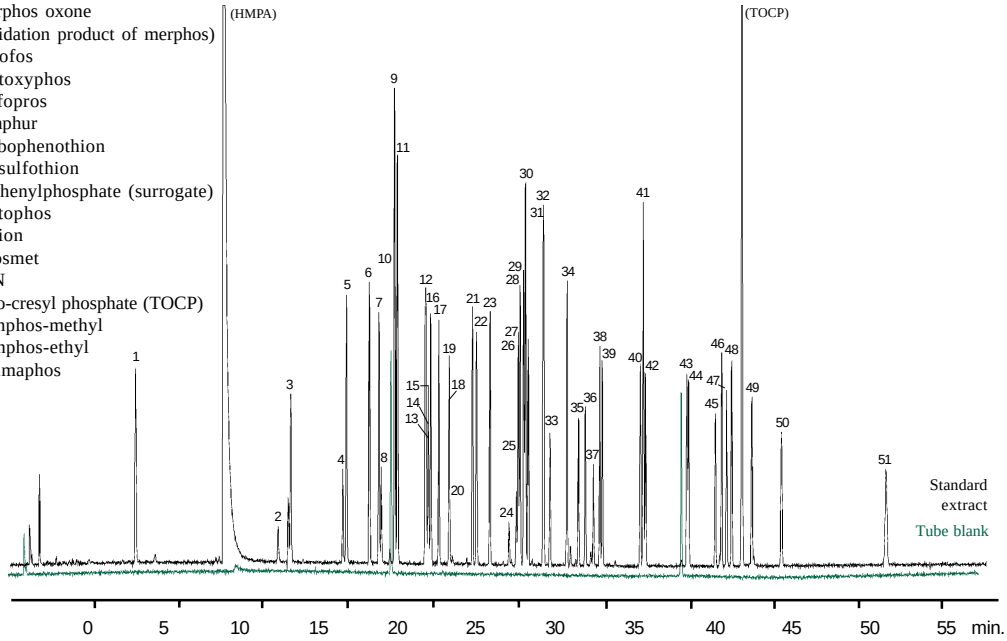
Analyte	% recovery avg. n=5	RSD
dichlorvos	91.6	5.8
mevinphos	90.7	8.9
ethoprop	103.1	8.7
phorate	101.7	10.2
naled	103.1	9.0
tributylphosphate (surrogate)	106.8	8.9
demeton-o	101.3	9.9
diazinon, demeton-s (coelution)	104.2	9.8
disulfoton	105.5	10.9
ronnel	101.9	10.1
merphos	109.3	8.9
chlorpyrifos	96.1	0.7
fenthion	103.6	9.8
parathion-methyl	103.1	10.1
trichloronate	101.6	14.7
tokuthion	98.8	11.1
stirofos	101.2	9.3
bolstar	98.1	7.9
fensulfothion	78.9	11.2
triphenylphosphate (surrogate)	92.9	9.1
azinphos-methyl	86.6	7.4
coumaphos	83.1	10.2
trichlorfon	55.0	12.9
thionazin	90.0	8.4
fonophos	94.6	8.1
dicrotophos	76.0	10.7
dichlorofenthion	95.3	9.1
chlorpyrifos methyl	101.8	9.9
aspon	91.9	7.9
fenitrothion	81.5	22.6
crotoxyphos	92.8	7.9
	avg. n=4	RSD
TEPP, sulfotepp (coelution)	84.7	0.4
dimethoate	82.3	17.4
monocrotophos	42.4	21.7
malathion	81.8	14.7
parathion-ethyl	80.3	13.9
EPN	64.4	14.7
terbufos	93.8	9.6
dioxathion	59.3	8.4
phosphamidon	68.7	10.0
chlorfenvinphos	80.4	10.5
carbophenothion	78.8	8.2
ethion	80.5	10.5
leptophos	91.3	1.7
famphur	78.5	7.1
phosmet	83.0	6.7
azinphos-ethyl	83.0	9.3

environmental

Figure 1

CarboPrep™ 90 SPE Cleanup for Method 8141A OPPs Provides Quantitation-Level Accuracy

1. dichlorvos
2. trichlorfon
3. mevinphos
4. demeton-o
5. thionazin
6. ethoprop
7. phorate
8. naled
9. sulfotepp
10. TEPP
11. tributylphosphate (surrogate)
12. fonophos
13. terbufos
14. demeton-s
15. dicrotophos
16. diazinon
17. disulfoton
18. dimethoate
19. dioxathion
20. monocrotophos
21. dichlorofenthion
22. chlorpyrifos methyl
23. ronnel
24. phosphamidon isomer
25. merphos
26. chlorpyrifos
27. fenthion
28. parathion-methyl
29. aspon
30. trichloronate
31. malathion
32. fenitrothion
33. phosphamidon
34. parathion-ethyl
35. chlorfenvinphos
36. tokuthion
37. merphos oxone (oxidation product of merphos)
38. stirofos
39. crotoxyphos
40. sulfopros
41. famphur
42. carbophenothion
43. fensulfothion
44. triphenylphosphate (surrogate)
45. leptophos
46. ethion
47. phosmet
48. EPN
49. tri-o-cresyl phosphate (TOCP)
50. azinphos-methyl
51. azinphos-ethyl
52. coumaphos



Rtx®-CLPesticides - 30m, 0.32mmID, 0.50µm cat.# 11139

Oven temp: 120°C (hold 10 min.) to 250°C (hold 15 min.) @ 4°C/min.
Inj.: 220°C, split/splitless sleeve
Det.: 295°C, Agilent (HP) 5890 FPD
Carrier gas: helium
Standards: OP Pesticide Calibration Mix A cat.# 32277, OP Pesticide Calibration Mix B cat.# 32278, OP Pesticide Mix C & D custom, Tributylphosphate surrogate standard cat.# 32280 Triphenylphosphate surrogate standard, cat.# 32281
Sample: 1.0mL hexane. Standards were spiked at 0.20µg/mL, injection volume 1µL.
Cartridge: 3mL, 250mg CarboPrep™ 90, cat.# 26091
Cartridge conditioning: Apply 3mL of hexane and pass through cartridge.
Extract clean-up: Prepare collection rack with vials, place under each cartridge. Add 1.0mL of extracted sample to cartridge and collect all solutions passing through. Add 20mL of CH_2Cl_2:hexane (20:80) to cartridge and allow it to elute using gravity feed.
Extract concentration: Solvent exchange extract to hexane. Do not allow extracts to dry completely. Concentrate extracts to 1.0mL. Sample is ready for analysis.

Product Listing:

Description	cat. #
Rtx®-CLPesticides, 30m, 0.32mm ID, 0.50µm	11139
Rtx®-OPPesticides, 30m, 0.32mm ID, 0.50µm	11239 ☆
CarboPrep™ 90 cartridge 3mL, 250mg	26091
Triphenylphosphate Standard	32281

Description	cat. #
Tributylphosphate Standard	32280
OP Pesticide Calibration Mix A	32277
OP Pesticide Calibration Mix B	32278

☆ Restek Recommended

RESTEK environmental

Applications note

Optimizing Massachusetts Volatile Petroleum Hydrocarbon GC Analysis

Total Petroleum Hydrocarbon (TPH) analysis allows the characterization of dozens of commercially available petroleum products, which are the most prevalent environmental pollutants. The two fractions of TPH—volatile gasoline range organic (GRO) compounds, also called petroleum volatile organic compounds (PVOC), and the semivolatile diesel range organic (DRO) compounds—are analyzed differently depending on their boiling point ranges.

Typical methods for the identification of gasoline use early and late eluting compounds to determine an analytical window for total gasoline quantitation. Then, GC analysis uses pattern recognition, the specific ratio of peaks that make up a particular compound, to identify a fuel. If a pattern falls within this window, it may be reported as gasoline and quantified. Difficult matrices can result in misidentification or poor quantitation of the sample, and environmental degradation (i.e., weathering) further complicates this analysis.

On January 1, 1998, the Massachusetts Department of Environmental Protection (MADEP) promulgated a new method, known as Volatile Petroleum Hydrocarbons (VPH) to better quantify gasolines. This method identifies and evaluates PVOCs by differentiating and characterizing the aromatic and aliphatic fractions of gasoline using a photo-ionization detector (PID) and a flame ionization detector (FID) in series. The data generated from this method will aid in evaluating human health hazards that may result from exposure to PVOCs. Other states in the US and provinces in Canada have adopted the VPH method for use in remediation, site characterization, and toxicity data (mixtures for other methods are listed in the UST Product Listing, lit. cat. #59617-A).

Difficulties with the Analysis

A large proportion of VPH samples are soil. The soil is weighed in the field and an equal amount of methanol is added at the time of sampling. 100uL of methanol extract is added to 4.9mL of water and then is purged. One problem with this analysis is that most purge-and-trap concentrators were not designed to have large amounts of methanol purged onto their absorbent beds. The VOCARB™ 3000 and VOCARB™ 4000 traps have difficulty retaining *n*-pentane and 2-methylpentane after repeated exposure to methanol, causing poor linearity of these compounds. We suggest using a BTEX trap because of its non-polar properties. You will experience a slight decrease in response for methyl-*tert*-butyl-ether, but it will not compromise your detection limit.

Column Selection

All purge-and-trap methods for this analysis result in broad, early eluting peaks. Therefore, choosing the right chromatographic column can prevent coelutions and poor quantitation. Many columns may drastically change the quantitation of aliphatic and aromatic compounds, or suffer from poor resolution of methanol and methyl-*tert*-butyl-ether or from poor separation of pentane and 2-methylpentane. For optimized separation of light hydrocarbons and light gas additives, use the Rtx®-502.2 column specified in the VPH method.

Figure 1 shows the separation of VPH compounds on an Rtx®-502.2 column, using a PID (Figure 1A) and an FID (Figure 1B). The first peak in the chromatogram is methanol. Notice that it is clearly separated from the methyl-*tert*-butyl-ether and *n*-pentane.

Figure 1

To achieve optimum VPH analysis using an Rtx®-502.2 column, use PID for aromatic compounds and FID for aliphatic compounds.

Figure 1A: PID

1. methanol
2. methyl-*tert*-butyl-ether (60ng)
3. benzene (20ng)
4. toluene
5. ethylbenzene (20ng)
6. *p*+*m*-xylene (40ng ea.)
7. *o*-xylene (40ng)
8. 1,2,4-trimethylbenzene (40ng)
9. naphthalene (40ng)
10. 2,5-dibromotoluene (ss)

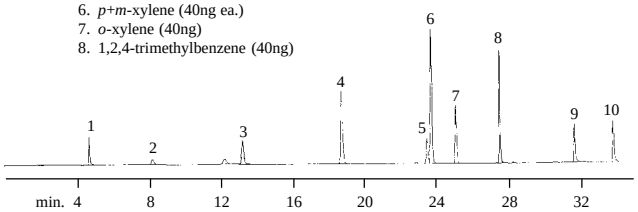
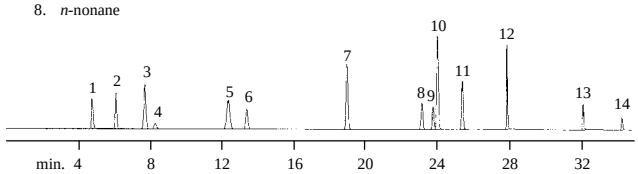


Figure 1B: FID

1. methanol
2. *n*-pentane (40ng)
3. 2-methylpentane (60ng)
4. methyl-*tert*-butyl-ether (60ng)
5. 2,2,4-trimethylpentane (60ng)
6. benzene (20ng)
7. toluene
8. *n*-nonane
9. ethylbenzene (20ng)
10. *p*+*m*-xylene (40ng ea.)
11. *o*-xylene (40ng)
12. 1,2,4-trimethylbenzene (40ng)
13. naphthalene (40ng)
14. 2,5-dibromotoluene (ss)



105m, 0.53mm ID, 3.0µm (cat.# 10910). **Concentration:** on-column at levels listed; **Oven temp:** 45°C to 90°C @ 3°C/min., to 140° @ 5°C/min., to 230°C @ 45°C/min. (hold 8 min.); **Carrier gas:** helium @ 15mL/min.; Tekmar® Model LSC 2000; **Trap:** BTEX; **Purge:** helium @ 40mL/min. for 11 min.; **Dry purge:** 2 min.; **Desorb preheat:** 245°C; **Desorb:** 2 min. @ 250°C; **Bake:** 6 min. @ 260°C.

Chromatograms courtesy of Severn Trent Laboratories, Burlington, VT.

environmental

The PID is used to identify target VPH analytes, defined as benzene, toluene, ethylbenzene, *m*-xylene, *p*-xylene, *o*-xylene, naphthalene, and methyl-*tert*-butyl-ether. The VPH method identifies all of the aromatic compounds after *o*-xylene to naphthalene. The reason for the distinction of aromatic from aliphatic compounds rests on current data, which suggests aromatic compounds are more toxic.

The FID detector is used only for reporting aliphatic compounds. This detector responds to all hydrocarbons, thereby necessitating the subtraction of aromatic and target compounds. The latter analytes subsequently are reported on the PID detector. There are two aliphatic ranges: C5 to C8, which elute from *n*-pentane to just before *n*-nonane; and C9 to C12, which elute from *n*-nonane to just before naphthalene.

Analytical Reference Materials

Running MA VPH method requires calibrating 13 separate compounds. These 13 compounds allow for the determination of three groups of analytes in the sample: total gasoline, aliphatic and aromatic hydrocarbons, and individual target compounds. Restek offers the required mixtures with a Certificate of Analysis or with complete data packs that can be used for audits and internal quality assurance. The VPH mixtures are made in the appropriate concentrations for spiking, ensuring accurate identification and quantitation of environmental samples in your laboratory. Restek also offers high concentration mixes that can be diluted down to a spiking concentration, useful for cost savings.

Product Listing

Rtx®-502.2 Columns (Fused Silica)

ID	df (µm)	20m	30m	40m	60m	105m
0.18mm	1.00	40914	—	40915	—	
0.25mm	1.40	—	10915	—	10916	—
0.32mm	1.80	—	10919	—	10920	10921
0.53mm	3.00	—	10908	—	10909	10910

MXT®-502.2 Columns (Silcosteel®)

ID	df (µm)	30m	60m	105m
0.28mm	1.60	70919	70920	70921
0.53mm	3.00	70908	70909	70910

MA VPH Matrix Spike Mix w/ Surrogate

benzene	*n*-pentane
ethylbenzene	toluene
isooctane	1,2,4-trimethylbenzene
2-methylpentane	*m*-xylene
methyl-*tert*-butyl-ether	*o*-xylene
naphthalene	*p*-xylene
n-nonane	2,5-dibromotoluene (surrogate)

2500µg/mL each in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30454	30454-510	
w/data pack	30454-500	30454-520	30554

MA VPH Standard

benzene	500µg/mL	*n*-pentane	1000µg/mL
ethylbenzene	500	toluene	1500
isooctane	1500	1,2,4-trimethylbenzene	1000
2-methylpentane	1500	*m*-xylene	1000
methyl-*tert*-butyl-ether	1500	*o*-xylene	1000
naphthalene	1000	*p*-xylene	1000
n-nonane	1000		

in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30434	30434-510	
w/data pack	30434-500	30434-520	30534

MA VPH Standard w/ Surrogate

benzene	500µg/mL	*n*-pentane	1000µg/mL
ethylbenzene	500	toluene	1500
isooctane	1500	1,2,4-trimethylbenzene	1000
2-methylpentane	1500	*m*-xylene	1000
methyl-*tert*-butyl-ether	1500	*o*-xylene	1000
naphthalene	1000	*p*-xylene	1000
n-nonane	1000	2,5-dibromotoluene*	1000

in P&T methanol, 1mL/ampul. **surrogate*

	each	5-pack	10-pack
	30452	30452-510	
w/data pack	30452-500	30452-520	30552

MA VPH Surrogate Standard

2,5-dibromotoluene

1000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30435	30435-510	
w/data pack	30435-500	30435-520	30535

10,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30453	30453-510	
w/data pack	30453-500	30453-520	30553

RESTEK pharmaceutical

Applications note

Analyzing Cardiac Medications by HPLC

In the United States, cardiovascular disease is the leading cause of death. In an effort to reduce mortality from heart disease, several classes of medications are used to decrease high blood pressure, control arrhythmias (abnormal heart rhythms), and treat congestive heart failure. Many of these cardiac medications include beta antagonists, ACE inhibitors, diuretics, or calcium channel blockers.

High performance liquid chromatography (HPLC) is the preferred technique to analyze many of the compounds used in these medications. To maximize the effectiveness of the separation, a chromatographer should choose the column and conditions that best allow amplification of structural differences between matrix components, related compounds, and analytes. Proper HPLC column selection is dictated by the analyte and the sample matrix. In fact, selecting the appropriate analytical column is critical when analyzing cardiac medications because many of them contain basic compounds, which tend to tail badly on poorly deactivated HPLC phases. Restek's fully end-capped Allure™ Basix, Ultra IBD (intrinsically base deactivated), and Ultra Cyano phases can use the basic nature of these compounds to achieve a separation that will not suffer from the problems normally resulting in peak tailing.

Angiotensin Converting Enzyme (ACE) Inhibitors

Ancient Egyptians used the ACE inhibitor, digoxin, as a poison. Ancient Romans used it as a wound dressing and heart stimulant. It is extracted primarily from the poisonous foxglove plant in a concentration of up to 0.4% by mass. A commercial digoxin standard claiming 100% purity is shown to be impure when the analysis is performed using the Ultra IBD column. The alternate selectivity of this phase to alkyl stationary phases results in the separation of two unknown impurity peaks in the digoxin standard (Figure 1).

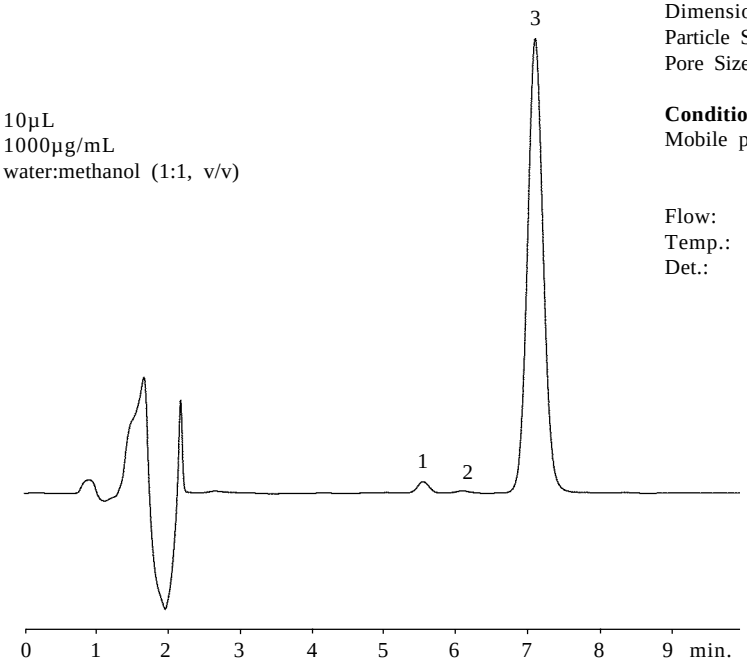
Figure 1

Ultra IBD column provides alternate selectivity, which separates impurities in a digoxin standard.

Peak List:
1. unknown
2. unknown
3. digoxin

Sample:

Inj.:	10µL
Conc.:	1000µg/mL
Solvent:	water:methanol (1:1, v/v)



Column:	**Ultra IBD**
Catalog #:	9175565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å
Conditions:	
Mobile phase:	water with 0.1% (v/v) acetic acid:acetonitrile (65:35, v/v)
Flow:	1.0mL/min.
Temp.:	27°C
Det.:	UV @ 220nm

LC_0068

pharmaceutical

Enalapril maleate, another common ACE inhibitor, can be separated by polar interaction using the Allure™ Basix phase. The Allure™ Basix column is able to interact with the basic amide and amine of enalapril to provide retention by a normal phase mechanism (Figure 2).

Calcium Channel Blockers

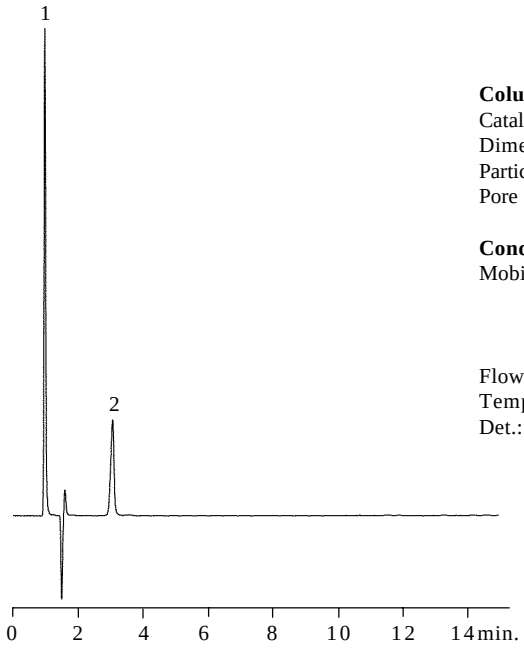
Verapamil, diltiazem, nicardipine, and nifedipine are a group of calcium channel blockers used to treat high blood pressure, angina (chest pain), and/or some arrhythmias. These four compounds all contain a basic amine group. Additionally, nifedipine and nicardipine contain more basic nitrophenol and pyridine functional groups. Figure 3 demonstrates how basic functional groups can be used to affect retention and separation of these compounds using the Ultra Cyano column. Also, the Allure™ Basix Column, in the reverse phase mode, easily retains verapamil (Figure 4).

Figure 2

The Allure™ Basix column provides retention by a normal phase mechanism.

Peak List:
1. maleate salt
2. enalapril

Sample:
Inj.: 2.5µL
Conc.: 1.1mg/mL
Solvent: methanol:water (30:70)



Column: **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile phase: 10mM ammonium formate, pH 2.5: acetonitrile (20:80, v/v)
Flow: 1.2mL/min.
Temp.: 25°C
Det.: UV @ 225nm

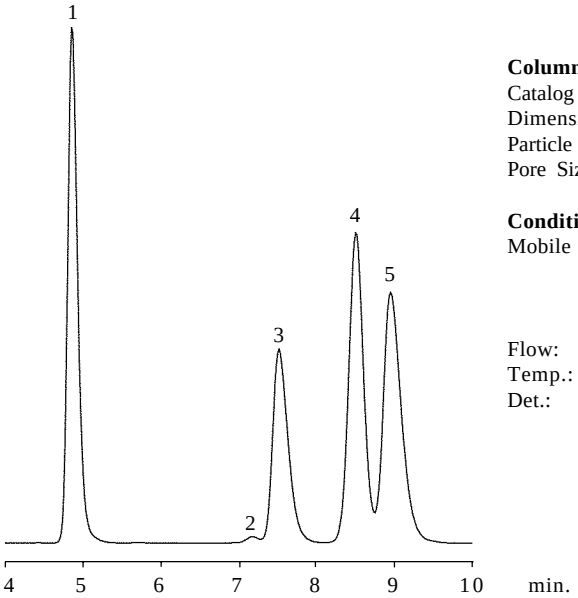
LC_0091

Figure 3

The Ultra Cyano column separates four of the most common calcium channel blockers.

Peak List:
1. diltiazem
2. nifedipine impurity
3. verapamil
4. nifedipine
5. nicardipine

Sample:
Inj.: 5µL
Conc.: 100µg/mL
Solvent: acetonitrile:water (1:1)



Column: **Ultra Cyano**
Catalog #: 9106565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile phase: 20mM potassium phosphate monobasic, pH 3.0:acetonitrile (70:30, v/v)
Flow: 1.2mL/min.
Temp.: 30°C (± 1°C)
Det.: 235nm

LC_0062

Beta Antagonists

Two of the more common beta antagonists are atenolol and metoprolol. The analytical techniques cited in some compendia methods for these compounds use a C18 phase with ion pairing agents. A simpler approach makes use of the nitrogen atom on these compounds as a key mechinism for separation. However, the basic amine groups allow analysis of these compounds using normal phase separation with the Allure™ Basix column.

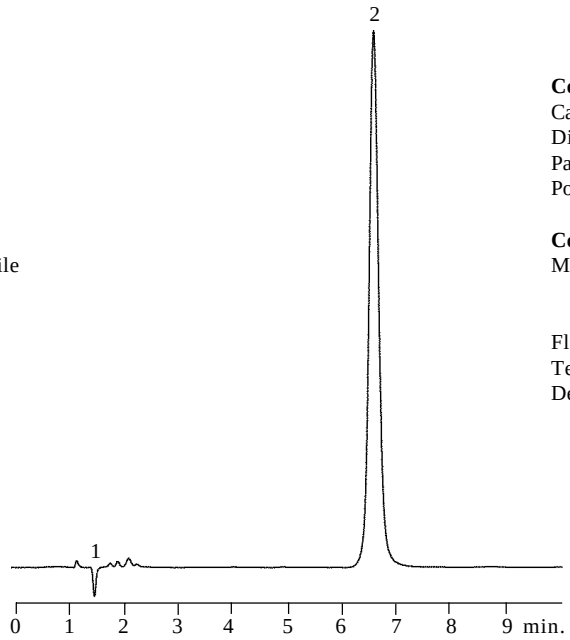
Because metoprolol is more lipid-soluble than atenolol, it is more hydrophobic. Therefore, an increase in the organic composition of the mobile phase actually will enhance the retention of metoprolol with the Allure™ Basix phase. The Allure™ Basix column performs separation of these components, provides alternate selectivity to alkyl stationary phases, and reveals an impurity in the metoprolol (Figures 5 and 6).

Figure 4

The Allure™ Basix column offers alternative selectivity for verapamil in the reverse phase mode.

Peak List:
1. toluene (marker)
2. verapamil HCL

Sample:
Inj.: 1µL
Conc.: 1mg/mL
Solvent: water:acetonitrile (70:30, v/v)



Column: **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile phase: 20mM ammonium acetate pH 4.5: acetonitrile (65:35, v/v)
Flow: 1.2mL/min.
Temp.: 25.0°C
Det.: UV @ 230nm

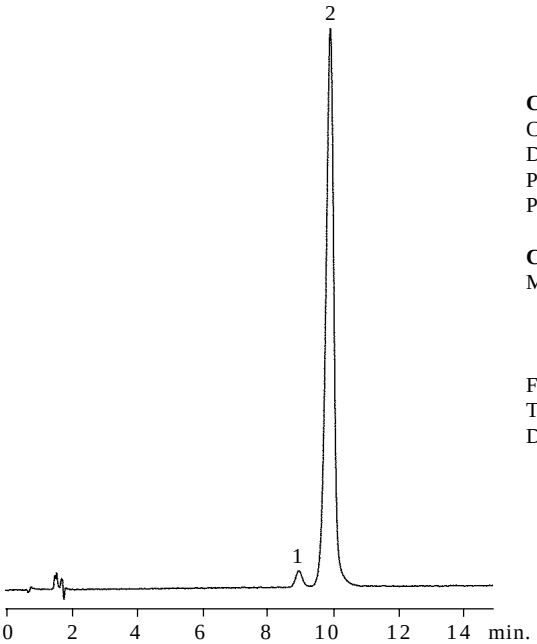
LC_0077

Figure 5

The Allure™ Basix column separates metoprolol and an impurity in 10 minutes.

Peak List:
1. unknown
2. metoprolol

Sample:
Inj.: 2.5µL
Conc.: 1.5mg/mL
Solvent: water:methanol (70:30)



Column: **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile Phase: 10mM ammonium formate, pH 2.5: acetonitrile (10:90, v/v)
Flow: 1.2mL/min.
Temp.: 25°C
Det.: UV @ 225nm

LC_0090

Diuretics

Another important class of cardiac and high blood pressure medications are diuretics. These compounds rid the body of excess fluids and salt (sodium). Diuretics such as furosemide are used for the management of edema associated with chronic heart problems. The furosemide molecule contains carboxylic acid and basic sulfa-amine groups. The zwitterionic nature of furosemide makes it an ideal candidate for analysis using an Ultra IBD column (Figure 7).

Enlargement of the baseline reveals that the furosemide standard is not a pure substance (Figure 7, inset). The impurities may possibly be a reason why the therapeutic mechanism of furosemide is not completely understood.

The diuretic admixture of triamterene and hydrochlorothiazide (HCTZ) is used to remove excess fluid while attempting to limit the amount of potassium displaced from the body. The highly-charged HCTZ contains numerous amine and sulfonic groups. The high charge is the reason it elutes in

Figure 6

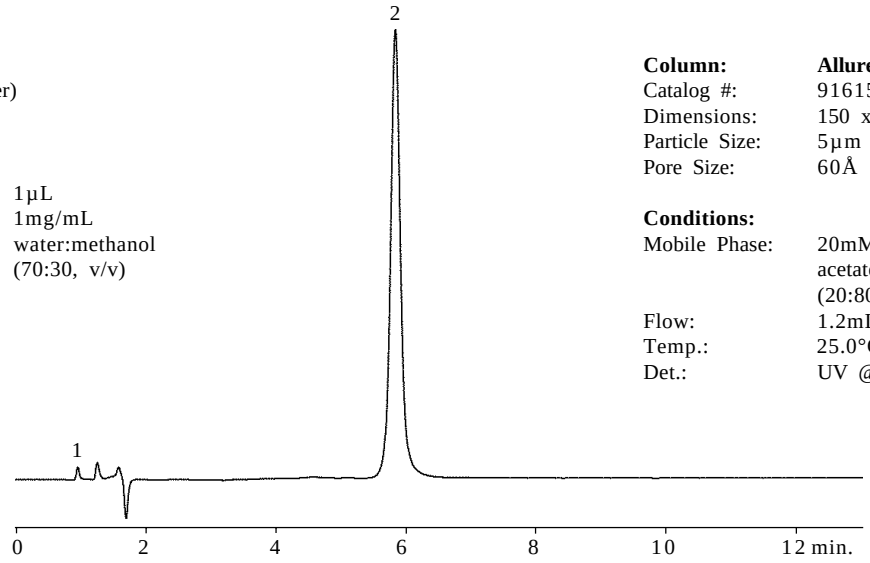
The Allure™ Basix column retains atenolol without ion pairing agents.

Peak List:
1. uracil (marker)
2. atenolol

Sample:
Inj.: 1µL
Conc.: 1mg/mL
Solvent: water:methanol (70:30, v/v)

Column: **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile Phase: 20mM ammonium acetate pH 4.5:acetonitrile (20:80, v/v)
Flow: 1.2mL/min.
Temp.: 25.0°C
Det.: UV @ 225nm



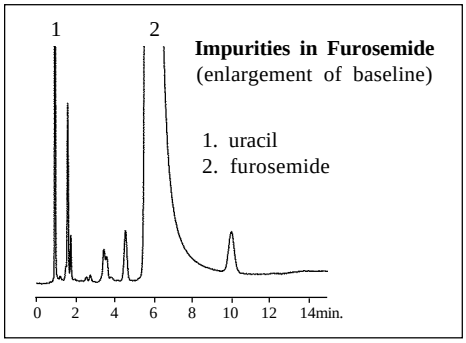
LC_0072

Figure 7

The Ultra IBD column separates impurities in furosemide.

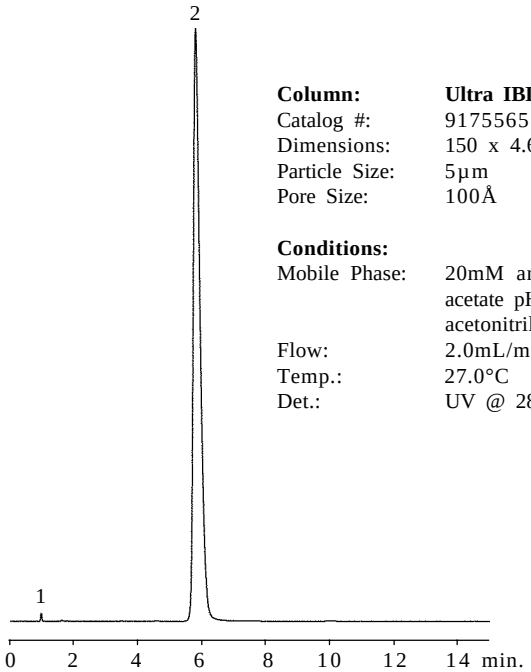
Peak List:
1. uracil (marker)
2. furosemide

Sample:
Inj.: 5µL
Conc.: 1000µg/mL
Solvent: acetonitrile:water (60:40, v/v)



Column: **Ultra IBD**
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 20mM ammonium acetate pH 4.5: acetonitrile (70:30, v/v)
Flow: 2.0mL/min.
Temp.: 27.0°C
Det.: UV @ 280nm



LC_0071

less than 3 minutes on the Allure™ Basix in reverse phase mode (Figure 8). The retention of HCTZ would increase if the organic modifier exceeded 50%, but solubility may become a concern. Triamterene is less charged than HCTZ. The amides present in the triamerene pteridine structures and the amino groups make this molecule fairly polar. Under the mobile phase conditions shown in Figure 8, triamterene is easily resolved from the HCTZ.

Summary

The Allure™ Basix, Ultra CN, and Ultra IBD columns can be used to achieve an alternate and effective selectivity to alkyl stationary phases for many types of cardiac medications.

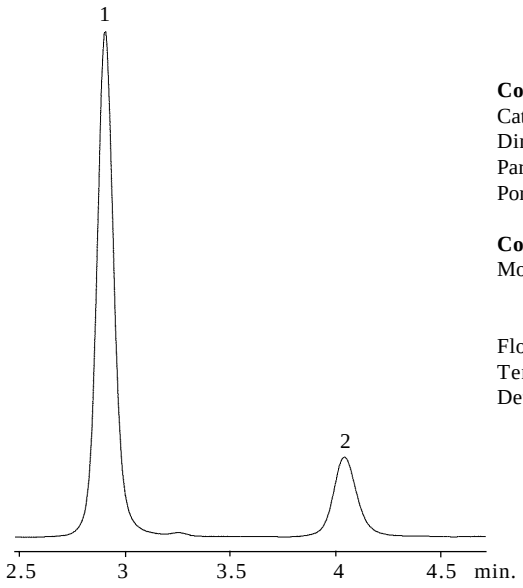
Because all three of these phases display a characteristic U-shaped profile for retention versus percentage of organic solvent in mobile phase, the mobile phase content can be modified to use either the normal or reverse phase mode if solubility problems arise. The selectivity of the Allure™ Basix and Ultra CN phases are based upon non-ionic polar interactions from basic functional groups as well as hydrophobic interaction. Basic molecules—especially those containing electron-deficient nitrogen complexes—can be retained readily by a normal phase mechanism. The selectivity for the IBD phase can be adjusted for acids, bases, zwitterionic, or neutral molecules.

Figure 8

The Allure™ Basix column easily resolves the diuretic admixture of triamterene and hydrochlorothiazide (HCTZ)

Peak List:
1. hydrochlorothiazide
2. triamterene

Sample:
Inj.: 1µL
Conc.: 500µg/mL
Solvent: methanol:water: tetrahydrofuran (30:35:35)



Column: **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile Phase: 20mM ammonium acetate, pH 4.5: acetonitrile (65:35, v/v)
Flow: 1.2mL/min
Temp.: 25°C
Det.: UV @ 225nm

LC_0086

Allure™ Basix, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9161531	9161532	9161533	9161535
50mm length	9161551	9161552	9161553	9161555
100mm length	9161511	9161512	9161513	9161515
150mm length	9161561	9161562	9161563	9161565
200mm length	9161521	9161522	9161523	9161525
250mm length	9161571	9161572	9161573	9161575

Ultra Cyano, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9106331	9106332	9106333	9106335
50mm length	9106351	9106352	9106353	9106355
100mm length	9106311	9106312	9106313	9106315

Ultra Cyano, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9106531	9106532	9106533	9106535
50mm length	9106551	9106552	9106553	9106555
100mm length	9106511	9106512	9106513	9106515
150mm length	9106561	9106562	9106563	9106565
200mm length	9106521	9106522	9106523	9106525
250mm length	9106571	9106572	9106573	9106575

Ultra IBD, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365

Ultra IBD, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

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Applications note

GC Analysis of Volatile Free Fatty Acids on the Stabilwax®-DA Column

Volatile free fatty acids are present in many foods, including eggs (AOAC Official Method 971.11), seafood (AOAC Official Method 973.26), and dairy products (AOCS Official Method Ca 5c-87). Gas chromatographic (GC) analysis is used to quantitate these fatty acids. Unlike fatty acids with longer hydrocarbon chain lengths, these volatile free fatty acids generally do not require methylation to obtain peak symmetry and reproducibility by GC. However, like all free fatty acids, they can be difficult to analyze because of their adsorptive nature. Therefore, a selective stationary phase that is specifically designed to analyze acidic compounds will provide the best results. The Restek Stabilwax®-DA column fits these criteria and provides good peak shape without methylation.

Preparing Free Fatty Acids

One can analyze existing free fatty acids in a matrix or saponify fats to obtain them. Saponification procedures are outlined in AOAC Methods 971.11D and 938.09D[1]. Samples are extracted with solvent and saponified by heating under a reflux with an excess of dilute aqueous ethanolic alkali. After saponification, the sample is neutralized with dilute hydrochloric acid or sulfuric acid. In many cases, an aqueous solution of phosphotungstic acid is added after mixing or shaking. The sample may be centrifuged and/or filtered, and then finally diluted to an aqueous solution.

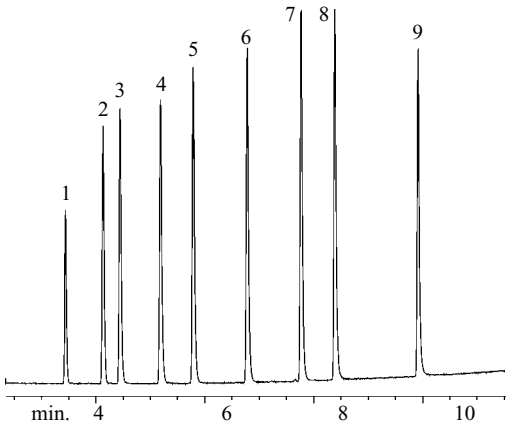
Figure 1

Stabilwax®-DA achieved excellent separation within 10 minutes.

15m, 0.53mm ID, 1.0 m Stabilwax®-DA (cat.# 11052)
0.2 L injection of a 10-20ng/ L free fatty acid standard in water. Direct injection using a Uniliner® liner.

Oven Temp.: 145 C isothermal
Inj./Det. Temp.: 250 C
Carrier gas: hydrogen
Linear Velocity: 80cm/sec. (flow rate: 10cc/min.)
FID Sensitivity: 4 x 10^{-11} AFS

1. acetic acid
2. propionic acid
3. isobutyric acid
4. *n*-butyric acid
5. isovaleric acid
6. *n*-valeric acid
7. isocaproic acid
8. caproic acid
9. heptanoic acid



Injecting Free Fatty Acids

Although free fatty acids can be injected by split or splitless techniques (especially when using smaller bore columns), a direct injection technique is recommended. Direct injection will reduce the risk of losing volatile low molecular weight fatty acids through the split vent, which improves quantitative reproducibility. Because free acids can be adsorbed, the analyst must make every effort to ensure an inert sample pathway by using properly deactivated direct injection liners and inert capillary columns. Regular preventive maintenance of the GC injection port is strongly recommended to prevent surfaces from becoming active over time.

Selecting a Column

Although the official methods listed previously indicate the use of packed column chromatography, capillary chromatography can provide much greater resolving power (i.e., column efficiency). We suggest using a Stabilwax®-DA capillary column for the analysis of volatile free fatty acids. The Stabilwax®-DA features a bonded Carbowax® phase that has been modified specifically for the analysis of acidic compounds. To prove its effectiveness, we analyzed a direct injection of straight chain and branched fatty acids ranging from acetic acid to heptanoic acid. Because the Stabilwax®-DA column has a strong affinity for free acids, excellent separation was achieved within 10 minutes (**Figure 1**). Also notice how the unique deactivation of the Stabilwax®-DA column produced sharp symmetrical peaks with minimal tailing.

Summary

An optimized GC system for the analysis of volatile free fatty acids requires direct injection for minimal discrimination and maximum inertness in the injection port, and requires an analytical column like the Stabilwax®-DA to provide selectivity and good peak shape.

Stabilwax®-DA Columns		
ID	**df**	**15-meter**
0.53mm	1.0 m	cat.# 11052
ID	**df**	**30-meter**
0.53mm	0.25 m	cat.# 11025

References:
1. AOAC International, Official Methods of the AOAC, 15th edition, 1990. Reference not available from Restek.

Order a FREE Foods, Flavors, and Fragrances Catalog! This 52-page document includes important analysis tips and chromatograms for the analysis of fats and oils, carbohydrates, vitamins, amino acids, organic acids, preservatives, flavors and fragrances, essential oils, and chiral compounds. Retention time indices and complete product listings for all of the relevant GC and HPLC products also are included (lit. cat.# 59260). Also, request Applications Note detailing food packaging testing (lit. cat.# 59348).

food & flavor

Product Listing

Stabilwax®-DA (Fused Silica)

(Crossbond® Carbowax® for acidic compounds) Stable to 250 C

Ideal for the analysis of free acids (no need for derivatization).
High thermal stability (250 C) and long column lifetime.
Crossbond® technology results in reduced bleed, increased column lifetime, and solvent rinsability.
100% bonded polyethylene glycol stationary phase.
Similar polarity to DB-FFAP, OV-351, NUKOL, and HP-FFAP columns.

ID	df (m)	Temp. Limits	15-Meter	30-Meter	60-Meter
0.25mm	0.10	40 to 250/260 C	11005	11008	11011
	0.25	40 to 250/260 C	11020	11023	11026
	0.50	40 to 250/260 C	11035	11038	11041
0.32mm	0.10	40 to 250/260 C	11006	11009	11012
	0.25	40 to 250/260 C	11021	11024	11027
	0.50	40 to 250/260 C	11036	11039	11042
	1.00	40 to 240/250 C	11051	11054	11057
0.53mm	0.10	40 to 250/260 C	11007	11010	11013
	0.25	40 to 250/260 C	11022	11025	11028
	0.50	40 to 250/260 C	11037	11040	11043
	1.00	40 to 240/250 C	11052	11055	11058
	1.50	40 to 230/240 C	11062	11065	11068

Uniliner® Liners

Uniliners®	each	5-pk.
Agilent Open-top Uniliner® w/ Wool	20843	20844
Varian Open-top Uniliner® w/ Wool	20845	20846
Shimadzu 94mm Uniliner® w/ Wool	21713	21719
CE/Thermoquest® Open-top Uniliner® w/Wool	20841	20842
PE Auto Sys Open-top Uniliner® w/ Wool	20837	20838
PE Auto Sys Cyclo-Uniliner®	20839	20840

Thermolite® Septa Green

Lowest bleed on FIDs, ECDs, and MSDs.
Each batch is tested to ensure lowest bleed.
Excellent puncturability.
Preconditioned/ready to use.
Does not adhere to hot metal surfaces.
Usable to 340 C inlet temperatures.
Packaged in non-contaminating glass jars.

Thermolite® Septum Diameters	25-pk.	50-pk.	100-pk.
9.5mm ($^3/_8$")	20359	20360	20361
10mm	20378	20379	20380
11mm ($^7/_{16}$")	20363	20364	20365
Shimadzu Plug	20372	20373	20374

Encapsulated Ferrules

Will not deform or stick in fittings.
Allows reuse of the ferrule.
Less torque needed to seal ferrule.
Unique blend of graphite provides less fragmentation and outgassing.

Encapsulated Ferrule ID	Fits Column ID	10-pk.
0.4mm	0.25mm	21036
0.5mm	0.32mm	21037
0.8mm	0.53mm	21038

Literature Request List

Lit. Cat. #	Title
59128	Application Note: Determination of Omega 3 and Omega 6 Fatty Acids
59136	Application Note: Analysis of Saw Palmetto using Rtx-Stabilwax
59177	Application Note: Ultra Aqueous C18 HPLC Column
59181	Application Note: Analysis of Vitamins Using Ultra C18 HPLC Columns
59186	Application Note: HPLC Analysis of Vanillin and Ethyl Vanillin in Vanilla Flavors
59241	HPLC Minicatalog
59546	Application Note: The Analysis of Chiral Flavor Compounds in Apple Juices using the Rt-bDEXsm & Rt-bDEXse Columns
59553	Application Note: Grape Flavor Analysis using Rt-bDEXsa Column
59579	Application Note: Analysis of Flavor Volatiles in Alcoholic Beverages
59580A	Application Note: Fast, Selective Triglyceride Analysis
59581	Application Note: Analysis of Cholesterol
59582	Application Note: Detection of Synthetic and Natural Antioxidants in Foods
59583	Application Note: Analyzing Free Fatty Acids
59584	Application Note: Analyzing Fatty Acid Methyl Esters (FAMEs)
59627B	Genuine Restek Replacement Parts Catalog for Agilent GCS
59889	Guide to the Analysis of Chiral Columns by GC
59890	Selection Guide for Polar Wax GC Column Phases
59199	Applications Note: Analyzing Heat Level of Peppers and Hot Sauces Using an Ultra C18 HPLC Column

APPLICATIONS NOTE

Sulfinert®-Treated Sample Cylinders

Increase Storage Time for Active Sulfur Compounds

Table I Minimum bend radius for Sulfinert®-treated tubing.

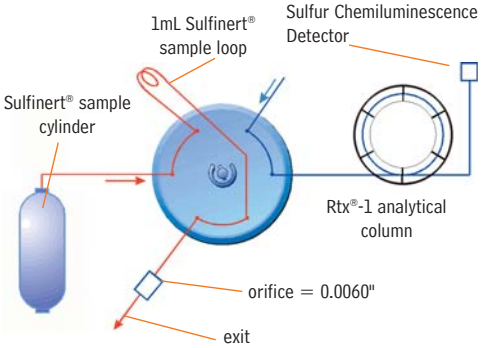
Tubing OD	Minimum Bend Radius
≤ 1/16"	1" (2.5cm)
1/8"	2" (5.1cm)
1/4"	4" (10.2cm)

Table II Sulfur compounds used to test the inertness of a Sulfinert® treated system.

Compound	Formula	Stock Conc. (ppbv)	Cylinder Conc. (ppbv)
hydrogen sulfide	H_2S	1000	17
carbonyl sulfide	COS	1000	17
methyl mercaptan	CH_3SH	1000	17
ethyl mercaptan	CH_3CH_2SH	1000	17
dimethyl sulfide*	CH_3SCH_3	1000	17
dimethyl disulfide	CH_3SSCH_3	1000	17

** internal standard*

Figure I Analytical system for detecting losses of active sulfur compounds.



Stainless steel sample cylinders commonly are used in the collection and analysis of refinery and natural gas samples. These samples often contain trace amounts of sulfur-containing compounds (e.g., hydrogen sulfide, mercaptans, and sulfides), which can interfere with reactions or poison catalysts in many petrochemical processes. Because sulfur compounds quickly react with stainless steel surfaces, accurate determination of these compounds is impossible when using untreated sample cylinders.

Restek's Sulfinert® passivation technique bonds an inert silica layer into the surface of the stainless steel. This layer acts as a barrier, preventing active compounds from reacting with or adsorbing to the stainless steel. Therefore, Sulfinert® products are ideal for storing and transferring reactive sulfur compounds. Most stainless steel products can be treated with Sulfinert® passivation, including tubing. Because the Sulfinert® layer is incorporated into the structure of the stainless steel, treated surfaces can be bent or flexed without affecting the inertness characteristics (Table I).

We developed a gas chromatographic analytical method to demonstrate the effects of using Sulfinert® transfer lines, sample loops, and sample cylinders for sampling, storing, and analyzing low-level reactive sulfur compounds. To characterize Sulfinert® surfaces, we tested the stability of sulfur compounds in three Sulfinert® sample cylinders over a 54-hour period. Table II lists the test compounds and their concentrations.

Analytical System

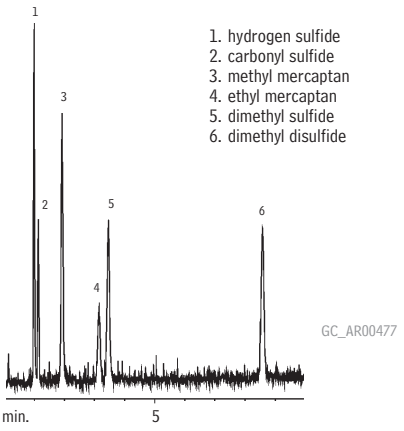
The analytical system was designed so that a 17ppbv standard could be detected with sufficient sensitivity to quantify compound loss. Sample introduction was with a 1mL Sulfinert® sample loop, Sulfinert® Valco® valve, and 1/16-inch Sulfinert® transfer lines (Figure 1). The analytical column was connected directly to the Valco® valve.

In order to control transfer of the sample to the 1mL sample loop, an orifice was attached to the exit of the sample loop. This allowed a controlled flow in the range of 60-120mL/min. during sample transfer (flow was pressure-regulated from the sample cylinder). An Rtx®-1 column (60m x 0.53mm, 7µm) and a Sievers model 355 sulfur chemiluminescence detector (SCD) were used.

1mL of a 1000ppbv standard was added to a 500cc sample cylinder and pressurized to 160psig. The sample was prepared "dry" (no water added to the cylinder) to simulate a petrochemical process. Dimethyl sulfide, which has been shown to be non-reactive in this mixture and is not adsorbed by stainless steel, was used as an internal standard.

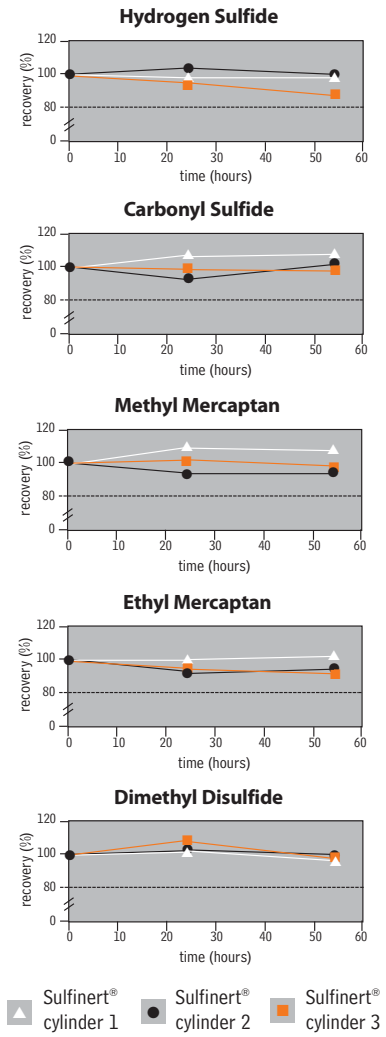
To introduce the sample onto the GC column, the sample loop was flushed with sample for 45 seconds, then the cylinder valve was closed and the sample loop was vented to atmospheric pressure. The Valco® valve was switched to introduce the sample from the loop to the analytical column, and the analysis was started. Figure 2 (page 2) shows the chromatogram.

Figure 2 17ppbv sulfur standards on an Rtx®-1 column with SCD detection.



Column: Rtx®-1 60m, 0.53mm ID, 7.0µm (cat.# 10193)
Oven temp.: 30°C (hold 1 min.) to 180°C @ 10°C/min. (hold 5 min.)
Carrier gas: helium
Flow rate: 8.5mL/min.

Figure 3 Stability of sulfur compounds is remarkable in Sulfinert®-treated cylinders.



Results

As shown in Figure 3, Sulfinert®-treated cylinders and accessories were inert to reactive sulfur compounds over the 54-hour test period. Hydrogen sulfide exhibited greater than 85% recovery over the test period; methyl mercaptan, ethyl mercaptan, carbonyl sulfide, and dimethyl disulfide exhibited greater than 90% recovery.

Conclusion

This investigation confirms the use of Sulfinert®-treated sample cylinders and transfer lines greatly increases the storage time for reactive sulfur compounds, ensuring more accurate analyses.

Sulfinert®-Treated Swagelok® Sample Cylinders

- Stable storage of sulfur compounds at ppb levels.
- D.O.T. rated to 1800psi at room temperature.
- High quality cylinders manufactured by Swagelok®.

Sulfinert®-treated gas sampling equipment is ideal for collecting and storing samples containing ppb levels of sulfur compounds, such as natural gas or beverage-grade carbon dioxide. Sulfinert® treatment ensures that sulfur compounds or other highly active compounds remain stable during transport from the field to the laboratory. These cylinders are made from 304 grade stainless steel with 1/4" female NPT threads on both ends.

Size	qty.	cat.#
75cc	ea.	24130
150cc	ea.	24131
300cc	ea.	24132
500cc	ea.	24133
1000cc	ea.	24134
2250cc	ea.	21394

Sulfinert®-Treated Alta-Robbins Sample Cylinder Valves

- All wetted parts are Sulfinert®-treated for inertness.
- Compatible with Sulfinert®-treated Swagelok® sample cylinders.
- Large, durable, Kel-F® seat ensures leak-free operation.

Description	qty.	cat.#
1/4" NPT Exit	ea.	21400
1/4" Compression Exit	ea.	21401
1/4" NPT with Dip Tube*	ea.	21402
1/4" NPT with 2850psi Rupture Disk	ea.	21403

*Specify dip tube length or % outage when ordering (maximum length = 5.25"/ 13.3cm)

Sulfinert®-Treated Rupture Disc Tee

2850 psig rating, 1/4" NPT connections.

Description	qty.	cat.#
Sulfinert® Rupture Disc Tee (1/4" NPT connections)	ea.	21396
Replacement Rupture Disc (not Sulfinert®-treated)	ea.	24298

Cleaning Sample Cylinders

Optimum performance of Silcosteel® or Sulfinert® treated surfaces can be maintained by cleaning them with a variety of neutral solvents. An increase in surface activity, with the potential of physical damage to the coating, can occur if the surface is exposed to oxygenated steam. Therefore, we do not recommend exposing Silcosteel® or Sulfinert® treated surfaces to steam environments in the presence of air or oxygen. However, Sulfinert® or Silcosteel® surfaces have been successfully cleaned using nitrogen-enriched steam.

Sulfinert®-Treated Gas Sampling Valves and Sample Loops

- Ideal for samples containing low concentrations of sulfur compounds.
- Sample loop sizes from 5µL to 5cc.

Use Sulfinert®-treated gas sampling valves for low-level sulfurs and other active compounds. Sulfinert® treatment eliminates active sites on the valve or in the loop, for better recovery of difficult-to-analyze compounds.

Sulfinert®-Treated Gas Sampling Valves (1/16" fittings, 0.40mm port diameter; "W Type" valve)

Description	qty.	cat.#
Sulfinert® Gas Sampling Valve; 4-Port	ea.	20584
Sulfinert® Gas Sampling Valve; 6-Port	ea.	20585
Sulfinert® Gas Sampling Valve; 10-Port	ea.	20586

Replacement Rotors

Description	qty.	cat.#
Replacement Rotor for 4-Port Sulfinert® Gas Sampling Valve	ea.	20587
Replacement Rotor for 6-Port Sulfinert® Gas Sampling Valve	ea.	20588
Replacement Rotor for 10-Port Sulfinert® Gas Sampling Valve	ea.	20589

Sulfinert®-Treated Gas Sample Loops (1/16" fittings, for "W Type" valves)

Size	qty.	cat.#
5µL	ea.	22840
10µL	ea.	22841
20µL	ea.	22842
25µL	ea.	22843
50µL	ea.	22844
100µL	ea.	22845
250µL	ea.	22846
500µL	ea.	22847
1cc	ea.	22848
2cc	ea.	22849
5cc	ea.	22850

please **note**

For Sulfinert®-treated fittings, see our general catalog or our website.

Coiled Siltek®/Sulfinert®-Treated† Welded 304 Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.011" (0.28mm)	0.022" (0.56mm)	22500				
0.021" (0.53mm)	0.029" (0.74mm)	22501				
0.010" (0.25mm)	1/16" (1.59mm)	22502				
0.020" (0.51mm)	1/16" (1.59mm)	22503	For prices please refer to our catalog or website			
0.030" (0.76mm)	1/16" (1.59mm)	22504				
0.040" (1.02mm)	1/16" (1.59mm)	22505				
0.085" (2.16mm)	1/8" (3.18mm)*	22506				
0.210" (5.33mm)	1/4" (6.35mm)*	22507				

*0.020" wall thickness

Coiled Siltek®/Sulfinert®-Treated† Seamless 316 Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)	22508	For prices please refer to our catalog or website			
0.180" (4.57mm)	1/4" (6.35mm)	22509				

**0.035" wall thickness

for **more** info

Call for availability of lengths greater than 1000ft.

Metric conversion:

6ft.	1.8m
25ft.	7.6m
50ft.	15.2m
200ft.	61m
>400ft.	>122m

Minimum Bend Radius

OD	Min. Bend Radius
≤1/16"	1" (2.5cm)
1/8"	2" (5.1cm)
1/4"	4" (10.2cm)

tech tip

†Siltek® and Sulfinert®: What's the Difference?

Siltek® is the name for our patented deposition process. When we developed the Siltek® process, the application that showed the greatest benefit, among many we investigated, was the storage and transfer of low ppb level active sulfur compounds, such as hydrogen sulfide and mercaptans. Because there was (and continues to be) demand for a reliable surface treatment for this application, we use the name Sulfinert® to describe Siltek® treated products created specifically for this purpose.

Got tape?

Restek offers three types of Teflon® thread-sealing tape. For information, please visit our website:
www.restekcoatings.com

Rtx®-1 Columns (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

fact

MXT® columns—rugged, flexible, Silcosteel®-treated stainless steel tubing; inertness comparable to fused silica tubing.

MXT®-1 Columns (Silcosteel®-treated stainless steel)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	15-Meter	30-Meter	60-Meter	105-Meter
0.53mm	3.00	-60 to 285°C	70182	70185	70188	70189
	5.00	-60 to 270°C	70177	70179	70183	
	7.00	-60 to 250°C	70191	70192	70193	

Rt-Sulfur™ Packed/Micropacked Columns for Sulfur Analysis

- Excellent reproducibility for low-level sulfur analysis.
- Eliminate the need for Teflon® tubing.

did you know?

Rt-XLSulfur™ columns are optimized for low ppb-level sulfur analysis!

Rt-XLSulfur™ Packed Columns

- The best packed columns available for low ppmv sulfur analyses.

OD	ID (mm)	1-Meter*	2-Meter*
1/8"	2.0mm	80484-	80485-
3/16"**	3.2mm	80482-	80483-

*Please add configuration suffix number to cat.# when ordering - see chart below.
**For installation kits for 3/16" columns, see our catalog or website.

for more info

about Siltek®/ Sulfinert® treatment, request information packet 59616.

Rt-Sulfur™ Micropacked Columns

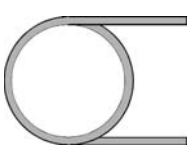
OD	ID	1-Meter	2-Meter
1/16"	1.0mm	19801	19800
0.95mm	0.75mm	19803	19802

Purchase installation kit separately; see our catalog, or website.

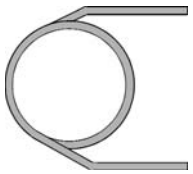
also available

For adapter kits for installing packed or micropacked columns, see our general catalog, or website.

Column Configurations



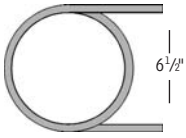
General Configuration
Suffix -800



Agilent 5880, 5890, 5987, 6890:
Suffix -810

Varian 3700, Vista Series, FID:
Suffix -820

PE 900-3920 Sigma 1,2,3:
Suffix -830



PE Auto System 8300, 8400, 8700 (Not On-Column):
Suffix -840

Note: Initial 2" of column will be empty, to accommodate a needle. For a completely filled column add suffix -901.

RESTEK
packed columns

Applications note

Rt-XLSulfur™ Packed GC Column for Analysis of Low-Level Sulfur Compounds in C1-C6 Hydrocarbon Streams

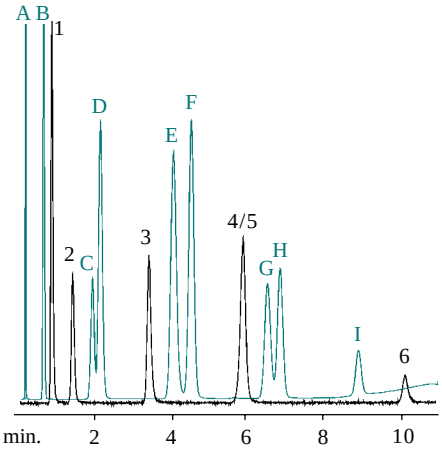
The analysis of sulfur compounds in C1-C6 hydrocarbon streams by gas chromatography (GC) is an important application in the petrochemical field. The presence of sulfur compounds in petroleum products can affect the longevity and performance of catalysts used in hydrocarbon processing. As requirements for sulfur detection become more stringent, the importance of good chromatographic separation of the hydrocarbons from the sulfur compounds and the inertness of the analytical columns increases.

This is the second generation of packing material for the analysis of sulfur compounds. The first packing material, in the Rt-Sulfur™ column, had inertness characteristics for low ppmv levels of sulfur compounds. Now, with the second generation, the Rt-XLSulfur™ column, it is possible to achieve low ppbv detection of sulfur compounds.

Detectors used for sulfur determination generally are specific (e.g., sulfur chemiluminescence detection) and help eliminate positive response from chromatographic interferences. Unfortunately, when high levels of hydrocarbons elute through the detector simultaneously with sulfur compounds, the signal for sulfur is quenched and area counts are decreased. For a successful analysis, the analytical column must resolve the hydrocarbons from the sulfurs listed in Figure 1. A packed, micropacked, or PLOT column can be used to achieve this requirement.

Figure 1

The Rt-XLSulfur™ micropacked column separates hydrocarbons from sulfur compounds.



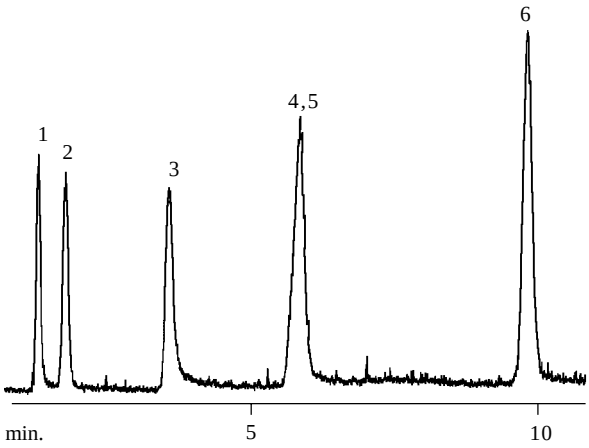
Sulfurs
1. hydrogen sulfide
2. carbonyl sulfide
3. methyl mercaptan
4. ethyl mercaptan
5. dimethyl sulfide
6. dimethyl disulfide

Hydrocarbons
A. methane
B. ethane
C. propylene
D. propane
E. isobutane
F. butane
G. isopentane
H. pentane
I. hexane

1m x 0.75mm ID Rt-XLSulfur™ column (cat.# 19806)
Oven temp.: 60°C to 230°C @ 15°C/min.; **Carrier gas:** helium;
Flow rate: 9mL/min.; **Detection:** FID/SCD

Figure 2

The Rt-XLSulfur™ column is sensitive enough for 50ppbv sulfur compounds.



1. hydrogen sulfide
2. carbonyl sulfide
3. methyl mercaptan
4. ethyl mercaptan
5. dimethyl sulfide
6. dimethyl disulfide

1m x 0.75mm ID Rt-XLSulfur™ column (cat.# 19806)
Sample conc.: 50ppb; **Oven temp.:** 60°C to 230°C @ 15°C/min.;
Carrier gas: helium; **Flow rate:** 9mL/min.; **Detection:** SCD

Hydrocarbons are non-reactive but sulfur compounds, especially hydrogen sulfide and methyl mercaptan, are easily adsorbed by undeactivated surfaces. Therefore, there are two areas of concern when performing this analysis with packed or micropacked columns: one is the inertness and selectivity of the solid support, and the other is the inertness of the tubing walls. Packed and micropacked columns typically use metal tubing for ruggedness but the surface is very adsorptive for sulfur compounds. So, for sulfur analysis, Teflon® tubing is often used. However, this tubing is permeable and will expand and contract during temperature changes.

Sulfinert™ and Silcosteel® treatments adhere a thin, inert layer onto stainless steel and other metal surfaces. The Sulfinert™ coating is the leading passivation technique for the storage and transfer of low-level, organo-sulfur-containing samples.

Restek designed the Rt-XLSulfur™ column to accomplish the sensitive separation of hydrocarbons from sulfur compounds. All components of the column have been tested for inertness. The packing material is extensively deactivated for the analysis of low ppbv-levels of hydrogen sulfide and methyl mercaptan, and then is prepared to achieve the proper selectivity and required resolution (Figure 1). Analysis of 50ppbv sulfur compounds using a 1mL gas loop and Sievers sulfur chemiluminescence detector (SCD) shows excellent sensitivity (Figure 2).

The interior tubing walls of the Rt-XLSulfur™ column are treated with a Sulfinert™ coating, a passivation technique designed to deactivate metal surfaces. This coating is found to be very inert for hydrogen sulfide and methyl mercaptan. (For more information on Sulfinert™ coating, request lit. cat.# 59203.) Another issue that is routinely overlooked with packed columns is that their end plugs are known to adsorb sulfur compounds. So, Restek also treated the end plugs in the Rt-XLSulfur™ column with Sulfinert™ passivation. The extra care taken with the surfaces in this column result in a more accurate analysis of sulfur compounds in hydrocarbon processes.

Product Listing

Rt-XLSulfur™ Packed & Micropacked Columns*

OD (in.)	ID (mm)	1-Meter	2-Meter
0.95mm	0.75	19806	19807
1/16	1.0	19804	19805
1/8	2.0	80484	80485
3/16	3.2	80482	80483

* *Installation kit must be purchased with column (no kit needed for 3/16" columns)*

For more information on Sulfinert™ coating, call 800-356-1688 or 814-353-1300, ext. 5, and request lit. cat.# 59203.

Installation Kits for 0.75mm ID columns

Description	cat.#
For valve applications	21062
For split applications	21063
For all HP GC split applications	21064

Installation Kits for 1.0mm ID columns

Description	cat.#
For valve applications	21065
For direct injections	21066

Installation Kits for 2mm ID columns

Description	cat.#
For valve applications	21067

RESTEK environmental

Applications note

GC/ECD Analysis of Haloacetic Acids in Water Samples Using Rtx®-CLPesticides and Rtx®-CLPesticides2 Columns

Haloacetic acids are a byproduct of chlorinated disinfection of drinking water. Recently, there has been some concern that these analytes may represent a chronic risk to human health, and toxicological evidence suggests that some of them are possible human carcinogens. Elevated levels of haloacetic acids in drinking water could pose acute human risk because of their corrosive natures. With the proper sample preparation technique and use of the Rtx®-CLPesticides and the Rtx®-CLPesticides2 columns, environmental chemists can achieve accurate analysis of these compounds. (Refer to US Environmental Protection Agency [EPA] Method 552).

Sample Extraction

Sample preparation requires a microextraction of a 40mL sample, methylation of the acids, and final neutralization of extract. The initial extraction of haloacetic acid compounds involves transferring 40mL of sample to a 60mL vial or separatory funnel, and adding the surrogate 2,3-dibromo-propionic acid.

The following steps are done in quick succession so that the heat generated from adding the acid to the sample helps dissolve the salts into the liquid phase: 1) Lower the sample pH to <0.5 using concentrated sulfuric acid. 2) Add two grams of copper II sulfate pentahydrate to the sample to color the water, making it is easy to distinguish the water phase from the organic phase. 3) Add 16gm of pre-cleaned sodium sulfate to the sample to increase the ionic strength of the aqueous phase. 4) Add 4mL of methyl *tert*-butyl ether (MTBE) to the sample and shake for two minutes.

Compound Methylation

To begin haloacetic acids methylation, transfer approximately 3mL of the MTBE extract to a 15mL graduated, conical centrifuge tube. Add 1mL of 10% sulfuric acid in methanol to the centrifuge tube. Cap the tube and heat at 50°C for two hours. After cooling, neutralize the extract with saturated sodium bicarbonate solution, adding it in 1mL increments. Continually vent the centrifuge tube because CO_2 will be generated during the neutralization process.

Transfer 1mL of the MTBE extract to an autosampler vial and spike 10uL of internal standard (25ppm 1,2,3-trichloropropane). Archive the remaining extract portion for later use if necessary.

Column Choice

The analysis of haloacetic acid compounds can be performed on a variety of GC column phases. An important criterion for column selection is the quality of resolution between the methylated haloacetic acid compounds and known interference compounds like bromoform. Bromoform may be present because of the partial decarboxylation of tribromoacetic acid in the methylation step using acidic methanol.

The Rtx®-CLPesticide and Rtx®-CLPesticide2 columns provide the necessary resolution for this analysis using GC/electron capture detection (ECD) (see chromatograms in **Figure 1**). These columns have historically been used for the analysis of chlorinated pesticides (US EPA Method 508), and chlorinated acids (Method 515), using the same analytical configuration.

Instrument Calibration

Analyze the laboratory performance check (LPC) solution to verify instrument performance. The LPC verifies three criteria: instrument sensitivity, chromatographic performance, and column performance. See **Table 1**, other side, for the results on the Rtx®-CLPesticide and Rtx®-CLPesticide2 columns. Monochloroacetic acid (MCAA) is used to verify instrument sensitivity. At a concentration of 6µg/L, MCAA must have a signal-to-noise ratio greater than three. Chromatographic performance is verified using a measure of peak symmetry called the Peak Gaussian Factor (PGF). The calculated PGF must be between 0.80 to 1.15 for optimum performance. The compound used for this is bromochloro- acetic acid (BCAA) at a concentration of 4µg/L (see below for calculation).

Peak Gausian Factor:

$PGF = (1.83 \times W_{1/2})/W_{1/10}$

where: $W_{1/2}$ = the peak width at half-height (in seconds)

$W_{1/10}$ = the peak width at one-tenth height (in seconds)

Peak Resolution:

Column performance is verified using the peak resolution between chlorodi-bromoacetic acid (CDBAA) and the surrogate (1,2,3-trichloropropane).

The criteria for resolution is greater than 0.5 using the following equation:

$R = t/W_{ave}$

where: t = the difference in elution time of the two peaks (in minutes)

W_{ave} = the average peak width of the two peaks measured at baseline (in minutes)

environmental

The GC is calibrated using standards that have been deriva- tized by the same procedure as the samples. This process helps reduce variability between extraction sets and laboratories. The low point of the curve should be near the detection limit and the high point should be 20 to 50 times higher. The last three standards should have concentrations evenly distributed between the low and high point in the curve.

Instrument stability is verified at every 10 sample injections by analyzing a mid-point calibration standard. All calculated compound concentrations in the standard must have a recovery of 70-130% of the theoretical value.

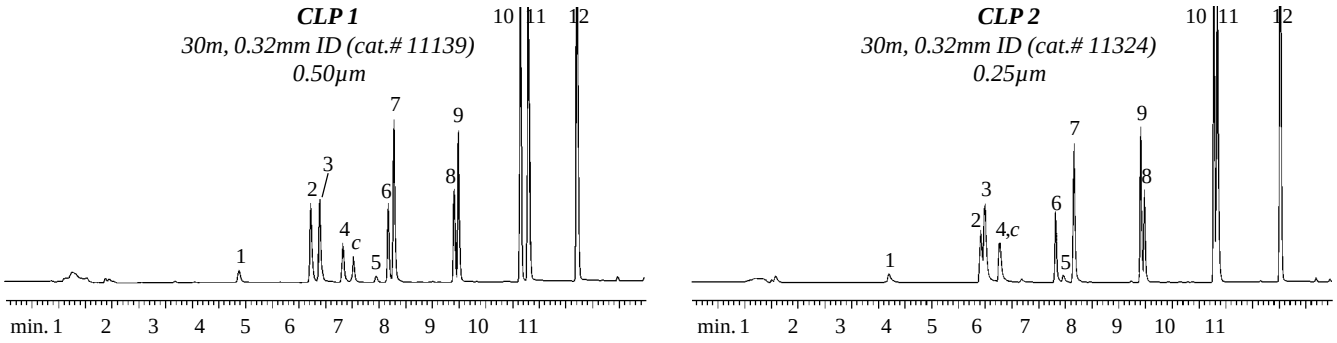
There are other quality controls that must be performed when analyzing samples by US EPA Method 552.2. They include laboratory duplicates, field duplicates, laboratory blanks, field blanks, laboratory fortified blank, laboratory fortified sample matrix spikes, and quality control samples. Read through the method and set-up a flowchart to determine when quality control samples should be performed. Doing so will reduce confusion and make the analysis straightforward.

Conclusion

The versatile Rtx®-CLPesticide and Rtx®-CLPesticide2 columns exhibit proper resolution of many compounds including haloacetic acids, chlorinated pesticides, chlorinated phenoxy herbicides, organophosphorus pesticides, and triazine herbicides. These columns help analysts reduce instrument downtime and increase sample throughput.

For more information, call Restek at 800-356-1688 or 814-353-1300, ext. 4, or your local Restek representative.

Figure 1: *The Rtx®-CLPesticide and Rtx®-CLPesticide2 columns provide the necessary resolution of haloacetic acids.*



c=solvent contaminant

1. monochloroacetic acid
2. monobromoacetic acid
3. dichloroacetic acid
4. dalapon
5. 1,2,3-trichloropropane
6. trichloroacetic acid
7. bromochloroacetic acid
8. dibromoacetic acid
9. bromodichloroacetic acid
10. chlorodibromoacetic acid
11. 2,3-dibromopropionic acid
12. tribromoacetic acid

Haloacetic acids, EPA Method 552.2 on Rtx-CLPesticides columns
Custom haloacetic acid methyl derivatives std. [20-200µg/mL] mix in MTBE.
Oven temp.: 40°C (hold 4 minutes) @ 14°C/min. to 200°C; **Inj. temp.:** 200°C, splitless, 2µL; **Carrier gas:** helium @ 46cm/sec.; **ECD Det.:** 300°C

Table 1: *Laboratory Performance Check Solution*

Parameter	Measurement	Analyte	Concentration	CLP Result	CLP2 Result	Acceptance Criteria
Instrument sensitivity	S/N	MCAA	0.006µg/mL	9	4	S/N>3
Chromatographic performance	PGF	BCAA	0.004µg/mL	0.92	1.05	PGF>0.08 and <1.15
Column performance	Resolution	CDBAA 2,3-DBPA	0.010µg/mL 0.010µg/mL	1.0	0.6	Resolution> 0.50

Product Listing

Rtx®-CLPesticides (Fused Silica) *Stable to 340°C*

ID	df (µm)	temp. limits	30-meter
0.32mm	0.50	-60 to 310/330°C	11139

Rtx®-CLPesticides2 (Fused Silica) *Stable to 340°C*

ID	df (µm)	temp. limits	30-meter
0.32mm	0.25	-60 to 310/330°C	11324

Restek Trademarks: Rtx.

RESTEK HPLC

Applications note

Analyze Polar Compounds by Reversed Phase HPLC Using Ultra Aqueous C18 Column

Short-chain organic acids, amino acids, and water-soluble vitamins are examples of highly polar compounds that are difficult to retain using conventional reversed phase columns, even with little or no organic solvent in the mobile phase. The Ultra Aqueous C18 column was designed for reversed phase applications that require highly aqueous mobile phases. The analysis of six organic acids, which are difficult to retain using many conventional C18 columns, shows the Ultra Aqueous C18 column provides enhanced retention and selectivity (Figure 1). The Ultra Aqueous C18 column also gives reproducible retention times, sharper peak shapes; allows use of highly aqueous mobile phases, and may eliminate the need for sample derivatization or ion pairing reagents.

Figure 1

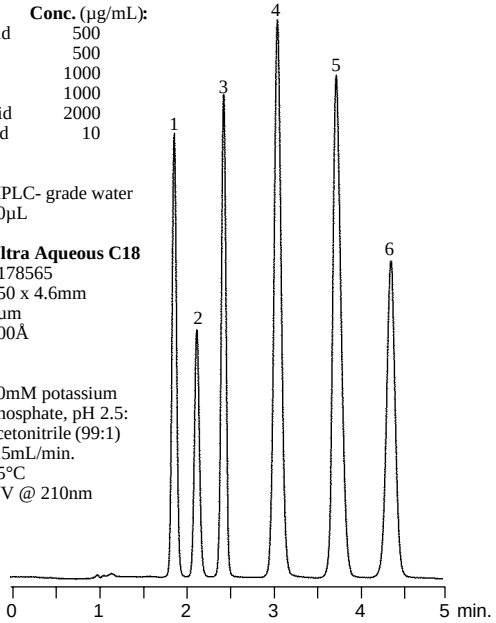
Ultra Aqueous C18 column provides enhanced retention and selectivity for organic acids.

Peak List: **Conc.** (µg/mL):
1. malonic acid 500
2. lactic acid 500
3. acetic acid 1000
4. citric acid 1000
5. succinic acid 2000
6. fumaric acid 10

Sample:
Solvent: HPLC- grade water
Inj.: 10µL

Column: **Ultra Aqueous C18**
Catalog #: 9178565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 50mM potassium phosphate, pH 2.5: acetonitrile (99:1)
Flow: 1.5mL/min.
Temp.: 25°C
Det.: UV @ 210nm



LC_0140

Reproducible Retention Times

When exposed to highly aqueous mobile phases, many conventional C18 columns will lose retention from run to run because of a phenomenon called “chain folding.” This loss of retention can be gradual or sudden and is attributed to the hydrophobic C18 chains self-associating (i.e., folding down onto the silica surface to avoid associating with a very hydrophilic mobile phase). While the chain folding process is reversible, it can make analyses of highly polar compounds difficult due to irreproducible retention times.

The Ultra Aqueous C18 column, however, avoids this problem—it provides stable and reproducible retention, even with 100% aqueous mobile phases. The Ultra Aqueous C18 column is designed using Type B, high-purity silica and a novel bonding chemistry that results in a true C18 phase (USP L1). Because polar groups on the silica surface keep the stationary phase wet, the alkyl chains remain totally extended even when continually exposed to a highly aqueous mobile phase. This unique secondary polar character prevents chain folding, and enhances the retention and selectivity of polar compounds without compromising the level of base deactivation.

Notice the highly reproducible separation of four small carboxylic acids using the Ultra Aqueous C18 column and a 100% aqueous mobile phase (Figure 2). No significant change in retention occurred over twenty injections, performed over a four-day period that included three days of continuous exposure of the column to the totally aqueous mobile phase.

Figure 2

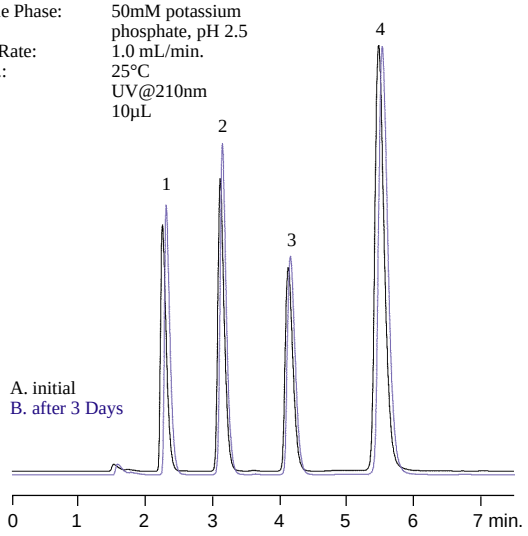
Ultra Aqueous C18 column achieves stable retention of organic acids in 100% aqueous mobile phase.

Peak List **Conc.** (mg/mL)
1. glycolic acid 5.4
2. malonic acid 4.2
3. acetic acid 7.8
4. maleic acid 0.06

Sample dissolved in mobile phase.

Column: **Ultra Aqueous C18 Column**
Catalog#: 9178565
Dimensions: 150x4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 50mM potassium phosphate, pH 2.5
Flow Rate: 1.0 mL/min.
Temp.: 25°C
Det.: UV@210nm
Inj.: 10µL



LC_0142

HPLC

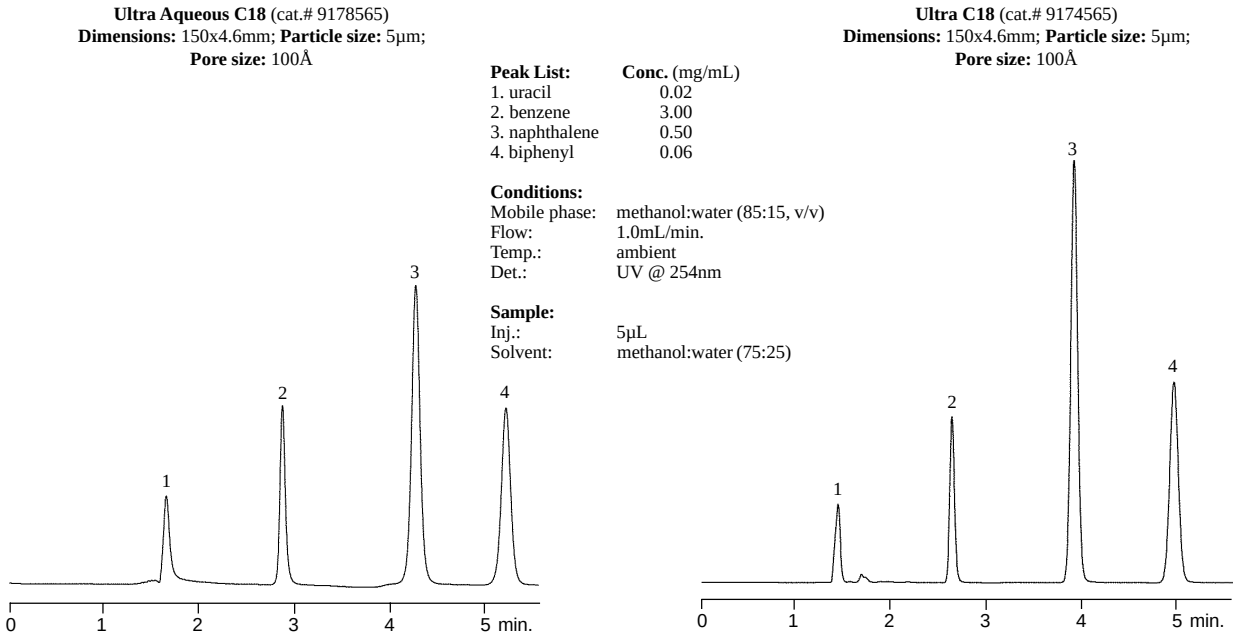
Greater Retention and Sharper Peak Shape of Polar Compounds

The Ultra Aqueous C18 column typically has very similar retention and selectivity to the more conventional Ultra C18 column when analyzing neutral, hydrophobic compounds (Figure 3). The Ultra C18 column is a highly retentive, well-deactivated, general purpose C18 column. The unique qualities of the Ultra Aqueous C18 column are apparent, however, when separating polar compounds (Figure 4). Although phenol, a neutral polar compound, is retained similarly by both columns, basic and more polar pyridine has approximately 2.5 times more retention on the Ultra Aqueous C18 column.

Also note the relatively symmetrical peak shape of pyridine using the Ultra Aqueous C18 column; the peak symmetry of pyridine is commonly used as a test probe for column base deactivation. Some older generation C18 columns enhanced their retention of polar compounds using low-coverage bonding, which left a large population of active silanols on the silica

Figure 3

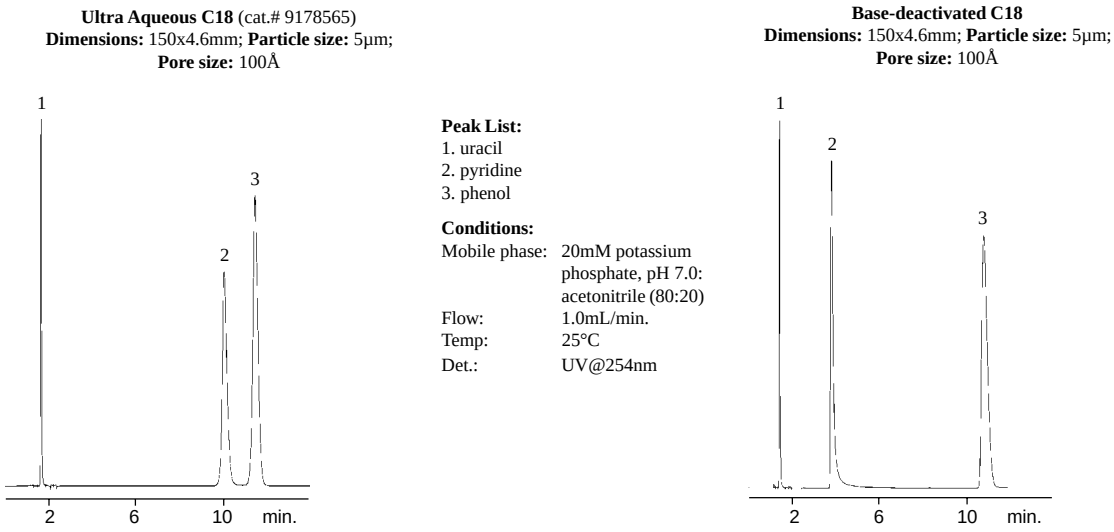
Hydrophobic compounds are retained similarly on the Ultra Aqueous C18 and the conventional Ultra C18 columns.



LC_0145

Figure 4

Ultra Aqueous C18 column provides enhanced retention of pyridine and shows a high level of base deactivation.



LC_0143

surface. Active silanols can enhance retention of polar compounds, but also can cause severe peak tailing for basic compounds like pyridine. The comparison in Figure 4 shows that the Ultra Aqueous C18 column achieves enhanced retention for polar compounds without sacrificing base deactivation. This combination of benefits sets the Ultra Aqueous C18 column apart from conventional C18 columns.

Maximum Retention Without Derivatization or Ion Pairing Reagents

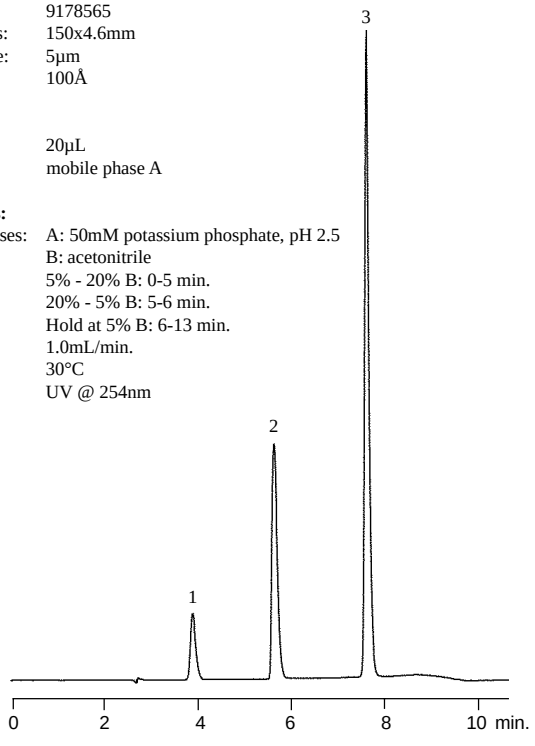
Amino acids frequently are derivatized in preparation for reversed phase HPLC analysis. Derivatization provides adequate retention and improved detection sensitivity for these compounds. The Ultra Aqueous C18 column retains and separates many amino acids without derivatization or ion pairing reagents (Figure 5). The amino acids in this example are three of the most hydrophobic because of their aromatic side groups, and thus they are not as difficult to retain as some of the more hydrophilic amino acids. However, please note the initial mobile phase contains 5% acetonitrile. For the more polar, hydrophilic amino acids, retention can be increased considerably by reducing the proportion of acetonitrile. To maximize retention, the acetonitrile can be eliminated without compromising reproducibility.

Figure 5

The Ultra Aqueous C18 column retains and separates many amino acids without derivitization or ion pairing reagents.

Peak List	**Conc.** (mg/mL)
1. tyrosine	0.2
2. phenylalanine	1.6
3. tryptophan	0.4

Column:	**Ultra Aqueous C18 Column**
Catalog#:	9178565
Dimensions:	150x4.6mm
Particle size:	5µm
Pore size:	100Å
Sample:	
Inj.:	20µL
Solvent:	mobile phase A
Conditions:	
Mobile phases:	A: 50mM potassium phosphate, pH 2.5 B: acetonitrile 5% - 20% B: 0-5 min. 20% - 5% B: 5-6 min. Hold at 5% B: 6-13 min.
Flow:	1.0mL/min.
Temp.:	30°C
Det.:	UV @ 254nm



LC_0146

The group of compounds known as **water-soluble vitamins** is chemically very diverse, including both acidic and basic molecules. Some of the water-soluble vitamins, such as thiamin and ascorbic acid, are very polar and thus difficult to retain by reversed phase HPLC. Many reversed phase methods for water-soluble vitamins require ion pairing reagents in order to retain the more polar analytes. The Ultra Aqueous C18 column analyzes six water-soluble vitamins with gradient elution and relatively simple mobile phases that contain no ion pairing reagents (Figure 6).

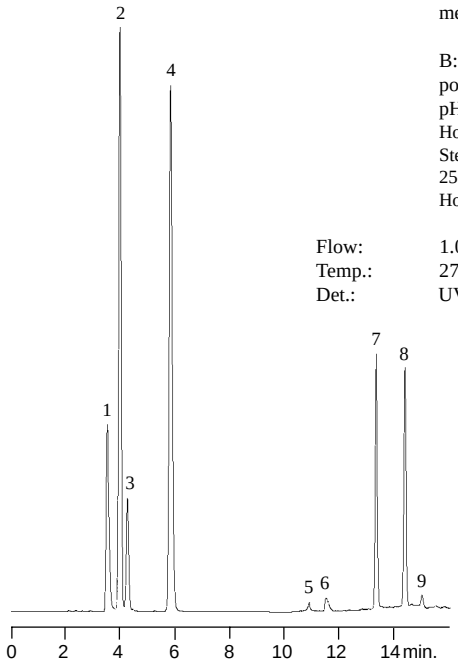
Figure 6

The Ultra Aqueous C18 column separates six water-soluble vitamins using a relatively simple mobile phase and no ion pairing reagents.

Peak List:	**Conc.** (µg/mL)
1. thiamin (B1)	250
2. ascorbic acid (C)	1,000
3. unknown	n/a
4. nicotinic acid (B3)	1,000
5. unknown	n/a
6. pantothenic acid (B5)	1,000,000
7. folic acid (B9)	500
8. riboflavin (B2)	250
9. methyl paraben	0.2

Initial dilutions of B1 and B2 basified with ammonium hydroxide

Column:	**Ultra Aqueous C18**
Catalog #:	9178575
Dimensions:	250 x 4.6mm
Particle size:	5µm
Pore size:	100Å
Sample:	
Inj.:	1µL
Solvent:	all analytes dissolved in HPLC-grade water
Conditions:	
Mobile phase:	A: 25mM potassium phosphate, pH 2.00: methanol (95:5) B: methanol:25mM potassium phosphate, pH 3.5 (60:40) Hold 0% B: 0-6 min Step to 25% B: 6.01min 25-100% B: 6.01-11min Hold 100% B: 11-16min
Flow:	1.0mL/min.
Temp.:	27°C
Det.:	UV @ 254nm



LC_0141

Conclusion

The Ultra Aqueous C18 column provides selectivity similar to a conventional C18 column, while maintaining a high level of base deactivation. In addition, the Ultra Aqueous C18 column can benefit your analysis by allowing 100% aqueous mobile phases; providing enhanced retention of polar compounds, better peak shape, and no need of sample derivatization or ion pairing reagents.

Product Listing

Ultra Aqueous, 3µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9178331	9178332	9178333	9178335
50mm	9178351	9178352	9178353	9178355
100mm	9178311	9178312	9178313	9178315

Ultra Aqueous, 3µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9178332-700	9178333-700	9178335-700
50mm	—	9178352-700	9178353-700	9178355-700
100mm	—	9178312-700	9178313-700	9178315-700

Ultra Aqueous, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9178531	9178532	9178533	9178535
50mm	9178551	9178552	9178553	9178555
100mm	9178511	9178512	9178513	9178515
150mm	9178561	9178562	9178563	9178565
200mm	9178521	9178522	9178523	9178525
250mm	9178571	9178572	9178573	9178575

Ultra Aqueous, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9178532-700	9178533-700	9178535-700
50mm	—	9178552-700	9178553-700	9178555-700
100mm	—	9178512-700	9178513-700	9178515-700
150mm	—	9178562-700	9178563-700	9178565-700
200mm	—	9178522-700	9178523-700	9178525-700
250mm	—	9178572-700	9178573-700	9178575-700

RESTEK HPLC

Applications note

HPLC Analysis of Vitamins

The market for nutritional and dietary supplements is growing tremendously. And, as these nutraceutical and dietary supplement markets grow, the need for simple, rugged, and accurate high performance liquid chromatography (HPLC) methods to analyze vitamins becomes increasingly important.

Vitamins encompass a wide range of organic compounds. They are required by the human body in only minute quantities. Yet, they have significant impact at the cellular level. Vitamins play a vital role in converting fat and carbohydrates to energy, regulating metabolism, and other bodily functions.

Vitamins can be broadly classified into two groups, water-soluble vitamins and fat-soluble vitamins. Fat-soluble vitamins include such constituents as Vitamin A (retinol) and Vitamin D3 (cholecalciferol). Vitamin A has been found to play an important role in proper growth and eye function, while Vitamin D is required for proper bone and tooth growth.

Fat-Soluble Vitamins

The Restek Ultra C18 column is an ideal first choice to separate a wide range of fat-soluble vitamins. Its very retentive, high-purity packing exhibits excellent peak shape. The silica has a carbon load of 20% and is fully end-capped, which eliminates any unwanted silanol interactions and improves column-to-column reproducibility.

The fat-soluble vitamins are extremely hydrophobic and must be dissolved in an organic solvent. A mixture of six fat-soluble vitamins were separated using an Ultra C18 column (Figure 1).

Figure 1

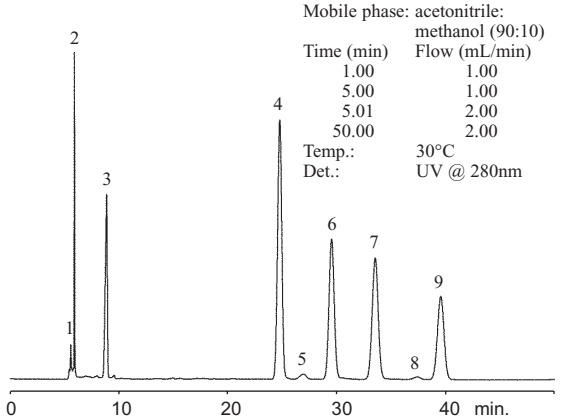
The Ultra C18 column provides excellent peak shape and resolution of fat-soluble vitamins.

Peak List:	Conc.: (mg/mL)
1. solvent front	
2. menadione (vitamin K3)	0.45
3. all-trans-retinol (vitamin A)	0.34
4. vitamin D3	0.4
5. unknown	
6. alpha tocopherol (vitamin E)	2.4
7. alpha tocopherol acetate (vitamin E Acetate)	2.4
8. unknown	
9. phylloquinone (vitamin K1)	0.84

Column:	**Ultra C18**
Catalog #:	9174575
Dimensions:	250 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å
Sample:	
Inj.:	1µL
Solvent:	all analytes dissolved in HPLC-grade diethyl ether.
Conditions:	
Mobile phase:	acetonitrile: methanol (90:10)

Time (min)	Flow (mL/min)
1.00	1.00
5.00	1.00
5.01	2.00
50.00	2.00

Temp.: 30°C
Det.: UV @ 280nm



LC_0144

The analytes were dissolved in diethyl ether. The sample solution was then analyzed using a simple isocratic mobile phase (acetonitrile:methanol [90:10]) with a stepped flow rate (1mL/min. from 1 minute to 5 minutes, then 2mL/min. from 5 minutes to 50 minutes). All six constituents are easily resolved and show excellent peak shape.

Water-Soluble Vitamins

The group of compounds known as water-soluble vitamins is very diverse chemically, including both basic and acidic molecules. Some of the water-soluble vitamins, such as thiamin and ascorbic acid, are very polar and thus difficult to retain by reversed phase HPLC. Many reversed phase methods for water-soluble vitamins require ion-pairing reagents in order to retain the more polar analytes. However, the Ultra Aqueous C18 column easily separates six water-soluble vitamins with a gradient elution and relatively simple mobile phases that contain no ion pairing reagents (Figure 2).

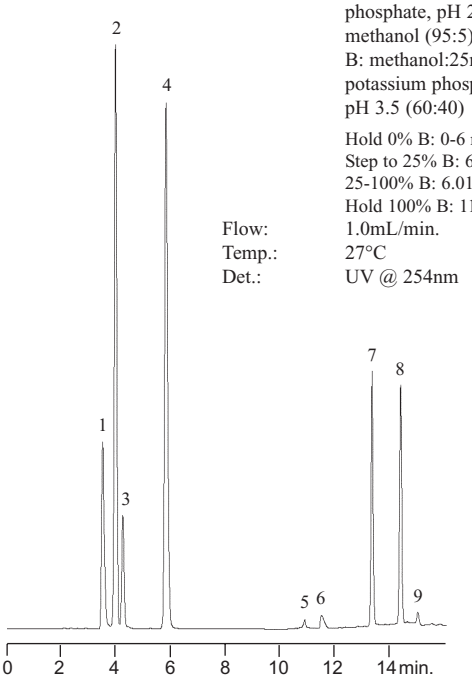
Figure 2

The Ultra Aqueous C18 column easily separates six water-soluble vitamins.

Peak List:	Conc. (µg/mL)
1. thiamin (B1)*	250
2. ascorbic acid (C)	1,000
3. unknown	n/a
4. nicotinic acid (B3)	1,000
5. unknown	n/a
6. pantothenic acid (B5)	1,000,000
7. folic acid (B9)	500
8. riboflavin (B2)*	250
9. methyl paraben (preservative)	0.2

*Initial dilutions of B1 and B2 basified with ammonium hydroxide.

Column:	**Ultra Aqueous C18**
Catalog #:	9178575
Dimensions:	250 x 4.6mm
Particle size:	5µm
Pore size:	100Å
Sample:	
Inj.:	1µL
Solvent:	all analytes dissolved in HPLC-grade water
Conditions:	
Mobile phase:	A: 25mM potassium phosphate, pH 2.00: methanol (95:5) B: methanol:25mM potassium phosphate, pH 3.5 (60:40)
	Hold 0% B: 0-6 min. Step to 25% B: 6.01min. 25-100% B: 6.01-11min. Hold 100% B: 11-16min.
Flow:	1.0mL/min.
Temp.:	27°C
Det.:	UV @ 254nm



LC_0141

For simple, rugged, and accurate high performance liquid chromatography (HPLC) methods and columns to analyze fat- and water-soluble vitamins, turn to Restek for products and service.

Ultra Aqueous C18, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9178531	9178532	9178533	9178535
50mm	9178551	9178552	9178553	9178555
100mm	9178511	9178512	9178513	9178515
150mm	9178561	9178562	9178563	9178565
200mm	9178521	9178522	9178523	9178525
250mm	9178571	9178572	9178573	9178575

Ultra Aqueous C18, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9178532-700	9178533-700	9178535-700
50mm	—	9178552-700	9178553-700	9178555-700
100mm	—	9178512-700	9178513-700	9178515-700
150mm	—	9178562-700	9178563-700	9178565-700
200mm	—	9178522-700	9178523-700	9178525-700
250mm	—	9178572-700	9178573-700	9178575-700

Ultra C18, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9174531	9174532	9174533	9174535
50mm	9174551	9174552	9174553	9174555
100mm	9174511	9174512	9174513	9174515
150mm	9174561	9174562	9174563	9174565
200mm	9174521	9174522	9174523	9174525
250mm	9174571	9174572	9174573	9174575

Ultra C18, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9174532-700	9174533-700	9174535-700
50mm	—	9174552-700	9174553-700	9174555-700
100mm	—	9174512-700	9174513-700	9174515-700
150mm	—	9174562-700	9174563-700	9174565-700
200mm	—	9174522-700	9174523-700	9174525-700
250mm	—	9174572-700	9174573-700	9174575-700

RESTEK HPLC

Applications note

Analysis of Vanillin and Ethyl Vanillin in Vanilla Flavors Using Ultra C8 Column

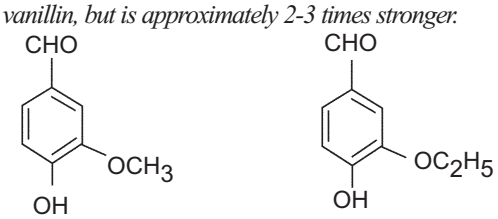
Introduction

Vanilla extracts and flavorings are used in a wide range of food products, including dairy products, beverages, baked goods, and confections. They also are used as a background note or flavor enhancer in products such as sauces, soups, and vegetables. In fact, vanilla is the only flavor with a US Federal Department of Agriculture (FDA) standard of identity (21 CFR 169), which states that vanilla extract must contain the extractive material from 13.35 oz. of vanilla beans per gallon and at least 35% alcohol by volume. If the alcohol content is less than 35%, the solution is "vanilla flavor." Imitation vanilla extract contains natural and artificial flavorings, including vanillin, and has alcohol as the solvent. Imitation vanilla flavors contain vanillin, ethyl vanillin, or other acceptable flavoring materials, with or without "real" vanilla.

The flavor profile of vanilla contains over 250 components, with vanillin present at levels between 0.5-2%. A curing process creates the characteristic odor and flavor by promoting an enzymatic reaction that transforms glucovanillin and other substances into vanillin and other aromatic compounds. In general, vanillin is the component of vanilla extract that is imitated in artificial flavorings. United States Pharmacopoeia (USP) defined vanillin can be synthesized from sources such as lignin, a byproduct of the paper industry. Ethyl vanillin also is synthesized for use in vanilla flavorings, and has a taste very similar to vanillin. Figure 1 shows the chemical structures for vanillin and ethyl vanillin. The aromatic rings on these compounds allow sensitive detection based on their UV absorbance at 254nm.

Figure 1

Ethyl vanillin is a synthetic molecule that has a similar taste to vanillin, but is approximately 2-3 times stronger.



Vanillin **Ethyl Vanillin**

Analysis

In the Association of Official Analytical Chemists (AOAC) Method 990.25, flavor compounds in vanilla extract and artificial vanilla flavor are analyzed using HPLC. The analytes are separated on a C8 column and quantitated by comparing their UV absorbance at 254nm to an external standard. The analysis is performed isocratically, with a mobile phase of acidified water:methanol (90:10) and a flow rate of 2.5mL/min. Even at this high flow rate, the run time is long (approximately 40 minutes) and there is significant broadening of the late-eluting ethyl vanillin peak.

An efficient separation can be performed using the Restek Ultra C8 reverse phase column (150 x 4.6mm, with 5µm particles) and a gradient pump program, such as acidified water:methanol (80:20 to 60:40). By using a gradient program, the run time can be reduced to 25 minutes at a flow rate of 1mL/min (Figures 2 and 3).

Figure 2 shows the analysis of a standard solution of vanillin and ethyl vanillin. Using these parameters, the k' values for vanillin and ethyl vanillin are 4.8 and 6.8, respectively. The resolution of these compounds is excellent under these run conditions. Figure 3 shows the injection of a solution of vanilla extract. In addition to vanillin, there are other aromatic compounds present in the extract. Vanillin is well resolved from these components, and the vanillin can be easily quantitated in this sample. Other flavor components present in vanilla extracts and flavorings also can be analyzed using this procedure.

Figure 2

The Ultra C8 column achieves excellent resolution of vanillin and ethyl vanillin.

Peak List: **Conc.:** (mg/mL)
1. vanillin 0.12
2. ethyl vanillin 0.04

Column: **Ultra C8**
Catalog #: 9103565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Sample:
Inj.: 10µL
Solvent: 40% ethanol

Conditions:
Mobile phase: A:1.2% acetic acid
B:methanol

Time (min.)	%B
0.0	20.0
5.0	20.0
15.0	40.0
20.0	40.0
25.0	20.0

Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm

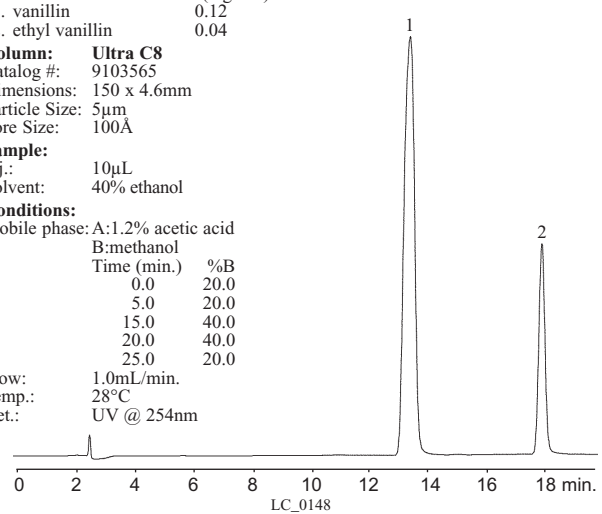


Figure 3

The analysis of a 5% solution of a vanilla extract allows easy quantitation of vanillin.

Peak List:
1.vanillin

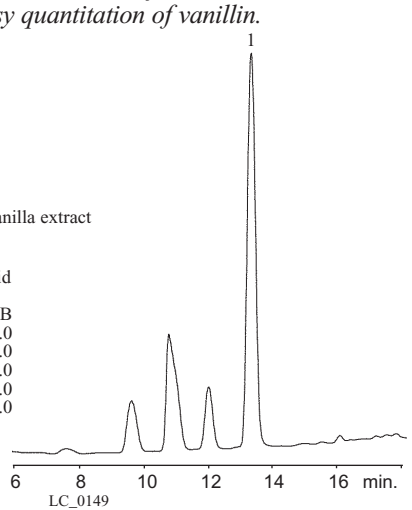
Column: Ultra C8
Catalog #: 9103565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Sample:
Inj.: 10µL
Conc.: 5% solution of vanilla extract
Solvent: 40% ethanol

Conditions:
Mobile phase: A:1.2% acetic acid
B:methanol

Time (min.)	%B
0.0	20.0
5.0	20.0
15.0	40.0
20.0	40.0
25.0	20.0

Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm



HPLC

Summary

The analysis of vanillin and ethyl vanillin in vanilla flavors can be performed efficiently by liquid chromatography using an Ultra C8 column and UV detection at 254nm. The separation can be carried out using either an isocratic or a gradient elution program; however, in the gradient mode the run time is significantly reduced and less solvent is needed. In addition, much less peak broadening is observed with the gradient program, resulting in higher sensitivity for the later-eluting compounds.

References

1. AOAC Official Methods of Analysis (2000), 17th edition, method 990.25.
2. Brandt, Laura. "The Creation and Use of Vanilla," Food Product Design (1996), editorial archives.

Ultra C8, 3µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9103331	9103332	9103333	9103335
50mm	9103351	9103352	9103353	9103355
100mm	9103311	9103312	9103313	9103315

Ultra C8, 3µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9103332-700	9103333-700	9103335-700
50mm	—	9103352-700	9103353-700	9103355-700
100mm	—	9103312-700	9103313-700	9103315-700

Ultra C8, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9103531	9103532	9103533	9103535
50mm	9103551	9103552	9103553	9103555
100mm	9103511	9103512	9103513	9103515
150mm	9103561	9103562	9103563	9103565
200mm	9103521	9103522	9103523	9103525
250mm	9103571	9103572	9103573	9103575

Ultra C8, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9103532-700	9103533-700	9103535-700
50mm	—	9103552-700	9103553-700	9103555-700
100mm	—	9103512-700	9103513-700	9103515-700
150mm	—	9103562-700	9103563-700	9103565-700
200mm	—	9103522-700	9103523-700	9103525-700
250mm	—	9103572-700	9103573-700	9103575-700

RESTEK environmental

Applications note

Techniques to Optimizing GC Analysis of Ethylene Glycol in Water

The analysis of ethylene glycol in water is a very common test in environmental laboratories. Many of these samples originate from water runoff at airports where ethylene glycol is used as a de-icing agent for airplanes during winter months. Because ethylene glycol is highly soluble in water, it is not easily concentrated by purge and trap. Therefore, the most frequently used sample introduction technique is direct aqueous injection. The direct aqueous injection of ethylene glycol can be challenging because, if not done properly, it can be difficult to attain reproducibility and good peak shape. The large expansion volume of water can cause backflash, carryover can cause inconsistent results, and excess water can extinguish the FID flame. These problems can prevent achieving the detection limit for ethylene glycol, which may vary in the 1-10ppm range.

Poor Peak Shape

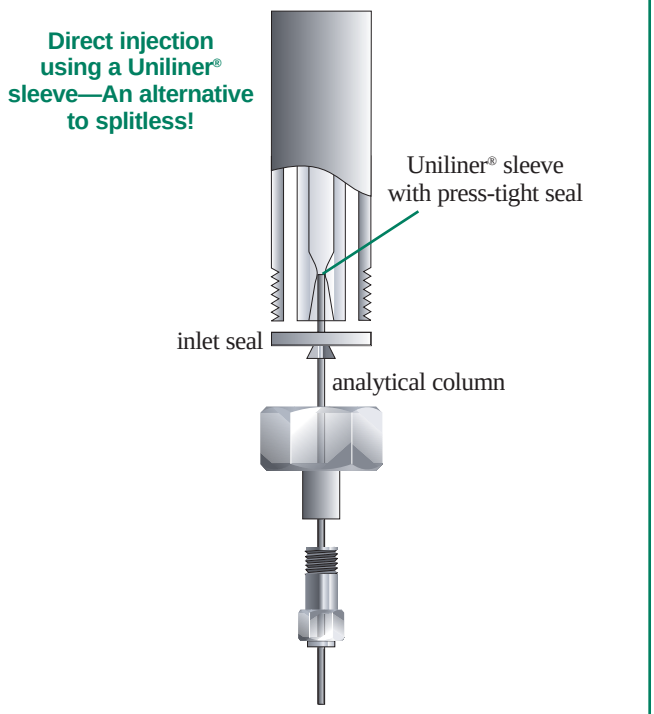
With a column head pressure of 10psig and an injection port temperature of 250°C, a 1µL injection of water will expand to 1420µL of vapor. This large vapor cloud exceeds the volume of most inlet liners, causing backflash. If backflash occurs, the vapor cloud can expand out of the liner and injection port and result in poor sample transfer onto the column. Also, the glycol compounds are not focused in a narrow band but, instead, are focused in the condensed water that beads onto the column walls, so the compounds of interest can elute as split peaks. This peak splitting effect is most apparent when performing a splitless injection because of the solvent focusing required. Split peaks and backflash compromise the analysis by causing irreproducible peak shapes.

One technique to reduce the effect of vapor expansion and poor solvent focusing is the use of a Uniliner® injection port sleeve. This sleeve forms a leak-free connection with the column end (**Figure 1**), thereby ensuring a complete sample transfer. Additionally, the Uniliner® sleeve requires operation at a higher pressure than traditional splitless liners, which forces the large vapor cloud to be focused into a narrow band when entering the column. This minimizes sample backflash and eliminates the need for solvent focusing. By using a Uniliner® sleeve, the aqueous ethylene glycol sample is completely vaporized and properly transferred to the column in a focused, narrow band, thereby achieving reproducible peak areas. Uniliner® sleeves are available for conversion of packed column injection systems and for split/splitless injection systems.

Sample Residue Carryover

Carryover is another problem associated with ethylene glycol analysis. When analyzing glycols, carryover can be caused by sample residue in the syringe being carried over from one injection to another. If the syringe is not properly cleaned between analyses, carryover will cause inconsistent results.

Figure 1
The Uniliner® sleeve forms a leak-free connection, minimizes backflash, and helps focus the sample.



Rinsing the syringe with either water or water/methanol (50:50) three to six times between each injection will eliminate sample residue and minimize the possibility of carryover.

FID Flameout

Column stationary phase choice is a critical consideration when analyzing glycols in water via direct injection. Water analyzed on a non-polar stationary phase, such as the Rtx®-1 column, or on a moderately polar stationary phase, such as the Rtx®-200 column, will cause the flame on the FID to be extinguished. This is because the water will not partition properly and will "bead up" on the phase, producing a large plug of water that passes through the detector and extinguishes the flame. The more commonly-used GCs will experience flameout under these circumstances while others will not.

To minimize the possibility of extinguishing the flame, select a polar stationary phase that is more compatible with water. The Stabilwax® stationary phase is one of the more polar phases, making it a good choice for water injections. It allows water to partition properly, which prevents it from beading up on the stationary phase and quenching the FID flame.

environmental

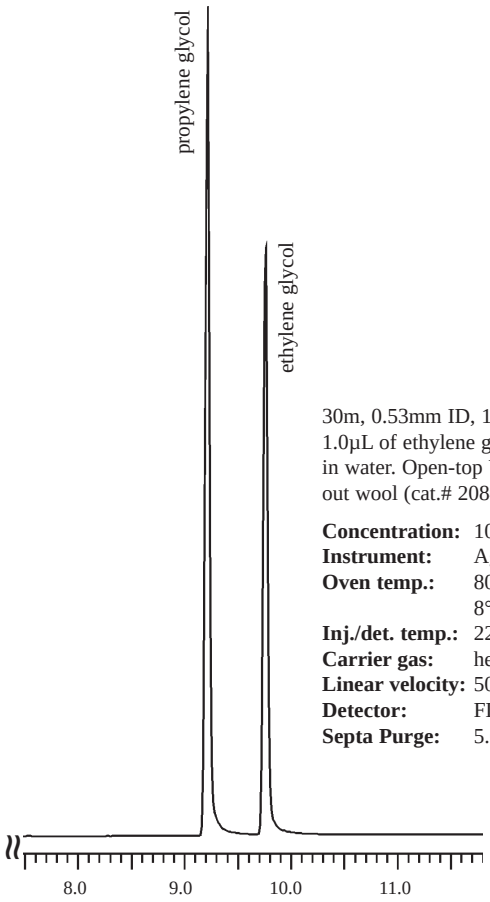
The Stabilwax® column can easily handle direct aqueous injections without showing any signs of degradation. Testing of the Stabilwax® column was performed by injecting 1µL of a water standard 100 times. Peak shape and response of ethylene and propylene glycol remained consistent throughout the analyses (**Figures 2 and 3**). The Stabilwax® column also allows sensitive detection of low ppm-levels of glycol compounds. Notice the 5ppm detection limit for ethylene glycol in water is easily achieved, and peak shape is maintained when compared to a 25ppm standard (**Figure 4**).

Conclusion

You can achieve better response and reproducibility for the GC analysis of ethylene glycol in water by using direct injection with a Uniliner® sleeve, a polar capillary column such as Stabilwax®, and multiple syringe washes between runs. Using these techniques can assist in attaining reproducible analyses with detection limits in the low ppm range.

Figure 2

The Stabilwax® column shows good peak shape and response for ethylene glycol after 100 injections.



30m, 0.53mm ID, 1.0µm Stabilwax (cat.# 10655)
1.0µL of ethylene glycol and propylene glycol sample in water. Open-top Uniliner direct injection liner without wool (cat.# 20843-205).

Concentration:	100ppm
Instrument:	Agilent 5890 w/7673 Autosampler
Oven temp.:	80°C (hold 1 min.) to 200°C @ 8°C/min. (5.0 min.)
Inj./det. temp.:	225°C/250°C
Carrier gas:	helium
Linear velocity:	50cm/sec.
Detector:	FID
Septa Purge:	5.0cc/min.

Figure 3

The Stabilwax® column combined with a Uniliner® sleeve shows remarkable response consistency.

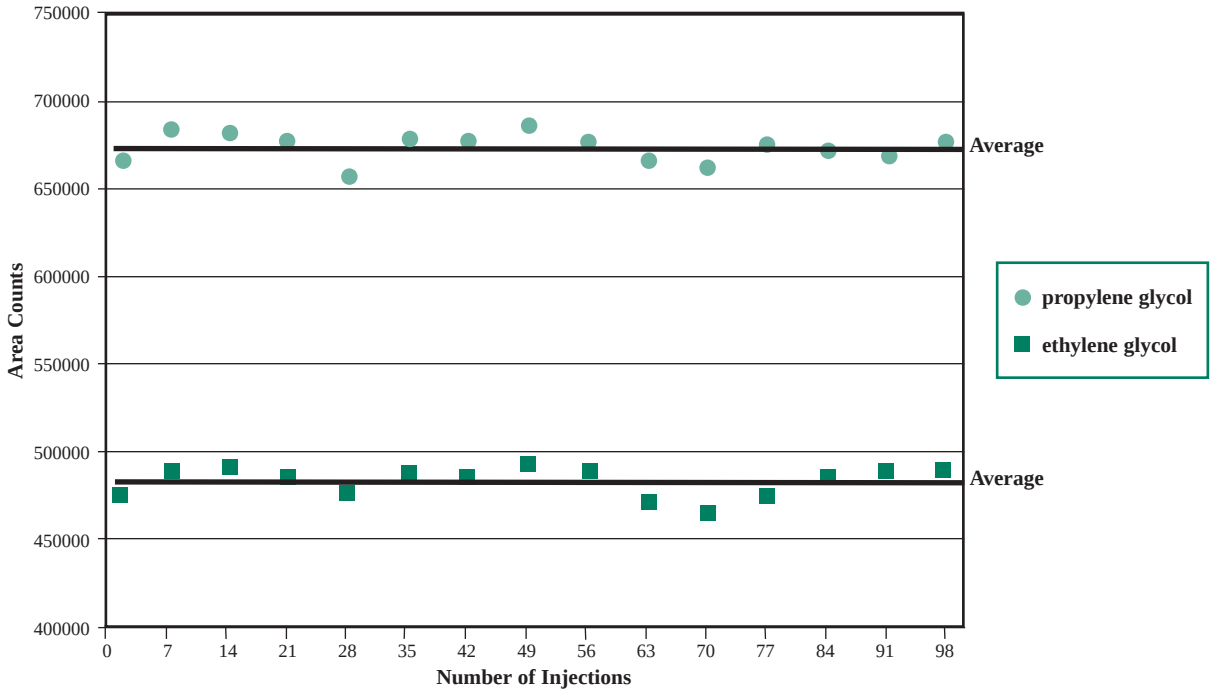
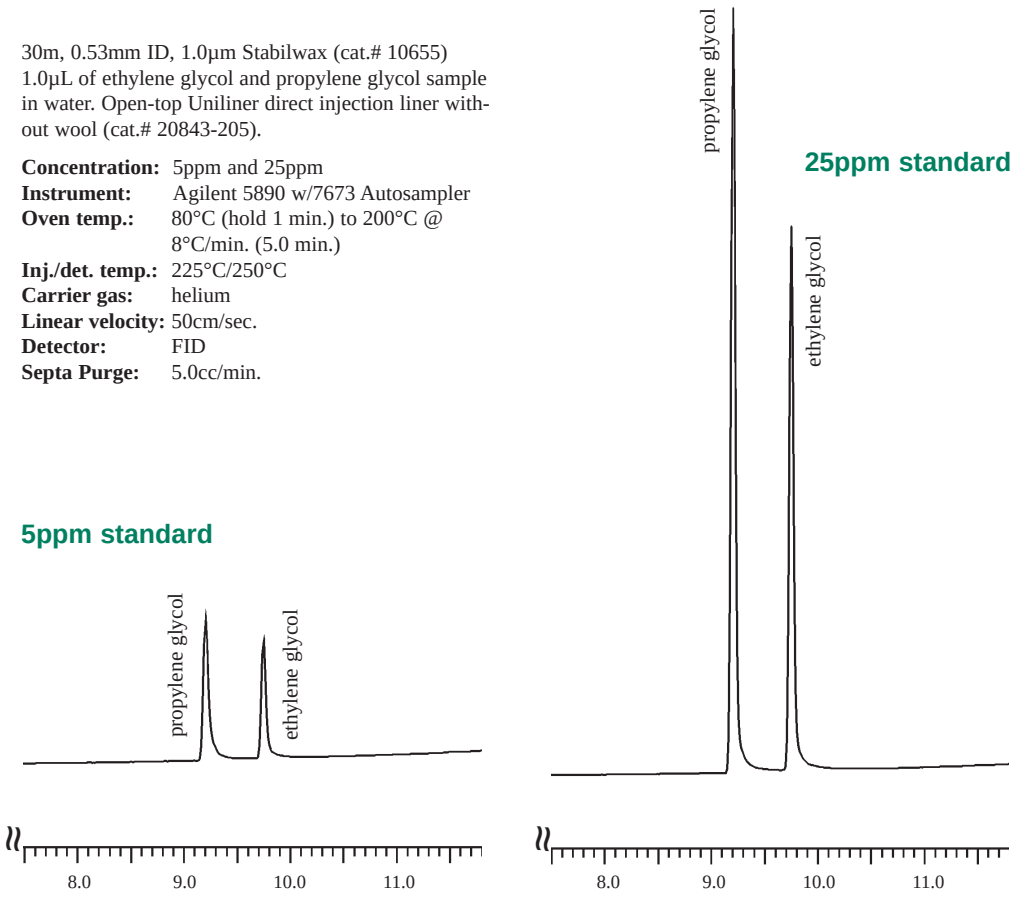


Figure 4

The Stabilwax® column can easily analyze 5ppm and 25ppm standards.

30m, 0.53mm ID, 1.0µm Stabilwax (cat.# 10655)
1.0µL of ethylene glycol and propylene glycol sample in water. Open-top Uniliner direct injection liner without wool (cat.# 20843-205).

Concentration: 5ppm and 25ppm
Instrument: Agilent 5890 w/7673 Autosampler
Oven temp.: 80°C (hold 1 min.) to 200°C @ 8°C/min. (5.0 min.)
Inj./det. temp.: 225°C/250°C
Carrier gas: helium
Linear velocity: 50cm/sec.
Detector: FID
Septa Purge: 5.0cc/min.



Product Listing:

Stabilwax® Columns

30m	0.32mm ID	1.0µm	cat.# 10654
30m	0.53mm ID	1.0µm	cat.# 10655

Uniliner® Sleeves

Description	Column ID Inj. Mode	Each*	5-pack
Uniliner® Sleeve *(large buffer volume chamber—85mm long for injections ≤4µL)*	0.32 & 0.53mm DI only	20308	20309
	0.53mm DI or OC	20301	20305
Cyclo-Uniliner® Sleeve *(for active dirty samples)*	0.32 & 0.53mm DI only	20319	20320
Open-Top Uniliner® Sleeve *(packed with fused silica wool)*	0.32 & 0.53mm DI only	20315	20316
Uniliner® Sleeve Adaptor *(required for installing Uniliner® sleeves in ¼" injection ports)*	*includes a ¼" SS nut and graphite ferrule, a 1/16" SS nut, and a 0.8mm ID graphite ferrule.* ***For injection ports <8cm:*** *cat.# 20310 ea.* ***For injection ports 8-15cm:*** *cat.# 20311 ea.* ***For Shimadzu:*** *cat.# 20312 ea.*		

**Add the suffix "-205" to the catalog number to order without wool.*

continued on back

Product Listing, continued:

Uniliner® Sleeves

Direct Injection Liners for Agilent & Finnigan GCs *(0.32/0.53mm ID)*	Benefits/Uses:	ID***/OD & Length (mm)	Each*	5-pack
Uniliner®†	trace, active samples, high recovery & linearity	4.0 ID, 6.3 OD x 78.5	20335	20336
Cyclo-Uniliner®†	trace, dirty, high MW active samples, high recovery & linearity	4.0 ID, 6.3 OD x 78.5	20337	20338
Open-top Uniliner® with Wool†	trace, dirty active samples, high recovery & linearity	4.0 ID, 6.3 OD x 78.5	20843	20844

Direct Injection Liners for Agilent 6890 GCs *(0.32/0.53mm ID)*	Benefits/Uses:	ID***/OD & Length (mm)	Each*	5-pack
Drilled Uniliner®†	allows direct injection when using an EPC-equipped GC	4.0 ID, 6.3 OD x 78.5	21054	21055

Direct Injection Liners for Varian GCs *(0.32/0.53mm ID)*	Benefits/Uses:	ID***/OD & Length (mm)	Each*	5-pack
Uniliner®	trace, active samples, high recovery & linearity	4.0 ID, 6.3 OD x 72	20345	20346
Cyclo-Uniliner®	trace, dirty, high MW, active samples, linearity	4.0 ID, 6.3 OD x 72	20347	20348
Open-top Uniliner® w/ Wool*	trace, dirty, active samples, high recovery & linearity	4.0 ID, 6.3 OD x 72	20845	20846

Direct Injection Liners for Shimadzu GCs *(0.32/0.53mm ID)*	Benefits/Uses:	ID***/OD & Length (mm)	Each*	5-pack
128mm Uniliner®	trace, active samples, high recovery & linearity	3.0 ID, 5.0 OD x 128	20872	20873
128mm Cyclo-Uniliner®	trace, dirty, high MW active samples, linearity	3.5 ID, 5.0 OD x 128	20874	20875
99mm Uniliner®	trace, active samples, high recovery & linearity	3.0 ID, 5.0 OD x 99	20876	20877
99mm Cyclo-Uniliner®	trace, dirty, high MW active samples, high recovery & linearity	3.0 ID, 5.0 OD x 99	20893	20894
94mm Uniliner® w/ Wool*	trace, dirty, high MW active samples, high recovery & linearity	3.0 ID, 5.0 OD x 94	21713	21719

Direct Injection Liners for Perkin-Elmer GCs *(0.32/0.53mm ID)*	Benefits/Uses:	ID***/OD & Length (mm)	Each*	5-pack
Uniliner®	trace, active samples, high recovery & linearity	3.5 ID, 5.0 OD x 100	20855	20856
Cyclo-Uniliner®	trace, dirty, active samples, linearity	3.5 ID, 5.0 OD x 100	20857	20858
Auto SYS Open-top Uniliner® w/ Wool*	trace, dirty, active samples, high recovery & linearity	4.0 ID, 6.2 OD x 92.1	20837	20838
Auto SYS Cyclo-Uniliner®	trace, dirty, high MW active samples, linearity	4.0 ID, 6.2 OD x 92.1	20839	20840

Split Liners for 5000-6000 Series GCs	Benefits/Uses:	ID***/OD & Length (mm)	Each*	5-pack
Open-top Uniliner® w/ Wool*	trace, dirty, active samples, high recovery & linearity	4.0 ID, 5.5 OD x 79.5	20841	20842

Direct injection Liners for 8000 & TRACE™ Series GCs	Benefits/Uses:	ID***/OD & Length (mm)	Each*	5-pack
Uniliner® w/ Wool	trace, active samples, high recovery & linearity	5.0 ID, 8.0 OD x 105	21005	21006

**Add the suffix "-205" to the catalog number to order without wool.*
***These liners are packed with fused silica wool. To order glass wool instead, add the suffix "-202" to the liner's catalog number.*
****Nominal ID at syringe needle expulsion point.*
†These Uniliner® sleeves are for split/splitless injection ports.

Trademarks: *Uniliner, Rtx, and Stabilwax (Restek). TRACE (ThermaQuest Corp.).*

APPLICATIONS NOTE

TO-Can™ Air Monitoring Canisters

Long-Term Stability of TO-14/TO-15 Compounds

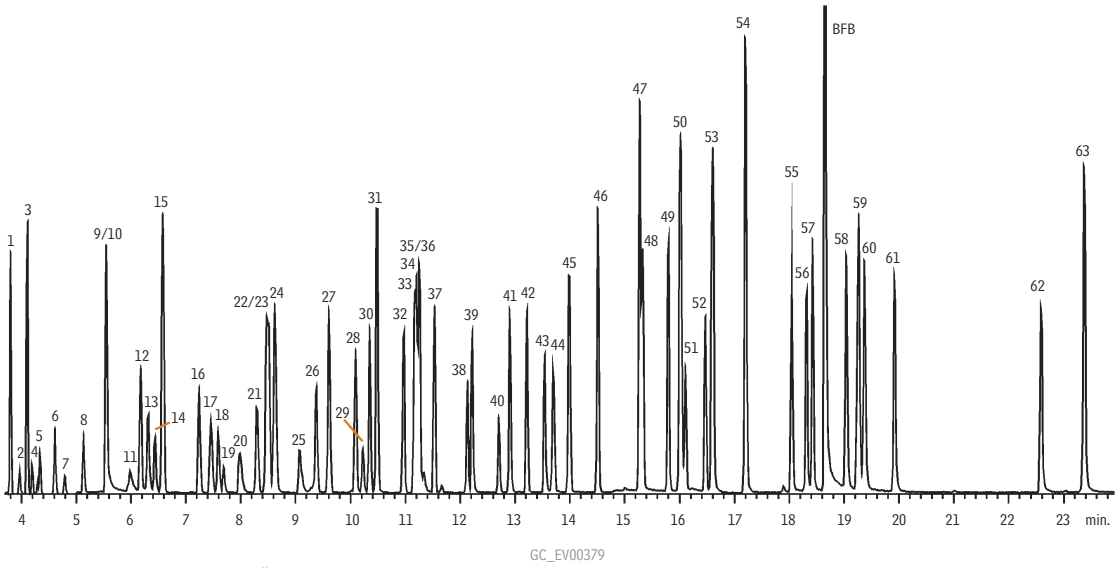
US Environmental Protection Agency (EPA) Compendium of Air Methods TO-14/TO-15 are designed to regulate the collection, storage, and analysis of atmospheric volatile organic compounds (VOCs) using treated air sampling canisters. Restek TO-Can™ canisters are electropolished using a proprietary process and extensively cleaned using an ultrasonic method—treatments that ensure a high-quality, passivated surface to maintain stability of the TO-14/TO-15 compounds during storage. The design of the frame surrounding the electropolished canister eliminates the need for weld marks on the sphere, which can be focal points for VOC breakdown or adsorption. A Parker Hannifin metal-to-metal diaphragm valve further ensures the reliable performance of the canister.

To collect VOCs in ambient air, TO-Can™ canisters should be cleaned and evacuated prior to being sent to the field. In the field, the sample is drawn through a sampling train that will regulate the rate and duration of sampling. The TO-Can™ canister is then sent to an analytical laboratory, where a known amount is drawn from the canister, concentrated on a concentrating trap, and analyzed according to Methods TO-14/TO-15, typically using a 60m, 0.32mm ID, 1.0µm Rtx®-1 capillary column in a GC/MS system.

TO-Can™ canisters meet the holding time criteria for Methods TO-14/TO-15

A 62-component TO-15 standard (10ppbv) was injected into a TO-Can™ canister and humidified to 70% relative humidity. The standard was analyzed on day 0, day 7, and day 14 of storage. Table I (page 2) shows the results of the study. The TO-Can™ canister ensured excellent stability for these polar and non-polar compounds. The analysis on an Rtx®-1 column shows excellent resolution and peak shapes (Figure 1).

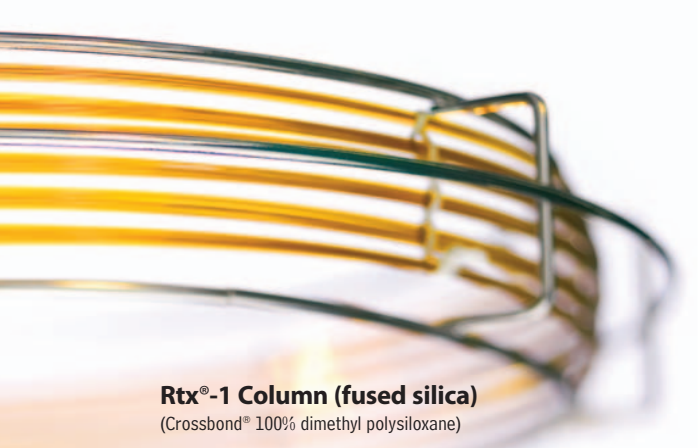
Figure 1 TO-Can™ canister maintains excellent stability for TO-14/TO-15 compounds at 10ppbv after 14 days.



GC_EV00379

Rtx®-1 60m, 0.32mm ID, 1.0µm (cat.# 10157).
Sample: 200mL of 10ppbv TO-15 standard, injected into TO-Can™ canister, humidified to 70% RH, stored 14 days.
Concentrator: Nutech 3550 Preconcentrator, 200mL of sample concentrated at -160°C, thermally desorbed at 150°C, and cryofocused at -185°C.
Oven temp.: 30°C (hold 4 min.) to 175°C @ 9°C/min. to 220°C @ 40°C/min.
Carrier gas: helium @ 1.2mL/min.
Det.: Agilent 5971 MS
Scan range: 35-265amu
(see Table I on page 2 for peak identifications)

Chromatogram courtesy of Gina Maio, Severn Trent Laboratories, Inc., Burlington, VT.



Rtx®-1 Column (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.00	-60 to 320/340°C	60-Meter	10157

The ultimate in controlled heating,
for reliably cleaning your air canisters!

Air Canister Heating Jacket

- Heats entire canister, and valve.
- Two temperature settings, 75°C and 150°C.*
- Prevents sample condensation, for accurate sub-sampling.
- Easily fits canister up to 6 liters.
- Lightweight; comfortable to the touch when heated.
- Connect up to five Canister Heating Jackets to one 15 amp circuit.

Description	qty.	cat.#
Air Canister Heating Jacket (110 volt)	ea.	24123

*Not CE certified.

TO-14A Internal Standard/Tuning Mix

bromochloromethane
1-bromo-4-fluorobenzene (4-bromofluorobenzene)
chlorobenzene-d5
1,4-difluorobenzene

In nitrogen, 104 liters @ 1800psig

1ppm	cat. # 34408	(ea.)
100ppb	cat. # 34425	(ea.)

Table I Holding time criteria for Methods TO-14/TO-15 are easily achieved using a TO-Can™ canister.

Compounds	RTs	Day 1 ppbv	Day 7 ppbv	Day 14 ppbv
1. dichlorodifluoromethane (Freon® 12)	3.794	10.0	10.0	11.0
2. chloromethane	3.952	9.2	9.0	10.0
3. dichlorotetrafluoroethane (Freon® 114)	4.096	9.7	9.7	11.0
4. vinyl chloride	4.193	10.0	9.8	11.0
5. 1,3-butadiene	4.327	10.0	9.9	12.0
6. bromomethane	4.601	9.3	8.5	9.7
7. chloroethane	4.774	9.4	8.2	10.0
8. bromoethene	5.117	9.8	8.8	9.7
9. acetone	5.436	9.1	9.4	9.9
10. trichlorofluoromethane (Freon® 11)	5.527	9.8	10.0	10.0
11. isopropyl alcohol	5.709	10.0	8.8	7.8
12. 1,1-dichloroethene	6.149	9.6	9.6	11.0
13. methylene chloride	6.271	9.1	9.5	9.8
14. 3-chloropropene	6.392	9.1	8.3	8.4
15. carbon disulfide	6.544	8.0	8.9	9.6
16. trichlorotrifluoroethane (Freon® 113)	6.544	9.3	10.0	11.0
17. *trans*-1,2-dichloroethene	7.196	9.5	10.0	10.0
18. 1,1-dichloroethane	7.394	9.3	9.9	10.0
19. methyl *tert*-butyl ether	7.500	12.0	8.3	8.2
20. methyl ethyl ketone	7.834	9.2	9.1	10.0
21. *cis*-1,2-dichloroethene	8.228	9.6	9.8	10.0
22. bromochloromethane (IS)	8.395	10.0	10.0	10.0
23. *n*-hexane	8.471	9.0	9.4	9.9
24. chloroform	8.532	9.3	10.0	10.0
25. tetrahydrofuran	8.972	8.2	7.2	7.3
26. 1,2-dichloroethane	9.291	9.0	8.1	8.9
27. 1,1,1-trichloroethane	9.549	8.5	7.8	8.6
28. benzene	10.019	9.1	8.7	9.8
29. carbon tetrachloride	10.171	7.3	7.4	7.5
30. cyclohexane	10.307	9.2	9.2	10.0
31. 1,4-difluorobenzene (IS)	10.399	10.0	10.0	10.0
32. 1,2-dichloropropane	10.884	9.8	8.3	8.9
33. bromodichloromethane	11.081	7.2	8.0	8.6
34. trichloroethene	11.127	8.0	8.5	9.3
35. 1,4-dioxane	11.157	7.4	7.9	9.9
36. 2,2,4-trimethylpentane	11.188	7.7	7.9	8.7
37. *n*-heptane	11.461	7.9	8.0	9.0
38. *cis*-1,3-dichloropropene	12.068	7.7	8.3	9.5
39. methyl isobutyl ketone	12.129	8.5	8.6	10.0
40. *trans*-1,3-dichloropropene	12.644	7.5	7.9	8.2
41. 1,1,2-trichloroethane	12.842	8.8	11.0	9.9
42. toluene	13.160	9.0	12.0	11.0
43. methyl butyl ketone	13.464	9.3	9.7	10.0
44. dibromochloromethane	13.631	8.5	8.6	9.2
45. 1,2-dibromoethane	13.919	9.3	9.0	11.0
46. tetrachloroethene	14.481	9.7	8.5	12.0
47. chlorobenzene-d5 (IS)	15.224	10.0	10.0	10.0
48. chlorobenzene	15.285	10.0	9.4	11.0
49. ethylbenzene	15.755	11.0	11.0	12.0
50. xylene (*m,p*)	15.983	20.0	19.0	23.0
51. bromoform	16.059	9.6	8.4	8.5
52. styrene	16.438	11.0	8.3	8.5
53. 1,1,2,2-tetrachloroethane	16.545	11.0	9.1	8.0
54. xylene (*o*)	16.575	12.0	8.9	7.8
55. 2-chlorotoluene	18.017	11.0	10.0	7.8
56. 4-ethyltoluene	18.290	11.0	9.7	7.7
57. 1,3,5-trimethylbenzene	18.396	11.0	10.0	8.4
58. 1,2,4-trimethylbenzene	19.018	11.0	11.0	10.0
59. 1,3-dichlorobenzene	19.246	10.0	10.0	9.9
60. 1,4-dichlorobenzene	19.352	10.0	9.9	10.0
61. 1,2-dichlorobenzene	19.898	10.0	10.0	10.0
62. 1,2,4-trichlorobenzene	22.569	12.0	11.0	10.0
63. hexachlorobutadiene	23.358	12.0	11.0	10.0

TO-15 62 Component Mix

acetone
benzene
benzyl chloride*
bromodichloromethane
bromoform
bromomethane
1,3-butadiene
2-butanone (MEK)
carbon disulfide*
carbon tetrachloride
chlorobenzene
chloroethane
chloroform
chloromethane
cyclohexane
dibromochloromethane
1,2-dichlorobenzene
1,3-dichlorobenzene
1,4-dichlorobenzene
1,1-dichloroethane
1,2-dichloroethane
1,1-dichloroethene
cis-1,2-dichloroethene
trans-1,2-dichloroethene
1,2-dichloropropane
cis-1,3-dichloropropene
trans-1,3-dichloropropene
1,4-dioxane
ethanol*
ethyl acetate
ethyl benzene
ethyl dibromide (1,1-dibromoethane)
4-ethyltoluene
trichlorofluoromethane (Freon® 11)
dichlorodifluoromethane (Freon® 12)
1,1,2-trichloro-1,2,2-trifluoroethane (Freon® 113)
1,2-dichlorotetrafluoroethane (Freon® 114)
heptane
hexachloro-1,3-butadiene
hexane
2-hexanone (MBK)
4-methyl-2-pentanone (MIBK)
methylene chloride
methyl *tert*-butyl ether (MTBE)
2-propanol
propylene
styrene
1,1,2,2-tetrachloroethane
tetrachloroethene
tetrahydrofuran
toluene
1,2,4-trichlorobenzene
1,1,1-trichloroethane
1,1,2-trichloroethane
trichloroethene
1,2,4-trimethylbenzene
1,3,5-trimethylbenzene
vinyl acetate
vinyl chloride
m-xylene
o-xylene
p-xylene

In nitrogen, 104 liters @ 1800psig

1ppm	cat. # 34436 (ea.)
100ppb	cat. # 34437 (ea.)

*Stability of this compound cannot be guaranteed.

cylinder
design

Aluminum construction.
Size: 8 x 24 cm.
Volume/Pressure: 104 liters of gas @ 1800psig.
Outlet Fitting: CGA-180.
Weight: 1.5 lbs.

TO-Can™ Air Monitoring Canisters

Optimized for US EPA Methods TO-14/TO-15

- High quality, metal-to-metal seal, 2/3-turn valve with metal diaphragms.
- Sizes from 1 to 15 liters.
- Optional 30" Hg/60psig vacuum/pressure gauge (other gauges available).

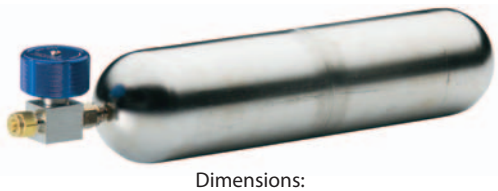
Description	qty.	cat.#
1L Volume		
TO-Can™ Canister, 1/4" Valve	ea.	24172
TO-Can™ Canister with Gauge, 1/4" Valve	ea.	24176
3L Volume		
TO-Can™ Canister, 1/4" Valve	ea.	24173
TO-Can™ Canister with Gauge, 1/4" Valve	ea.	24177
6L Volume		
TO-Can™ Canister, 1/4" Valve	ea.	24174
TO-Can™ Canister with Gauge, 1/4" Valve	ea.	24178
15L Volume		
TO-Can™ Canister, 1/4" Valve	ea.	24175
TO-Can™ Canister with Gauge, 1/4" Valve	ea.	24179

Miniature Air Sampling Canisters

These 1000cc canisters are suitable for sampling volatile organic compounds in air according to US EPA Methods TO-14/TO-15.

Description	Volume	qty.	cat.#
With Quick-Connect Stem Fittings			
Electro-Polished Miniature Canister with Quick-Connect Stem Fitting	1000cc	ea.	24194
Sulfinert®-Treated Miniature Canister with Quick-Connect Stem Fitting	1000cc	ea.	24195
Sulfinert®-Treated Miniature Canister with Sulfinert®-Treated Quick-Connect Stem Fitting	1000cc	ea.	24196
With Metal-Seated Diaphragm Valve			
Electro-Polished Miniature Canister with Metal-Seated Diaphragm Valve	1000cc	ea.	24197
Sulfinert®-Treated Miniature Canister with Metal-Seated Diaphragm Valve	1000cc	ea.	24198
Sulfinert®-Treated Miniature Canister with Sulfinert®-Treated Diaphragm Valve	1000cc	ea.	24199
With Nut & Ferrule			
Electro-Polished Miniature Canister with Nut & Ferrule	1000cc	ea.	24206
Sulfinert®-Treated Miniature Canister with Nut & Ferrule	1000cc	ea.	24208

Also available: 400cc canisters. See our catalog or website.



Dimensions:
2.75" diameter, 11.92" long
(7 x 30.3cm)

free literature

For detailed information about using, cleaning, and certifying passive sampling trains in air sampling applications, request our technical guide *A Guide to Passive Air Sampling*.

Call Restek at 800-356-1688 or 814-353-1300, ext. 5, or contact your Restek representative, to request your free copy!

lit. cat.# 59977B

Passive Air Sampling Kits

- Improved design eliminates leaks at the filter.
- Excellent for sampling times from 1 hour to 125 hours, or grab sampling.

Available in six sampling flow ranges, Restek's passive air sampling kit incorporates all hardware necessary to collect air samples, and is easy to assemble for field sampling.* The stainless steel kit is ideal to partner with the Restek TO-Can™ air sampling canister for methods TO-14A/TO-15. Use the Siltek®-treated version with the Restek SilcoCan® air sampling canister when collecting low-level volatile sulfur compounds, or other active compounds.

Air Sampling Kits

Canister Volume					Flow	Orifice	Siltek®-Treated	Stainless Steel
400cc	1 Liter	3 Liter	6 Liter	15 Liter	(sccm)	size	Complete Sampling Kits	Complete Sampling Kits
8 hour	24 hour	48 hour	125 hour	—	0.5–2	0.0008"	24217	24216
2 hour	4 hour	12 hour	24 hour	60 hour	2–4	0.0012"	24160	24165
1 hour	2 hour	6 hour	12 hour	30 hour	4–8	0.0016"	24161	24166
—	1 hour	4 hour	8 hour	20 hour	8–20	0.0020"	24162	24167
—	—	2 hour	3 hour	8 hour	20–40	0.0030"	24163	24168
—	—	—	1 hour	3 hour	40–80	0.0060"	24164	24169

*Air sampling canisters sold separately.

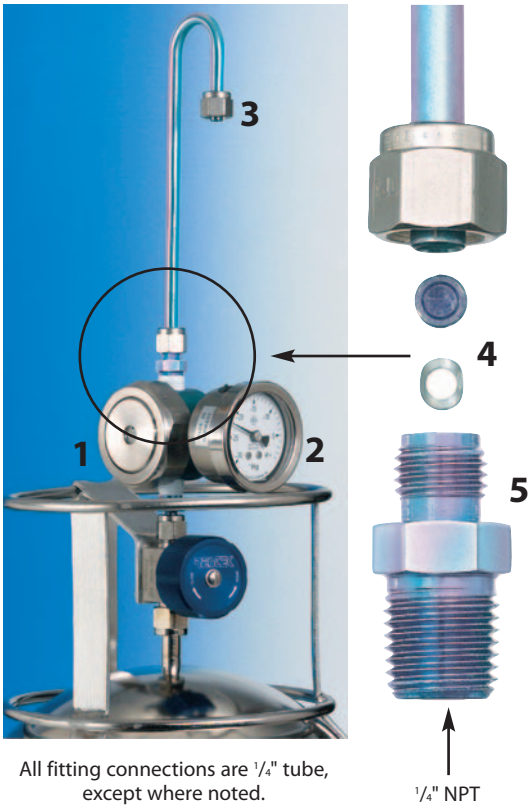
did you know?

We also offer sampling bags, thermal desorption tubes, and a range of gas reference standards to meet your environmental air sampling requirements.

1. Veriflo™ SC423XL flow controller
Designed to maintain a constant mass flow as the pressure changes from 30" Hg to 5" Hg. All wetted parts of the flow controller can be Siltek®-treated.

2. Stainless steel vacuum gauge
Monitors canister pressure change during sampling.

3. 1/4-inch Siltek® sample inlet
Stainless steel nut prevents water droplets from accumulating at the edge of the tubing, where they could be pulled into the sampling train.



All fitting connections are 1/4" tube, except where noted.

4. 2-micron frit filter and washer
Replaceable. Available in stainless steel, or Siltek®-treated for optimum inertness.

5. Interchangeable critical orifice
Sapphire critical orifice controls the flow with very high precision. Available in stainless steel, or Siltek®-treated for optimum inertness.

For individual components, see our catalog or website.

RESTEK 20 YEARS

Lit. Cat.# 59189A

RESTEK environmental

Applications note

Separating *m*- and *p*-Xylene Isomers by US EPA Method 8260 Using an Rtx®-200 GC Column

Xylenes are aromatic hydrocarbons that naturally occur in petroleum and coal tar; they also can be commercially derived from these substrates. Three xylene isomers exist: *meta*-, *para*-, and *ortho*-xylene, usually referred to as *m*-, *p*-, and *o*-xylene, respectively. Mixed xylenes produced from petroleum contain 20% *o*- and *p*-xylene, with 44% *m*-xylene.[1] The isomers of *m*- and *p*-xylene are difficult to resolve using gas chromatography (GC) and most capillary columns. Although limited data exists suggesting toxicological differences between *m*- and *p*-xylene, there is still interest in resolving them.

The US Environmental Protection Agency (EPA) does not require separation of the xylene isomers, but rather requests their calculation as totals or sums.[2] Some states such as New York have action limits based on *m*- and *p*-xylene separately. For example the action limit in drinking water is 5µg/L for *m*-xylene and 5µg/L for *p*-xylene. A drinking water sample that has 9µg/L of total xylenes could, in fact, exceed the limits by having 9µg/L of *m*-xylene and no *p*-xylene present in the sample.[3] However, other states and agencies would consider the action limit for these two isomers as 10µg/L total.

A recent performance evaluation from New York state contained one of the isomers of xylene, which required the contracted environmental laboratories to determine actual concentrations of *m*- and *p*-xylene separately. The most common way to perform a GC separation of *m*- and *p*-xylene is by using a polyethylene glycol (PEG) stationary phase, such as the Restek Stabilwax® column. Chromatographically, baseline separation is possible; however, bleed levels are unacceptable for a mass spectrometer (MS) and sample matrices containing organic acids can contribute to bleed from the stationary phase.

The more ideal column choice for this particular separation is the Rtx®-200 column. The Crossbond® trifluoropropylmethyl polysiloxane stationary phase (**Figure 1**) features exceptionally low bleed at common volatile application working temperatures because its maximum operating temperature is 360° C.

The Rtx®-200 column provides unique separation of volatile organic compounds (VOCs) listed in US EPA Methods 524 and 8260 (**Figure 2**), making it the best column to separate xylene isomers for specific state requirements (**Figure 3**). The Rtx®-200 column's only limitation is the resolution of the gases—peaks 2, 3, 5, and 6 (**Figure 2**).

This column also is a good choice for analyzing samples having complex matrices; analyses where coelutions of several compounds can make identification of tentitively identified compounds (TICs) nearly impossible on a "624" phase. Trifluoropropyl stationary phases like that of the Rtx®-200 column have a unique selectivity because of the electrophilic nature of the fluorine-containing polymer. This creates interactions with electron-rich molecules like ketones and halogenated compounds. This unique selectivity results in different elution orders and resolves compounds that phenyl, cyano and methyl phases cannot. In this analysis, the Rtx®-200 column can be used to confirm TICs and resolve multiple coelutions.

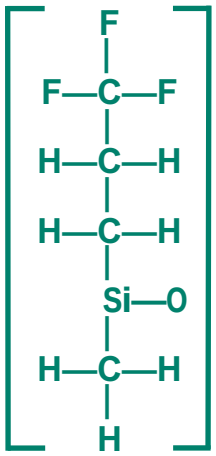
References

1. Toxicological Profile for Total Xylenes. Prepared by Clements Associates, Inc.. under Contract No. 205-88-0608. Prepared for Agency for Toxic Substances and Disease Registry, US Public Health Services, Atlanta, GA. December 1990.

2. US EPA Method 8000B, Rev. 2. Determinative Chromatographic Separations. Page 7 Section 3.3.3. Washington, DC. December 1996.

3. Consumer Confidence Report. New York Water Service Corporation. 60 Brooklyn Avenue Merrick, New York, NY. September 1999.

Figure 1—Phase Structure
Rtx®/MXT®-200 trifluoropropylmethyl polysiloxane



Polarity: selective for lone pair electrons.

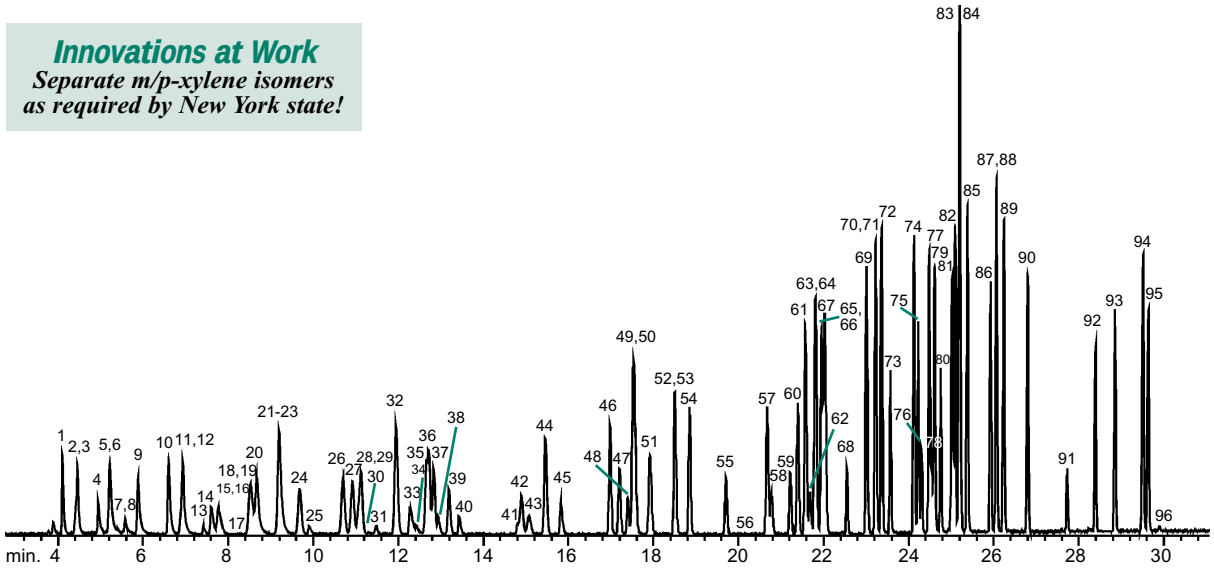
Uses: environmental samples, solvents, Freon® samples, drugs, ketones and alcohols.

Figure 2

The Rtx®-200 column provides unique separation of the VOCs listed in US EPA Method 8260.

Innovations at Work

Separate m/p-xylene isomers as required by New York state!



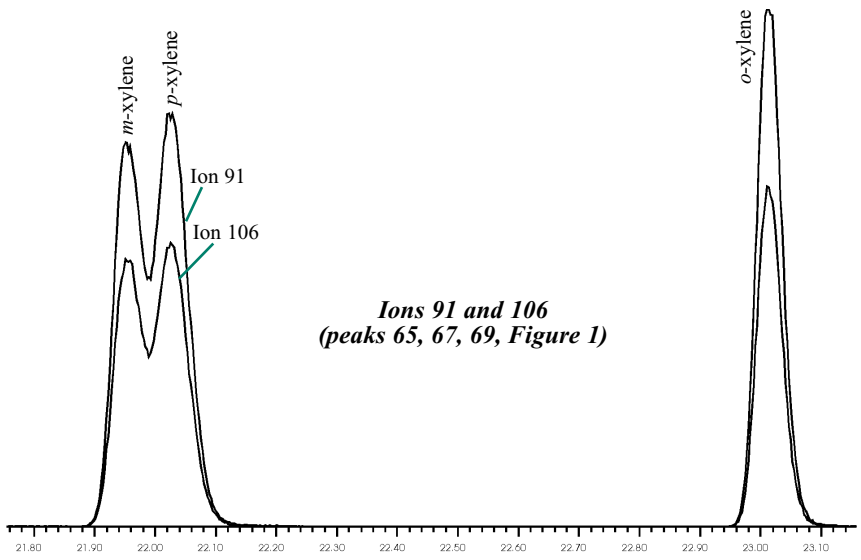
60m, 0.25 mm ID, 1.0µm Rtx-200 (cat.# 15056)
Compounds in at 10 ppb in 5mL of RO water.
Ketones, alcohols in at 2x (unless otherwise noted).

Concentrator:	Tekmar LSC-3100 Purge and Trap
Trap:	Vocarb 3000 (type K)
Purge:	11 min. @ 40mL/min. @ ambient temperature
Dry Purge:	1 min. @ 40mL/min.
Desorb Preheat:	245°C
Desorb:	250°C for 2 min., flow 10mL/min.
Bake:	260°C for 8 min.
Interface:	transfer line 0.53mm ID Silcosteel MXT tubing
Oven Program:	40°C (hold 10 min.) to 100°C @ 6°C/min. (hold 1 min.) to 210°C @ 30°C/min. (hold 7 min.)
Carrier Gas:	helium @ ~1.3mL/min. constant flow Adjust dichlorodifluoromethane to a retention time of 4.1 min. @ 40°C.
Detector:	Agilent 5973 MS, scan range 35 to 300 AMU

1. dichlorodifluoromethane
2. chloromethane
3. vinyl chloride
4. bromomethane
5. chloroethane
6. trichlorofluoromethane
7. carbon disulfide
8. ethanol (2500ppb)
9. 1,1-dichloroethene
10. methylene chloride
11. allyl chloride
12. trans-1,2-dichloroethene
13. *tert*-butyl alcohol (100ppb)
14. methyl *tert*-butyl ether
15. allyl alcohol (250ppb)
16. diisopropyl ether
17. propargyl alcohol (500ppb)
18. 1,1-dichloroethane
19. ethyl-*tert*-buyl ether
20. chloroform
21. dibromofluoromethane
22. *cis*-1,2-dichloroethene
23. bromochloromethane
24. carbon tetrachloride
25. acetone
26. 2,2-dichloropropane
27. 1,1,1-trichloroethane
28. vinyl acetate
29. 1,1-dichloropropene
30. isobutyl alcohol (500ppb)
31. acrylonitrile
32. benzene
33. *tert*-amyl-methyl ether
34. tetrahydrofuran
35. 1,2-dichloroethane
36. trichloroethene
37. bromodichloromethane
38. methyl acrylate
39. dibromomethane
40. ethyl acetate
41. 2-butanone
42. 1,4-difluorobenzene
43. pentafluorobenzene
44. 1,2-dichloropropane
45. isopropyl acetate
46. *cis*-1,3-dichloropropene
47. dibromochloromethane
48. methyl methacrylate
49. toluene
50. toluene-d8
51. tetrachloroethene
52. 1,1,2-trichloroethane
53. *n*-propyl acetate
54. *trans*-1,3-dichloropropene
55. 1,2-dibromoethane
56. pyridine (250ppb)
57. 1,3-dichloropropane
58. ethyl methacrylate
59. bromoform
60. 1,1,1,2-tetrachloroethane
61. ethylbenzene
62. 4-methyl-2-penanone
63. chlorobenzene
64. chlorobenzene-D5
65. *m*-xylene
66. 2-picoline (250ppb)
67. *p*-xylene
68. *n*-butyl acetate
69. *o*-xylene
70. stryrene
71. 2-hexanone
72. isopropylbenzene
73. 1,1,2,2-tetrachloroethane
74. *n*-propylbenzene
75. bromobenzene
76. 4-bromo-1-fluorobenzene
77. 1,3,5-trimethylbenzene
78. pentachloroethane
79. 2-chlorotoluene
80. 1,2,3-trichloropropane
81. 4-chlorotoluene
82. *tert*-butylbenzene
83. 1,2,4-trimethylbenzene
84. *sec*-butylbenzene
85. *p*-isopropyltoluene
86. 1,3-dichlorobenzene
87. 1,2-dichlorobenzene-d4
88. 1,4-dichlorobenzene
89. *n*-butylbenzene
90. 1,2-dichlorobenzene
91. 1,2-dibromo-3-chloropropan
92. hexachlorobutadiene
93. 1,2,3-trichlorobenzene
94. naphthalene
95. 1,2,4-trichlorobenzene
96. nitrobenzene (250ppb)

Figure 3

An extracted ion chromatogram shows the Rtx®-200 column is the best column choice to separate m/p-xylene isomers.



60m, 0.25 mm ID, 1.0µm Rtx-200 (cat.# 15056)
Compounds in at 10 ppb in 5mL of RO water.
Ketones, alcohols in at 2x (unless otherwise noted).

Concentrator:	Tekmar LSC-3100 Purge and Trap
Trap:	Vocarb 3000 (type K)
Purge:	11 min. @ 40mL/min. @ ambient temperature
Dry Purge:	1 min. @ 40mL/min.
Desorb Preheat:	245°C
Desorb:	250°C for 2 min., flow 10mL/min.
Bake:	260°C for 8 min.
Interface:	transfer line 0.53mm ID Silcosteel MXT tubing
Oven Program:	40°C (hold 10 min.) to 100°C @ 6°C/min. (hold 1 min.) to 210°C @ 30°C/min. (hold 7 min.)
Carrier Gas:	helium @ ~1.3mL/min. constant flow. Adjust dichlorodifluoromethane to a retention time of 4.1 min. @ 40°C.
Detector:	Agilent 5973 MS, scan range 35 to 300 AMU

Product Listing:

Coiled Silcosteel® Tubing

Silcosteel®-Treated Welded/Drawn Grade 304 Stainless Steel Tubing *Sold by the foot—5 ft. minimum.*	
cat.#	**ID, OD**
20590	0.011" ID (0.28mm ID), 0.022" OD (0.56mm OD)
20591	0.021" ID (0.53mm ID), 0.029" OD (0.74mm OD)
20592	0.010" ID (0.25mm ID), 1/16" OD (1.59mm OD)
20593	0.020" ID (0.51mm ID), 1/16" OD (1.59mm OD)
20594	0.030" ID (0.76mm ID), 1/16" OD (1.59mm OD)
20595	0.040" ID (1.02mm ID), 1/16" OD (1.59mm OD)
0.020" wall:	
20596	0.085" ID (2.16mm ID), 1/8" OD (3.18mm OD)
0.020" wall:	
20597	0.210" ID (5.33mm ID), 1/4" OD (6.35mm OD)

Silcosteel®-Treated Seamless 316 Grade Stainless Steel Tubing *Sold by the foot—5 ft. minimum.*	
cat.#	**ID, OD**
0.035" wall:	
20598	0.055" ID (1.40mm ID), 1/8" OD (3.18mm OD)
0.035" wall:	
20599	0.180" ID (4.57mm ID), 1/4" OD (6.35mm OD)

Call for availability of lengths greater than 1000 ft.

***Metric conversion:** 6 ft. (1.8m), 25 ft. (7.6m), 50 ft. (15.2m), 200 ft. (61m), >400 ft. (>122m)

Other lengths and sizes of Silcosteel® tubing are available on a custom basis!

Straight Silcosteel® Tubing

0.085" ID (2.16mm), **1/8" OD** (3.18mm)		
Length	**Individual**	**5-Pack**
18" (457mm)	20575	20576

0.210" ID (5.33mm), **1/4" OD** (6.35mm)		
Length	**Individual**	**5-Pack**
18" (457mm)	20577	20578

Minimum Bend Radius
(dependent on OD)

OD	Min. Bend Radius
£1/16"	1"
1/8"	2"
1/4"	4"

Column product listing continued on back.

Restek Corporation
(800) 356-1688 • (814) 353-1300
www.restekcorp.com

Product Listing:

Rtx®-200 (Fused Silica)

(Crossbond® trifluoropropylmethyl polysiloxane) Stable to 360°

ID	df (µm)	temp. limits*	15-Meter	30-Meter	60-Meter	105-Meter
0.25mm	0.10	-20 to 320/340°C	15005	15008	15011	
	0.25	-20 to 320/340°C	15020	15023	15026	15029
	0.50	-20 to 310/330°C	15035	15038	15041	15044
	1.00	-20 to 290/310°C	15050	15053	15056	15059
0.32mm	0.10	-20 to 320/340°C	15006	15009	15012	
	0.25	-20 to 320/340°C	15021	15024	15027	15030
	0.50	-20 to 310/330°C	15036	15039	15042	15045
	1.00	-20 to 290/310°C	15051	15054	15057	15060
	1.50	-20 to 280/300°C	15066	15069	15072	15075
0.53mm	0.10	-20 to 310/330°C	15007	15010	15013	
	0.25	-20 to 310/330°C	15022	15025	15028	
	0.50	-20 to 300/320°C	15037	15040	15043	
	1.00	-20 to 290/310°C	15052	15055	15058	
	1.50	-20 to 280/300°C	15067	15070	15073	
	3.00	-20 to 260/280°C	15082	15085	15088	15091
ID	**df (µm)**	**temp. limits**	**10-Meter**	**20-Meter**	**40-Meter**	
0.18mm	0.20	-20 to 310/330°C	45001	45002	45003	
	0.40	-20 to 310/330°C	45010	45011	45012	

MXT®-200 (Silcosteel®)

(Crossbond® trifluoropropylmethyl polysiloxane) Stable to 360°

ID	df (µm)	temp. limits*	15-Meter	30-Meter	60-Meter
0.25mm	0.50	-20 to 330°C	75035	75038	
	1.00	-20 to 310°C	75050	75053	
0.53mm	1.00	-20 to 290/310°C	75052	75055	75058
	1.50	-20 to 280/300°C	75067	75070	75073
	3.00	-20 to 260/280°C	75082	75085	75088
ID	**df (µm)**	**temp. limits***	**10-Meter**	**20-Meter**	**40-Meter**
0.18mm	0.20	-20 to 310/330°C	71881	71882	71883
	0.40	-20 to 310/330°C	71884	71885	71886

**The maximum temperatures listed are for 15- and 30-meter lengths. Longer lengths may have a slightly reduced maximum temperature.*

RESTEK environmental

Applications note

Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) Using Rtx®-5Sil MS and Rtx®-CLPesticides2 Capillary Columns

Analysis of polycyclic aromatic hydrocarbons (PAHs) is a very common method in environmental laboratories. US Environmental Protection Agency (EPA) Method 8100 requires gas chromatograph/flame ionization detection (GC/FID) to quantitate PAHs found in extracts from soil, water, or biological samples. This method requires the use of a dual-column system, because most samples often contain hydrocarbon interferences. Confirmational analysis increases the confidence of proper identification and quantitation of the PAHs. Good resolution is necessary for proper quantitation; the most difficult compound pairs to resolve are benzo(b)/benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene/dibenzo(a,h)anthracene. Short analysis time is another key consideration for most laboratories. By decreasing analysis time, sample throughput is increased and the lab benefits from a cost savings.

Primary Analysis

For this analysis, the primary analytical stationary phase is a 5% diphenyl/95% dimethyl-polysiloxane polymer. The Restek Rtx®-5Sil MS column is an equivalent phase and is recommended for this analysis (Fig. 1). While selectivity is similar to 5% diphenyl/95% dimethyl-polysiloxane columns, the proprietary silarylene stationary phase of the Rtx®-5Sil MS column is designed to produce very low bleed.

Confirmational Analysis

Confirmational analysis is a technique that requires two analytical columns of different selectivities, resulting in different retention times of target or interfering compounds. These differences can improve quantitative and qualitative reliability through peak verification. The confirmational column recommended by Restek for this analysis is the Rtx®-CLPesticides2 column (Fig. 2). Quantitative reliability for this analysis is maintained because the stationary phases differ in selectivity, resulting in retention time shifts of both PAHs and interference compounds.

Resolution of PAHs

Resolution between benzo(b)fluoranthene and benzo(k)fluoranthene and indeno(1,2,3,-cd)pyrene and dibenzo(a,h)anthracene is essential for quantitation when using an FID. To achieve excellent resolution of these peak pairs, the carrier gas, column flow rate, and temperature program must all be optimized. And, to achieve even better quantitative reliability, it is recommended to clean sample extracts following EPA Method 3630 (silica gel) prior to analysis.

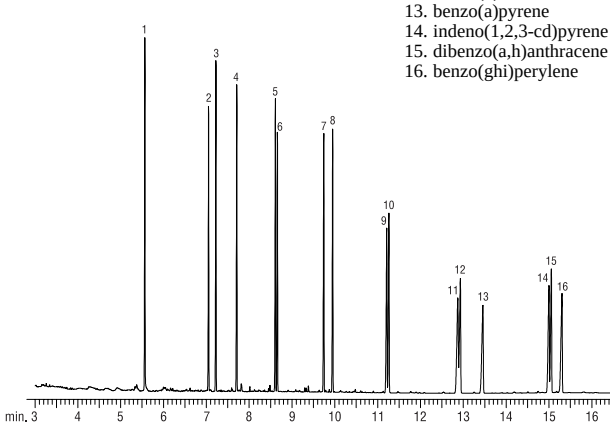
Optimizing Carrier Gas Flows

The resolution of PAHs can be increased and the overall analysis time reduced by using hydrogen carrier gas at high flow rates. For this application hydrogen is a better choice than helium because it is more efficient at higher flow rates. And, if used in the constant flow mode, the best separation and fastest analysis time can be achieved. (Constant pressure mode is not recommended because the flow rate will decrease as the oven temperature is increased. This could result in a loss of resolution for the later eluting PAHs and a longer analysis time.)

The optimum carrier gas flow rate for the 30m, 0.25mm ID, 0.25µm Rtx®-5Sil MS column is less than 1mL/min. However, by increasing the flow rate to 4mL/min. for the analysis of PAHs, the separation of the isomer pairs is increased and the analysis time is reduced to less than 17 minutes (Figure 1). The Rtx®-CLPesticides2 confirmation column can separate these compounds under identical conditions (Figure 2). Again, the faster flow rate (4mL/min.) improves separation and reduces analysis time to less than 18 minutes.

Figure 1–The Rtx®-5Sil MS column exhibits excellent resolution of polycyclic aromatic hydrocarbons including benzo(b)/benzo(k)fluoranthene in less than 16 minutes.

1. naphthalene
2. acenaphthylene
3. acenaphthene
4. fluorene
5. phenanthrene
6. anthracene
7. fluoranthene
8. pyrene
9. benzo(a)anthracene
10. chrysene
11. benzo(b)fluoranthene
12. benzo(k)fluoranthene
13. benzo(a)pyrene
14. indeno(1,2,3-cd)pyrene
15. dibenzo(a,h)anthracene
16. benzo(ghi)perylene



GC_EV00595

Rtx®-5Sil MS 30m, 0.25mm ID, 0.25µm (cat.# 12723)
Sample: 50µg/mL 610 PAH Mix standard (cat.# 31011) in carbon disulfide
Inj.: 1.0µL splitless (hold 2 min.), 2mm splitless w/ wool inlet liner (cat.# 20829)
Inj. temp.: 330°C
Carrier gas: hydrogen, 4mL/min constant flow
Oven temp.: 40°C (hold 2 min.) to 250°C @25°C/min. to 265°C @5°C/min. to 300°C (hold 4 min.) @25°C/min.
Det.: FID @350°C

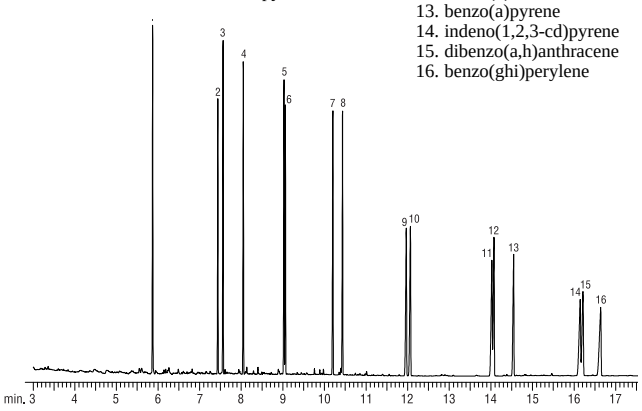
environmental

Optimizing Temperature Program

Optimizing the temperature program also contributes to better resolution of closely eluting peak pairs and shortens analysis times. The temperature program in Figures 1 and 2 achieves baseline resolution of indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene, and excellent resolution of benzo(b)fluoranthene and benzo(k)fluoranthene, while still keeping the analysis time under 18 minutes. Because the column flow rate and temperature program for both columns is the same, the analysis can be run simultaneously on the primary and confirmation columns.

Figure 2–The Rtx®-CLPesticides column is an excellent confirmational column for polycyclic aromatic hydrocarbon analysis.

1. naphthalene
2. acenaphthylene
3. acenaphthene
4. fluorene
5. phenanthrene
6. anthracene
7. fluoranthene
8. pyrene
9. benzo(a)anthracene
10. chrysene
11. benzo(b)fluoranthene
12. benzo(k)fluoranthene
13. benzo(a)pyrene
14. indeno(1,2,3-cd)pyrene
15. dibenzo(a,h)anthracene
16. benzo(ghi)perylene



GC_EV00596

Rtx®-CLPesticides2 30m, 0.25mm ID, 0.20µm (cat.# 11323)
Sample: 50µg/mL 610 PAH Mix standard (cat.# 31011) in carbon disulfide
Inj.: 1.0µL splitless (hold 2 min.), 2mm splitless w/ wool inlet liner (cat.# 20829)
Inj. temp.: 330°C
Carrier gas: hydrogen, 4mL/min constant flow
Oven temp.: 40°C (hold 2 min.) to 250°C @25°C/min. to 265°C @5°C/min. to 300°C (hold 4 min.) @25°C/min.
Det.: FID @350°C

Reducing Discrimination

Reduced response of the higher molecular weight PAHs is caused by discrimination in the injection port. In extreme cases the response of the last three PAH compounds may be lost completely. Discrimination will vary by injection port design. Chromatograms generated using a Perkin Elmer Autosys GC system exhibit minimal discrimination (Figures 1 and 2). The area ratio of naphthalene (peak 1) is ~2:1 compared to benzo(ghi)perylene (peak 16).

Several modifications can be made to reduce discrimination: increasing injection port temperature to improve vaporization; increasing the splitless hold time; and using a drilled Uniliner® inlet sleeve. The design of the drilled Uniliner® creates a seal between the liner and the column, which reduces the loss of high molecular weight compounds and thereby improves their response. The drilled Uniliner® has a small hole drilled at the top of the liner that allows it to work with small-diameter columns and electronic pressure control (EPC) injection systems.

Conclusion

PAH analysis by US EPA Method 8100 can be improved by choosing the appropriate analytical columns and by optimizing the temperature program, carrier gas type, and column flow rates. When operating under the conditions listed for Figures 1 and 2, the Rtx®-5Sil MS and the Rtx®-CLPesticides2 columns yield excellent resolution and short analysis times for PAHs.

Rtx®-5Sil MS Columns (-60 to 330/350°C)

30m	0.25mm ID	0.25µm	cat.# 12723
30m	0.32mm ID	0.25µm	cat.# 12724

Rtx®-CLPesticides2 Columns (-60 to 310/330°C)

30m	0.25mm ID	0.20µm	cat.# 11323
30m	0.32mm ID	0.25µm	cat.# 11324

Method 610–Polynuclear Aromatic Hydrocarbons Mix

acenaphthene
acenaphthylene
anthracene
benzo(a)anthracene
benzo(a)pyrene
benzo(b)fluoranthene
benzo(k)fluoranthene
benzo(ghi)perylene
chrysene
dibenzo(a,h)anthracene
fluoranthene
fluorene
indeno(1,2,3-cd)pyrene
naphthalene
phenanthrene
pyrene

2,000µg/mL each in CH_2Cl_2, 1mL/ampul

	Each	5-pk.	10-pk.
	31011	31011-510	
w/data pack	31011-500	31011-520	31111

Inlet Liners

For Agilent GCs

Drilled Uniliner® *(4.0mm ID, 6.3mm OD, 78.5mm length)*

21054 (ea.)	21055 (5-pk.)

Siltek™ Drilled Uniliner® *(1.0mm ID, 6.3mm OD, 78.5mm length)*

21390-214.1 (ea.)	21391-214.5 (5-pk.)

For PE GCs

Auto SYS Splitless w/Wool *(2.0mm ID, 6.2mm OD, 92.1mm length)*

20829 (ea.)	20830 (5-pk.)	20831 (25-pk.)

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RESTEK food & flavor

Applications note

Analyzing the Heat Level of Spicy Foods Using an Ultra C18 HPLC column

Introduction

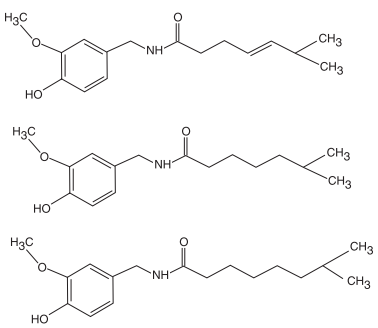
Spicy foods and sauces can be used to add zest to bland foods and to build more complex flavors in a variety of food types. They provide a nutritious way to add flavor to "healthy foods," such as low-salt, low-fat alternatives. Chili pepper extracts also are being explored for their medicinal uses, including rubdown liniments, arthritis pain lotions, and counter-irritant balms. As society's taste in spicy foods grows, so does the need to test and classify products and raw materials for their pungency (i.e., heat levels). Pungency is defined as the hot, sharp, stinging sensations experienced by the nasal and oral cavities and the tongue when certain flavor compounds contact them.[1] The trigeminal nerves in these areas are stimulated, tricking the body into thinking it is in pain. Then the body releases endorphins, which give a pleasant sensation.

In chili peppers, black pepper, and ginger, the pungent species are not volatile. Flavorings such as mustard, garlic, and horseradish, on the other hand, contain volatile compounds that contribute aroma as well as pungency. Pungency can differ both in intensity and duration, depending on the chemical species present.

There are over 200 chili pepper varieties, with a wide range of pungency levels and flavor profiles.[2] Chili peppers belong to the genus Capsicum, which contains five domesticated species. In chili peppers, pungency is a result of capsaicinoids, or vanillylamides of monocarboxylic acids. The capsaicinoid content, and thus the heat level, depends on the type of pepper, maturity, growing conditions, and processing methods. Most of the capsaicinoids can be found in the inner layer of the plant, including the placenta where the seeds attach. Only small amounts of capsaicinoids are found in the seeds themselves. There are seven generally recognized capsaicinoids in chili peppers, all of which evoke different responses in the consumer: The most famous and the hottest is capsaicin (CAP). In chili peppers, CAP and dihydrocapsaicin (DHC) are present in the greatest amounts and are the largest contributors to the heat level (Figure 1). The minor capsaicinoids include nordihydrocapsaicin (NDC), homocapsaicin, norcapsaicin, nornorcapsaicin, and homodihydrocapsaicin.

Figure 1

CAP and DHC are considered major contributors to the heat level. NDC is a minor capsaicinoid, which also contributes to the heat level.



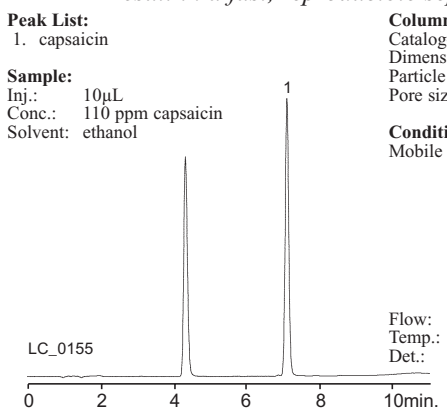
capsaicin (CAP)

dihydrocapsaicin (DHC)

nordihydrocapsaicin (NDC)

Figure 2

The Ultra C18 column and a gradient elution program result in a fast, reproducible separation.



Column: Ultra C18
Catalog#: 9174565
Dimensions: 150x4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: 1% acetic acid in water
B: acetonitrile

Time (min.)	%B
0	50
2	50
9	80
12	80
13	50

Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 280nm

Historical Perspectives

In 1912, Wilbur Scoville developed a method for determining heat levels. In this organoleptic (i.e., affecting the qualities of substances that stimulate the sense organs) procedure, a Scoville Heat Unit (SHU) is defined as the number of parts sugar water needed to neutralize the heat of one part sample extract. For example, if the heat of a cayenne pepper is 30,000 SHU, that means 30,000 parts of sugar water are needed to dilute one part of cayenne pepper extract to the last point that hotness can be detected. However, there are several problems with the organoleptic procedure for determining heat levels. The test is somewhat subjective because it relies on the tasters' palates and sensitivity. The geographic area or culture from which the taster originates can affect the results—different groups of people have different physical perceptions of heat. In addition, tasters can handle only a limited number of samples at one time, before "fatiguing of the palate" occurs.[3] This can make it difficult to process a large number of samples in a reasonable amount of time, such as in a quality control (QC) environment, and can affect the reproducibility of the tests.

Analytical Methods

Starting in the 1970s, several analytical methods for heat level measurement were introduced to overcome the limitations of the organoleptic procedure.[4] These included wet chemical methods; spectrometry; and paper, gas (GC), and liquid chromatography (HPLC). Of these, the HPLC procedures have provided the greatest specificity while requiring the least amount of sample preparation. The American Spice Trade Association (ASTA) and the Association of Official Analytical Chemists (AOAC) have published methods for the determination of capsaicinoids by HPLC. AOAC Method 995.03[5] specifies the separation of three target capsaicinoids using reversed phase HPLC on a C18 column, and quantitation using either UV or fluorescence detection. This method is performed isocratically with a 1% acetic acid:acetonitrile (60:40) mobile phase. Standardization is performed using synthetic capsaicin, N-vanillyl-*n*-nonanamide; and the relative amounts of CAP, DHC, and NDC are calculated by applying the specified factors. Using the appropriate calculations, the heat index can then be calculated, where 1ppm of total capsaicinoids is approximately equal to 15 SHU.

food & flavor

The selectivity and lot-to-lot reproducibility of the Restek Ultra C18 column make it an excellent choice for performing heat level measurements by HPLC. The analysis of capsaicinoids using an Ultra C18 column and a gradient elution program results in an efficient separation that can be performed in less than 8 minutes (Figure 2). In addition, the Ultra C18 column achieves good resolution of the three target capsaicinoids in an extract of habanero nuggets (Figure 3). The high percentage of organic at the end of the run helps to elute any strongly absorbing species present in the samples and improves analysis reproducibility.

Several brands of hot sauce were analyzed to determine their heat levels using this HPLC procedure. The samples were extracted in ethanol, filtered, and injected onto the analytical column. After determining the amounts (in ppm) of the three target capsaicinoids, the SHUs were calculated as described in AOAC Method 995.03 (Table 1). Dried habanero nuggets also were analyzed, as seen in Figure 3. This extract had a sizzling hotness rating of 348,000 SHU.

Figure 3

The Ultra C18 achieves good resolution of three target capsaicinoids in less than 9 minutes.

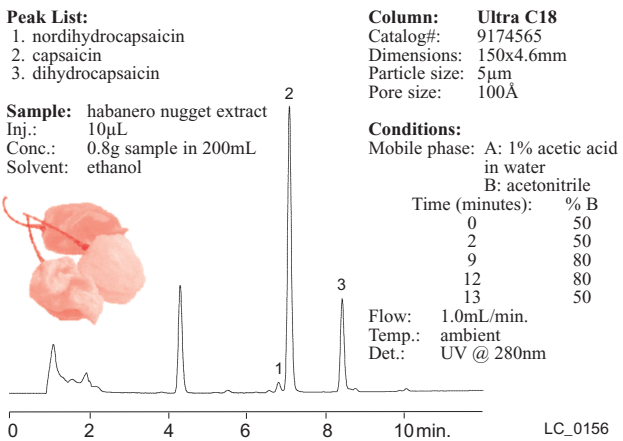


Table I

Comparison of five commercially available hot sauces.

Sample	SHU	Hotness Ranking
sauce #1	8,530	Super Hot
sauce #2	380	Mild
sauce #3	400	Mild
sauce #4	1,100	Hot
sauce #5	1,870	Hot
habanero nuggets	348,000	Off the chart

Conclusion

With the rapidly increasing popularity of Capsicum-based products, there is a need to quantitatively evaluate the heat content of both raw materials and finished products. The HPLC analysis described above gives an objective measurement of the heat level of a wide range of samples. By using a Restek Ultra C18 column and a gradient elution program, the analysis can be performed quickly and reproducibly with only minimal sample preparation.

References

1. Fennema, O. R. Food Chemistry (1996), 3rd edition, pp. 736-738.
2. Uhl, S. "Fire and Spice" in Food Product Design (1996), editorial archives.
3. Bensinger, M. "How Hot is that 'Devil' Sauce?" in Fiery Foods Magazine (1997), Sept/Oct.
4. Chiang, G. H. J. Food Science (1986), 51(2), pp. 499-503.
5. AOAC Official Methods of Analysis (2000), Method 995.03.

Contact Restek for other column dimensions available 800-356-1688

Ultra C18, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9174531	9174532	9174533	9174535
50mm	9174551	9174552	9174553	9174555
100mm	9174511	9174512	9174513	9174515
150mm	9174561	9174562	9174563	9174565
200mm	9174521	9174522	9174523	9174525
250mm	9174571	9174572	9174573	9174575

Ultra C18, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9174532-700	9174533-700	9174535-700
50mm	—	9174552-700	9174553-700	9174555-700
100mm	—	9174512-700	9174513-700	9174515-700
150mm	—	9174562-700	9174563-700	9174565-700
200mm	—	9174522-700	9174523-700	9174525-700
250mm	—	9174572-700	9174573-700	9174575-700

RESTEK HPLC

Applications note

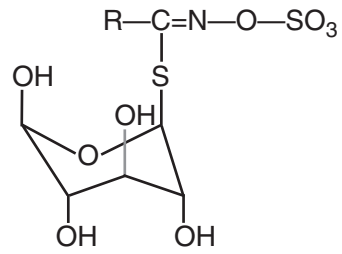
HPLC Analysis of Glucosinolates in Vegetable Extracts without Ion Pairing Using an Ultra Aqueous C18 Column.

Glucosinolates are a naturally occurring set of compounds found in a variety of edible plants, most notably in broccoli, radish, and cabbage. Agriculturally, the degradation compounds of glucosin-olates have been shown to act as natural pesticides and fungicides (this breakdown occurs in the soil). These toxic compounds then further degrade into harmless compounds. Research on glucosinolates is continuing in hopes of bringing a more environmentally friendly approach to pest control.

Nutritionally, human consumption of these compounds is associated with a significantly reduced risk for a variety of malignant cancers along the alimentary canal. They also have been shown to suppress existing tumor growth. Glucosinolates are precursors to isothiocyanates, such as sulforaphane (4-methylsulfinylbutyl isothiocyanate), which regulates mammalian enzymes of xenobiotic metabolism.

Phenethyl glucosinolate (gluconasturtiin) is one of the glucosinolates widely found in cruciferous vegetables. It is one of the least polar glucosinolates, making it relatively easy to retain by reverse phase high performance liquid chromatography (HPLC). However, there are a number of glucosinolates with hydrophilic "R-" groups, such as 3-methylsulfinylpropyl glucosinolate, that are very difficult to retain by conventional reverse phase HPLC. Additionally, the "R-" group of glucosinolates can vary greatly, resulting in a large number of glucosinolates with widely differing polarities (**Figure 1**). Thus many analysts resort to reverse phase ion-pairing methods to analyze glucosinolates. The addition of ion-pairing reagents is less convenient, and makes the analyses inherently less reproducible. Ion-pairing reagents also make gradient elution very impractical, due to long equilibration times.

Figure 1



Glucosinolates:
ß-thioglucoside
N-hydroxysulfates

The analysis of a phenethyl glucosinolate standard using an Ultra Aqueous C18 column shows good peak shape without the use of ion-pairing reagents (**Figure 2**). Extracts of cabbage and watercress were analyzed using the same conditions (**Figures 3 and 4).** Gradient elution from 0 to 75% acetonitrile was used to retain and elute analytes having a wide range of polarities. The Ultra Aqueous C18 column allows the use of simple reverse phase conditions for the analyses of glucosinolates, saving time as compared to reverse phase ion-pairing methods.

Figure 2: Phenethyl Glucosinolate on Ultra Aqueous C18

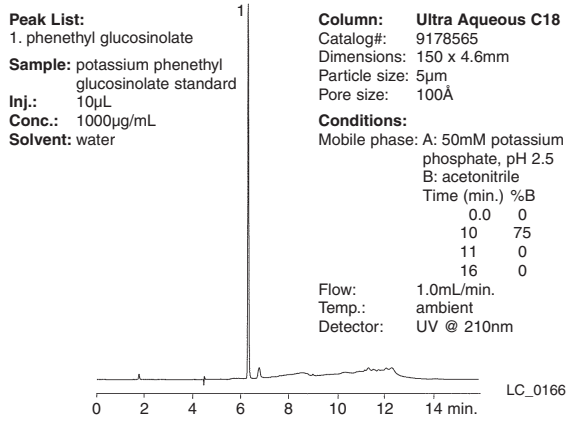


Figure 3: Cabbage Extract on Ultra Aqueous C18

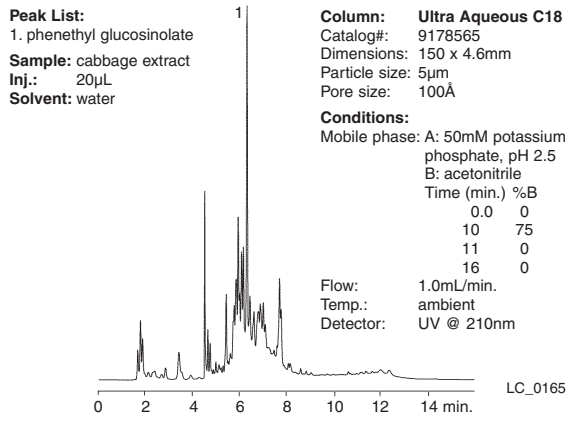
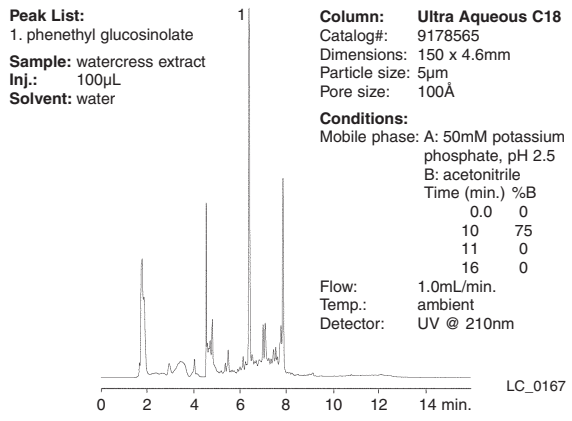


Figure 4: Watercress Extract on Ultra Aqueous C18



Ultra Aqueous C18, 3µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9178331	9178332	9178333	9178335
50mm	9178351	9178352	9178353	9178355
100mm	9178311	9178312	9178313	9178315

Ultra Aqueous C18, 3µm Columns with Trident™ Inlet Fitting

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9178332-700	9178333-700	9178335-700
50mm	—	9178352-700	9178353-700	9178355-700
100mm	—	9178312-700	9178313-700	9178315-700

Ultra Aqueous C18, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9178531	9178532	9178533	9178535
50mm	9178551	9178552	9178553	9178555
100mm	9178511	9178512	9178513	9178515
150mm	9178561	9178562	9178563	9178565
200mm	9178521	9178522	9178523	9178525
250mm	9178571	9178572	9178573	9178575

Ultra Aqueous C18, 5µm Columns with Trident™ Inlet Fitting

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9178532-700	9178533-700	9178535-700
50mm	—	9178552-700	9178553-700	9178555-700
100mm	—	9178512-700	9178513-700	9178515-700
150mm	—	9178562-700	9178563-700	9178565-700
200mm	—	9178522-700	9178523-700	9178525-700
250mm	—	9178572-700	9178573-700	9178575-700

Ultra Aqueous C18, Guard Cartridges

Dimensions	cat.#	Qty.
10 x 2.1mm	917850212	3
10 x 4.0mm	917850210	3
20 x 4.0mm	917850220	2

Acknowledgement: *The phenyl glucosinolate standard and extracts of cabbage and watercress were generously provided by Dr. Gerard Engelen-Eigles, University of Minnesota, Horticulture Department.*

RESTEK

clinical/forensic

Applications note

GC Analysis of Phenylpropanolamine in Cold Medications Using an Rtx®-5Amine Column

Until recently, phenylpropanolamine was an ingredient in prescription and over-the-counter medications. It primarily was used as a nasal decongestant, but also was used in over-the-counter appetite suppression preparations for weight control. However, a study by researchers at the Yale University School of Medicine[1] reported that phenylpropanolamine could increase the risk of hemorrhagic stroke in women. In November 2000, the Food and Drug Administration (FDA) issued a public health advisory requesting that all manufacturers of products containing phenylpropanolamine voluntarily discontinue manufacturing and marketing them. Although the risk of stroke is very low, the FDA determined that the serious medical effects associated with stroke outweighed the benefits derived from phenylpropanolamine. In addition, its use is not so important because a number of other medications can be substituted for phenylpropanolamine, especially for treating colds and sinus congestion.

Cold and sinus medication can be analyzed for the presence of phenylpropanolamine using a simple extraction procedure followed by analysis using capillary gas chromatography (GC). We surveyed the most commonly available cold medications, and chose two for testing. Brand A contained phenylpropanolamine at a concentration of 1.25mg/mL and guaifenesin at a concentration of 10mg/mL. Brand B contained only guaifenesin at a concentration of 20mg/mL. Each medication was supplied as a syrup. Extraction was performed by adding 100uL of syrup to a screw-cap culture tube and making it basic by adding 1.4mL of 2.5% ammonium hydroxide. The sample was mixed thoroughly before adding 3mL of extraction solvent (methylene chloride:isopropanol, 90:10). Each sample was mixed by gently rocking the tube for 5 minutes. The layers were allowed to separate and the top aqueous layer was aspirated to waste. The bottom organic layer was transferred to a clean glass culture tube and evaporated to dryness under a stream of dry nitrogen at room temperature. The extracts were reconstituted for analysis with 100µL of methanol.

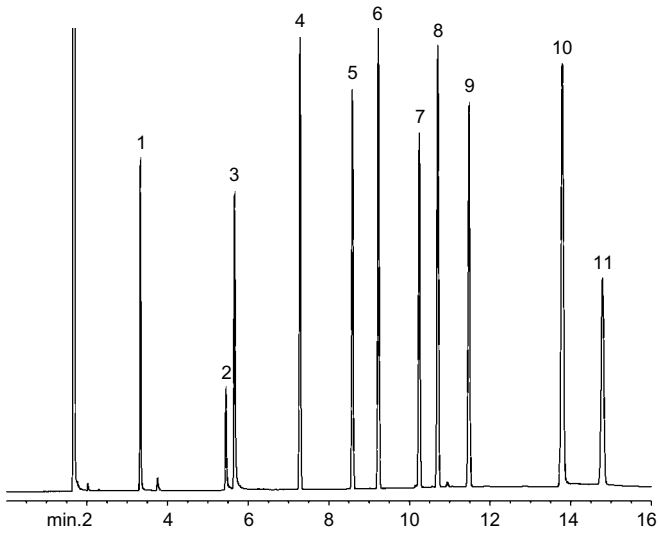
Most of the compounds in the target list, including phenylpropanolamine, are basic compounds that have a pKa greater than 8.0. After passing through the extraction protocol described above, these compounds are in the free base form in the reconstituted extract. Free bases can exhibit tailing peak shapes and reduced response on columns that are poorly deactivated or not designed specifically for use with basic compounds. Because of its superior performance analyzing free bases, an Rtx®-5 Amine column was chosen for this separation. A wide bore, thick-film column was needed for increased sample capacity because most of the cold medications had analyte concentrations well above 1mg/mL.

The Rtx®-5 Amine column provides excellent resolution of all the compounds commonly found in most cold medications (see **Figure 1**). Phenylpropanolamine (peak 1) is separated easily from the rest of the compounds. All of the target analytes exhibit good peak shape, even when in the free base form. Additionally, the analysis is complete in less than 15 minutes, which allows for quick turn-around of multiple samples.

Figure 1: The Rtx®-5Amine column provides excellent resolution of compounds in cold medications.

Rtx®-5Amine Column
30m, 0.53mm ID, 1.00µm df
(cat.# 12355)

Oven temp.:	175°C to 280°C @ 10°/min. (hold 5.5 min.)
Inj./det. temp.:	250°C/280°C
Carrier gas:	helium
Linear velocity:	40cm/second
Sample size:	1uL
Split vent flow:	88mL/min.
Split ratio:	20:1



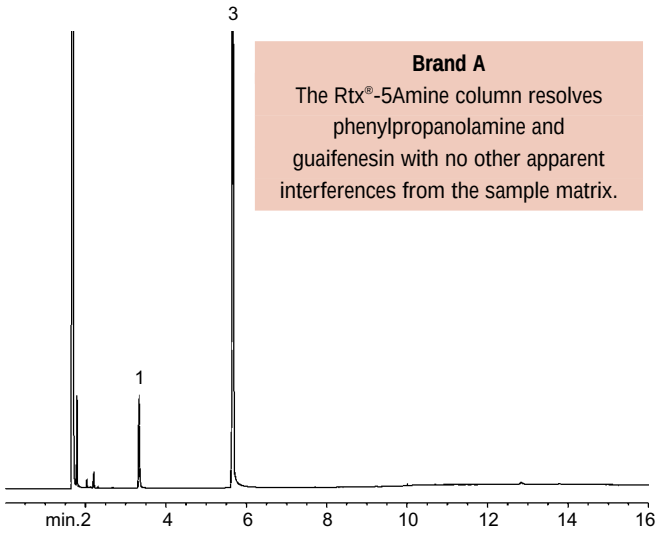
Peak List:
1. phenylpropanolamine
2. phenylephrine
3. guaifenesin
4. pheniramine
5. phenyltoloxamine
6. chlorpheniramine
7. brompheniramine
8. dextromethorphan
9. pyrilamine
10. codeine
11. hydrocodone

clinical/forensic

Figure 2 shows the analysis of Brand A and Brand B medications, respectively, using an Rtx®-5Amine column. Phenylpropanolamine and guaifenesin are well resolved from each other with no apparent interferences from the sample matrix. Based on the response of phenylpropanolamine in Brand A, concentrations as low as 10µg/mL can be detected by adjusting the sample size in the extraction procedure.

Cold and sinus medications can be checked quickly and easily for the presence of phenylpropanolamine using the procedure described above. Complete resolution of all analytes, and optimized peak shape and response can be achieved by selecting the appropriate column phase and dimensions.

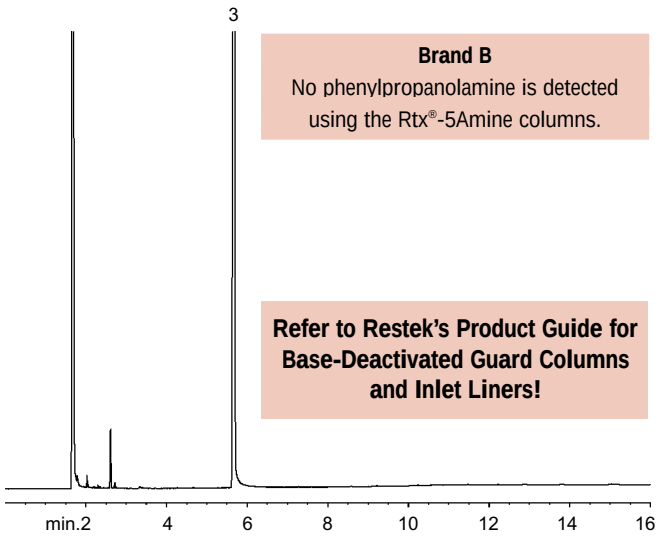
Figure 2: The analysis of Brand A and Brand B medications using an Rtx®-5Amine column.



Brand A
The Rtx®-5Amine column resolves phenylpropanolamine and guaifenesin with no other apparent interferences from the sample matrix.

Brand A and Brand B:
Rtx®-5Amine Column
30m, 0.53mm ID, 1.00µm df (cat.# 12355)

Oven temp.:	175°C to 280°C @ 10°/min. (hold 5.5 min.)	**Linear velocity:**	40cm/second
		Sample size:	1uL
Inj./det. temp.:	250°C/280°C	**Split vent flow:**	88mL/min.
Carrier gas:	helium	**Split ratio:**	20:1



Brand B
No phenylpropanolamine is detected using the Rtx®-5Amine columns.

Refer to Restek's Product Guide for Base-Deactivated Guard Columns and Inlet Liners!

Brand A and Brand B:
Peak List:
1. phenylpropanolamine
2. phenylephrine
3. guaifenesin
4. pheniramine
5. phenyltoloxamine
6. chlorpheniramine
7. brompheniramine
8. dextromethorphan
9. pyrilamine
10. codeine
11. hydrocodone

Rtx-5Amine (Fused Silica) (Crossbond® 5% diphenyl/95% dimethylpolysiloxane) Stable to 340°C

ID:	df (µm)	temp. limits	15-meter	30-meter
0.25mm	0.50	-60 to 300/315°C	12335	12338
	1.00	-60 to 300/315°C	12350	12353
0.32mm	1.00	-60 to 300/315°C	12351	12354
	1.50	-60 to290/305°C	12366	12369
0.53mm	1.00	-60 to 290/305°C	12352	12355
	3.00	-60 to 280/295°C	12382	12385

1. R. Horwitz, MD, L. Brass, MD, W. Kernan, MD, C. Viscoli, PhD, "Phenylpropanolamine and Risk of Hemorrhagic Stroke: Final Report of The Hemorrhagic Stroke Project", May 10, 2000.

RESTEK environmental

Applicationsnote

Fast Analysis of Dioxin and Related Compounds Using an Rtx®-5MS Column

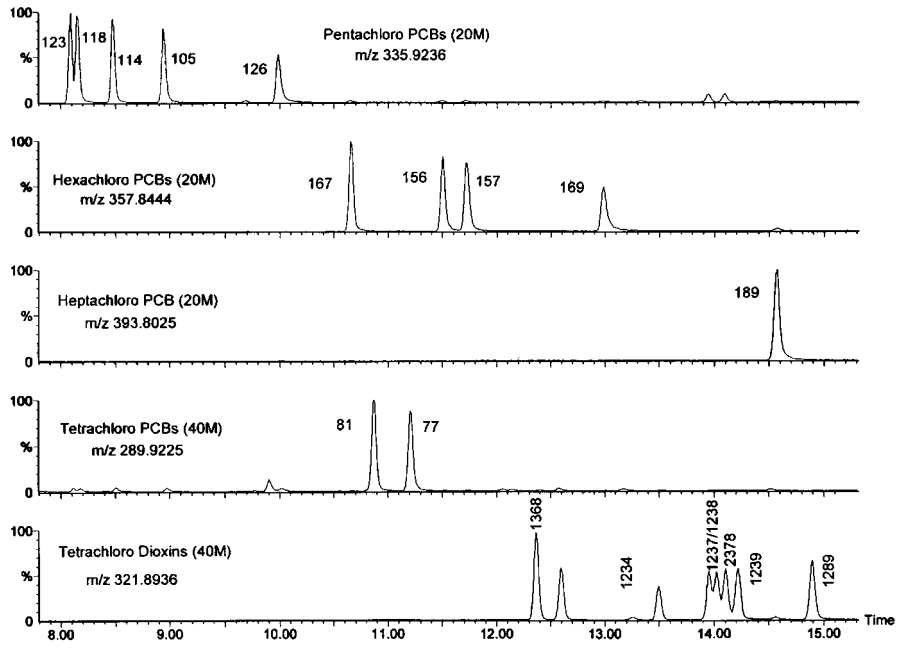
Dioxin and furan testing can be very time consuming and costly. Total analyses times can easily exceed one hour per sample, and instrument time on high-resolution mass spectrometers (MS) is quite valuable. In addition, many samples analyzed for dioxins and furans require analysis for polychlorinated biphenyls (PCBs) as well. Researchers at the Ontario Ministry of the Environment (MOE) and Restek have recently developed a method for more rapid dioxin, furan, and PCB analysis.

Historically, chlorinated dioxins and furans have been analyzed by gas chromatography (GC) separately from PCBs. In 1998, the World Health Organization (WHO) reported toxic equivalent factors (TEFs) for the 12 dioxin-like PCB congeners.[1] This enabled concentrations of PCBs to be expressed in terms of 2,3,7,8-TCDD, the most toxic form of dioxin. Using similar methods to analyze dioxins and PCBs allows detection limits up to three orders of magnitude lower than that of conventional PCB congener methods. The toxicity of a single sample now can be reported in toxic equivalents of 2,3,7,8,-TCDD (i.e., toxic equivalent quantities [TEQ]) by summing the toxic equivalents of each of the 17 toxic dioxin congeners and 12 dioxin-like PCB congeners.

Extracts were prepared according to Canada's MOE Method 3418, which is similar to the combination of US Environmental Protection Agency (EPA) Methods 1613 and 1668. The extracts are further cleaned using activated carbon.[2] This allows for the collection of two sample extract fractions: one containing the dioxins, furans, and coplanar PCBs; and the other containing the remaining PCBs, chlorinated and brominated diphenyl ethers, and other non-planar organic compounds. The chlorinated diphenyl ethers interfere with the furans and, therefore, they need to be analyzed separately. Normally, dioxins and furans, and PCBs (congeners) are analyzed separately on a 60m analytical column using GC/high resolution mass spectrometry (GC/HRMS) with analysis times of 50 to 90 minutes each.

Figure 1

The Rtx®-5MS column and a combined system can separate dioxin-like PCBs and dioxins/furans within 30 minutes.



GC_EV00392

Inj. temp.: 280°C; **Carrier gas:** helium; **Det.:** Agilent 6890 GC coupled to a Micromass Ultima HRMS @10,000RP.

20m, 0.1mm ID, 0.1µm Rtx®-5MS (custom cat.# 58136). **Column head pressure:** 100psi; **Oven program:** 100°C (hold 1 min.) to 200°C @ 100°C/min, to 235°C @ 13°C/min., to 300°C @ 27°C/min. (hold 4 min.); **Inj. volume:** 0.2mL

40m, 0.18mm ID, 0.18µm Rtx®-5MS (custom cat.# 550590). **Column head pressure:** 61psi; **Oven program:** 100°C (hold 0.62 min.) to 200°C @ 64.5°C/min, to 235°C @ 4.8°C/min. (hold 6.2 min.), to 300°C @ 9.7°C/min.(hold 5.6 min.); **Inj. volume:** 1.0mL

environmental

Table I

Mass windows used for parallel column system.

Mass Group	M/Z	Species	Fraction	Column (m)
1	289.9225,291.9195*	Cl_4CB	PCDD/F/COP	40
	301.9626,303.9598*	$13C_{12}$- Cl_4CB	PCDD/F/COP	40
	323.8834,325.8805*	Cl_5CB	MONO-ORTHO	20
	330.9792,330.9792	PFK Lock Mass, Lockmass Check		
	335.9236,337.9207*	$13C_{12}$- Cl_5CB	MONO-ORTHO	20
	357.8444,359.8415*	Cl_6CB	MONO-ORTHO	20
	371.8817*,373.8788	$13C_{12}$- Cl_6CB	MONO-ORTHO	20
	393.8025*,395.7996	Cl_7CB	MONO-ORTHO	20
2	303.9016,305.8987*	Cl_4CDF	PCDD/F/COP	40
	315.9419,317.9389*	$13C_{12}$- Cl_4CDF	PCDD/F/COP	40
	318.9792,318.9792	PFK Lock Mass, Lockmass Check		
	319.8965,321.8936*	Cl_4CDD	PCDD/F/COP	40
	327.8847	$^{37}Cl_4CDD$	PCDD/F/COP	40
	331.9368,333.9339*	$13C_{12}$- Cl_4CDD	PCDD/F/COP	40
	323.8834,325.8805*	Cl_5CB	PCDD/F/COP	40
	335.9236,337.9207*	$13C_{12}$- Cl_5CB	PCDD/F/COP	40
	357.8444*,359.8415	Cl_6CB	PCDD/F/COP	40
	371.8817*,373.8788	$13C_{12}$- Cl_6CB	PCDD/F/COP	40
	393.8025*,395.7996	Cl_7CB	MONO-ORTHO	20
	375.8364	Cl_6DPE	MONO-ORTHO	20
	405.8428*,407.8398	$13C_{12}$- Cl_7CB	MONO-ORTHO	20
3	339.8597*,341.8567	Cl_5CDF	PCDD/F/COP	40
	351.9000*,353.8970	$13C_{12}$- Cl_5CDF	PCDD/F/COP	40
	366.9792,366.9792	PFK Lock Mass, Lockmass Check		
	353.8576,355.8546*,357.8517	Cl_5CDD	PCDD/F/COP	40
	357.8444,359.8415*,361.8385	Cl_6CB	PCDD/F/COP	40
	367.8949*,369.8919	$13C_{12}$- Cl_5CDD	PCDD/F/COP	40
	371.881*,373.8788	$13C_{12}$- Cl_6CB	PCDD/F/COP	40
	405.8428*,407.8398	$13C_{12}$- Cl_7CB	PCDD/F/COP	40
	409.7974	Cl_7DPE	PCDD/F/COP	40
	427.7635	Cl_8CB	PCDD/F/COP	40
4	373.8208*,375.8178	Cl_6CDF	PCDD/F/COP	40
	383.8639,385.8610*	$13C_{12}$- Cl_6CDF	PCDD/F/COP	40
	389.8157*,391.8127	Cl_6CDD	PCDD/F/COP	40
	380.976,380.976	PFK Lock Mass, Lockmass Check		
	401.8559*,403.8829	$13C_{12}$- Cl_6CDD	PCDD/F/COP	40
	445.7555	Cl_8DPE	PCDD/F/COP	40
5	407.7818*,409.7789	Cl_7CDF	PCDD/F/COP	40
	417.8250,419.8220*	$13C_{12}$- Cl_7CDF	PCDD/F/COP	40
	423.7766*,425.7737	Cl_7CDD	PCDD/F/COP	40
	435.8169*,437.8140	$13C_{12}$- Cl_7CDD	PCDD/F/COP	40
	430.9728,430.9728	PFK Lock Mass, Lockmass Check		
	479.7165	Cl_9DPE	PCDD/F/COP	40
6	441.7428,443.7400*	Cl_8CDF	PCDD/F/COP	40
	457.7377,459.7348*	Cl_8CDD	PCDD/F/COP	40
	469.7779,471.7750*	$13C_{12}$-O_8CDD	PCDD/F/COP	40
	454.9728,454.9728	PFK Lock Mass, Lockmass Check		

* - ion occurs at 100% intensity in molecular ion cluster. All ions (m/z) monitored for detection of native species had a dwell time of 50ms. Detection of corresponding $13C_{12}$-labelled specie ions had dwell times of 25ms. Delay times were set at 10ms.

Because an MS is used for detection, many analysts want a column with the lowest bleed possible. Some laboratories may use silarylene columns (e.g., Rtx®-5Sil MS or DB-5MS® columns) due to their low bleed feature. However, these columns yield a coelution between 2,3,7,8-TCDD and 1,2,3,9-TCDD; and their elution orders and retention times will differ from the phase for which the window performance mixtures were designed. The Rtx®-5MS (5% diphenyl/95% dimethyl polysiloxane) column is better suited to meet the performance standards for this analysis. It separates all of the important compounds within 30 minutes, and each one is individually tested to provide low bleed levels for MS detection.

Chromatographic resolution and analysis time also are dependent on column dimensions (i.e., length, ID, phase thickness). Experimentally, we have found 175,000 plates are required to obtain separation of 2,3,7,8-TCDD from its nearest neighbors (1,2,3,7- and 1,2,3,8-TCDD the unresolved pair eluting before; and 1,2,3,9-TCDD the compound eluting after).[3] A 40m, 0.18mm ID, 0.18 m Rtx®-5MS column meets this criteria, and can complete the analysis in approximately half as much time as a 60m column. A 20m, 0.10mm ID, 0.10 m Rtx®-5MS column is capable of meeting these requirements in about one-quarter the time of a 60m column; however, there is little tubing length available for trimming to maintain column performance. Therefore, we suggest using a 40m column.

To minimize the number of ions that must be monitored simultaneously, elute the bulk of PCB compounds prior to eluting dioxin and furan compounds. Accomplish this by injecting the non-coplanar PCB fraction into a 20m Rtx®-5MS column that is set up parallel (i.e., two separate injectors) to a 40m Rtx®-5MS column, which is used for the separation of the dioxin/furan/coplanar PCB fraction. Both fractions are injected simultaneously. The columns are installed into the MS ion source in parallel. The resulting analysis time is less than that for a single fraction on a conventional 60m column (Figure 1). Table I summarizes the high resolution MS conditions and which masses are monitored for which compound.

For the analysis of dioxin-like PCBs and dioxins/furans, method consolidation and throughput increase is possible when using a parallel, dual-column system with GC/HRMS. This method allows the combination of several different analytical methods to a single system, and results in a total analysis time of less than 30 minutes for elution of octachlorodibenzodioxin.

References

1. Berg, M. V., L. Birnbaum, A.T.C. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X.R. Leeuwen, A.K.D. Liem, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern, and T. Zacharewski, Environmental Health Perspectives, 106 (1998), p. 775.
2. Kolic T.M., K. A. MacPherson, E.J. Reiner, T. Gobran, and A. Hayton, Organohalogen Compounds, 46 (2000), p. 562.
3. Reiner E.J., K.A. MacPherson, R. Brunato, T. Chen, M.A. Bogard, A.R. Boden, and G. Ladwig, Organohalogen Compounds, 45 (2000), p. 17.

Product Listing

Rtx®-5MS Columns				
ID	**df (m)**	**temp. limits**	**20-Meter**	**40-Meter**
0.10mm	0.10	-60 to 330/350 C	58136	
0.18mm	0.18	-60 to 330/350 C		550590

*Karen MacPherson and Dr. Eric Reiner, Ontario Ministry of the Environment..

RESTEK environmental

Applications note

Resolving Oxygenates from Gasoline Additives Using an Rtx®-VGC GC Column

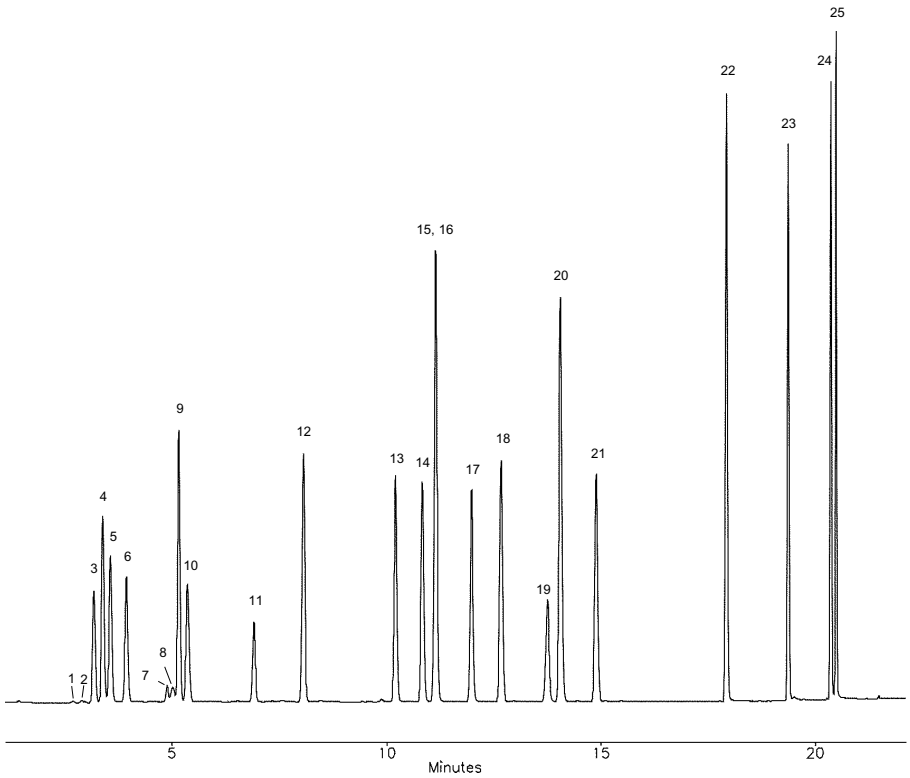
With over one million underground fuel tanks in the United States, contamination of ground and surface waters by gasoline in leaking tanks has been an environmental problem for years. Recent events have focused attention on a new class of compounds associated with gasoline leaks. These compounds, known as oxygenates—the most common of which is methyl-*tert*-butyl ether (MTBE), are added to gasoline to reduce overall emissions. Because oxygenates are polar compounds and are soluble in water, they move through aquifers easily. This poses a risk to drinking water supplies.

A variety of methods have been used by environmental laboratories to report oxygenates in gasoline, such as EPA Methods 8015, 8260, 8240, and 8020. Some of these methods recommend flame ionization (FID) or photoionization (PID) detection, while others recommend gas chromatography/mass spectrometry (GC/MS). Environmental samples contaminated with gasoline can contain both hydrocarbons and oxygenates, and to identify oxygenates it is necessary to chromatographically separate them from the hydrocarbons.

The success of these methods is based on the ability of the analytical column to resolve oxygenates from the early-eluting alkanes, alkenes, and, to a lesser extent, the alkynes. To minimize false positive results for MTBE, it is important to separate it from 2-methylpentane and 3-methylpentane. Nonpolar phases (e.g., Rtx®-1 and DB-MTBE columns) have been recommended for separating these compounds. However, these phases are incompatible with polar compounds, which can result in broader peaks and lower capacity for the alcohols. Another potential interference for MTBE is *tert*-butyl alcohol (TBA). The EPA recommends adding TBA to the target list for contaminated sites known to contain MTBE because it is a breakdown product of MTBE and an additive in gasoline. Both of these compounds respond on the photoionization detector (PID) and they share ions using mass spectrometry (MS) detection, so MTBE and TBA must be resolved regardless of which detector is used.

The Restek Rtx®-VGC column is coated with a medium polarity phase, which makes it ideal for the analysis of both hydrocarbons and oxygenates. The unique polarity of this column improves the separation of oxygenates, which results in more accurate detection when using PID or FID. A 30m, 0.45mm ID, 2.55µm Rtx®-VGC column helps determine low concentrations of oxygenates in the presence of aliphatic compounds, resolving MTBE from 2-methylpentane, 3-methyl pentane, and TBA (Figure 1, peaks 1 to 4). These column dimensions allow for correct desorb flow rates from

Figure 1–Using purge and trap concentration, the Rtx®-VGC column resolves oxygenates and other target gasoline compounds better than other columns specifically designed to resolve MTBE.



1. 2-methylpentane
2. 3-methylpentane
3. methyl-*tert*-buyl ether
4. *tert*-butanol
5. diisopropyl ether
6. ethyl-*tert*-butyl ether
7. isooctane
8. *n*-heptane
9. benzene
10. *tert*-amyl-methyl ether
11. a,a,a-trifluorotoluene
12. toluene
13. 1-chloro-3-fluorobenzene
14. ethylbenzene
15. *m*-xylene
16. *p*-xylene
17. *o*-xylene
18. isopropylbenzene
19. ethyl-methylbenzene
20. 1,3,5-trimethylbenzene
21. 1,2,4-trimethylbenzene
22. 4-bromochlorobenzene
23. naphthalene
24. 2-methylnaphthalene
25. 1-methylnaphthalene

30m, 0.45mm, 2.55µm Rtx®-VGC (cat.# 19408); each component 100ppb in 5mL of RO water, except *tert*-butanol 5000ppb; 2/1-methynapthalene 150ppb; ethyl methyl benzene 50ppb. **GC:** Finnigan 9001; **Oven program:** 40°C (hold 2 min.) to 130°C @ 6°C/min. (hold 0 min.) to 230°C @ 30°C/min. (hold 2 min.). **Carrier gas:** helium @ ~8mL/min.; **Detector**: Finnigan PID, make up 7mL min., purge 7mL/min., set @ 0.35mV, base temperature 200°C.

environmental

the purge and trap, faster analyses times, and better resolution of closely eluting peaks compared to traditional 0.53mm ID columns (Figure 2).

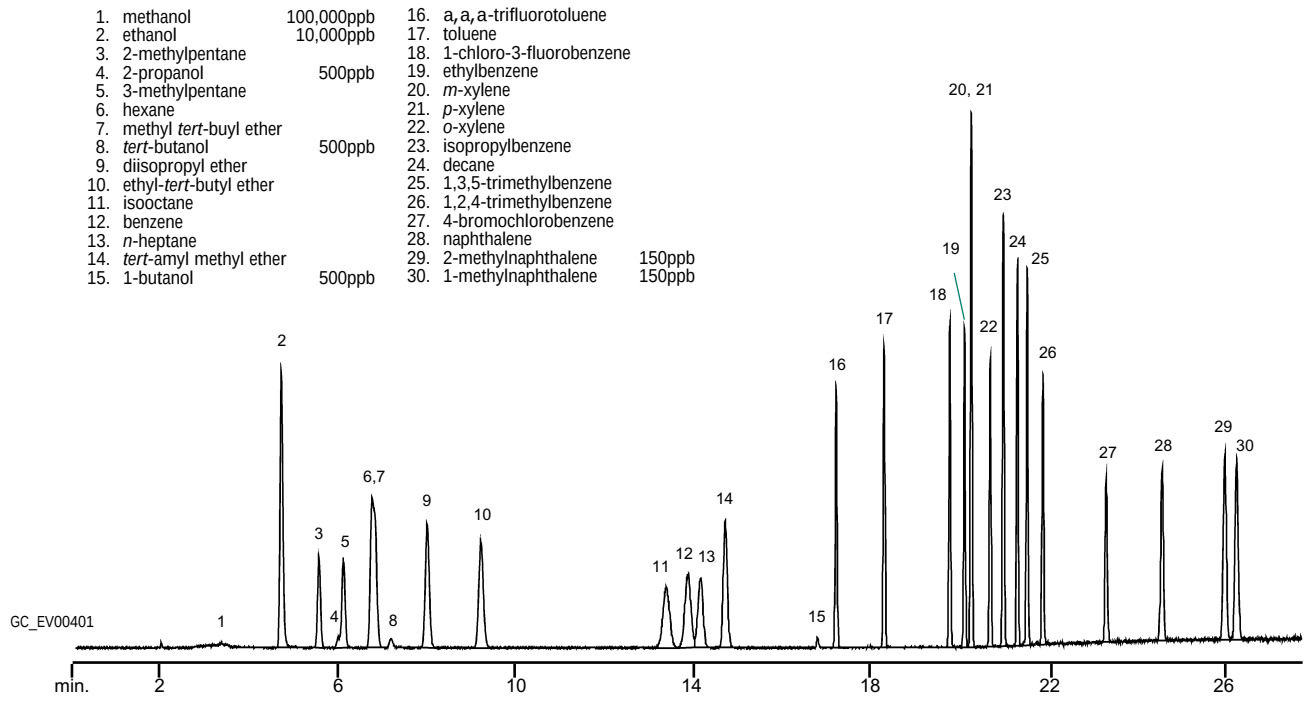
Oxygenates also can be analyzed by GC/MS following the protocol defined in US EPA Method 8260B. GC/MS is a common way to increase the level of confidence in chromatographic data over the GC methods. Using a 30m, 0.25mm ID, 1.4µm Rtx®-VGC column with a quadrapole MS can identify oxygenates and alcohols with a high degree of certainty because the compounds that share ions are well resolved using this column (Figure 2). The MS was used to positively identify oxygenates and pentanes from their spectra (Figure 3). Peak shapes are symmetrical for all these compounds using the Rtx®-VGC column, regardless of detector.

Purge and trap conditions such as purge time and temperature must be optimized to achieve good purging efficiency of oxygenates and hydrocarbons. You can review optimized conditions in the figures shown in this Applications Note. The selection of the trap adsorbent material also is critical to achieving accurate quantitative results. The most demanding compounds to analyze are the alcohols because they purge poorly and can interfere with other target analytes. A 'J' trap (e.g., BTEXTRAP™ trap) can handle a heavy sample load with percent levels of methanol, but has a lowered ability to retain the more polar analytes like ethers and alcohols. So, if the majority of your samples are highly contaminated soils requiring methanol extract and you are analyzing for MTBE, it is best to use a 'J' trap. For cleaner samples where sensitivity is an issue, then you will achieve better results with a 'K' trap (e.g., VOCARB 3000™ trap). Additionally, GC analysis conditions such as temperature program rate and flow rate are critical for achieving good separation of oxygenates from hydrocarbons.

To confirm the ability of the Rtx®-VGC to provide accurate quantitative results, a composite gasoline standard was analyzed using both PID and GC/MS. The GC/MS analysis was used to confirm the identity of the analytes that matched target compound retention times on the PID. Any compound that was found within 0.10 minute of a target compound could be identified as a possible oxygenate. The GC/MS confirmed that only one target compound, diisopropyl ether, gave a false positive retention time match with 2-methyl-1-pentene. Although 2-methyl-1-pentene is found at low concentrations relative to the methylpentanes, it responds well on the PID. Using the composite gasoline standard, no other oxygenates matched within the 0.10 minute retention time window, thereby making positive identification for most of the oxygenates possible using the PID (Figure 4). Because gasoline composition can vary from state to state, the use of a confirmation column or MS detection is strongly recommended because alkenes such as 2-methyl-1-pentene can interfere with positive identification of oxygen-containing compounds.

Chlorobenzene also is a common contaminant in drinking water and is commonly analyzed in addition to gasoline using purge and trap with PID detection. Because the boiling point and retention time of chlorobenzene are similar to ethylbenzene, *m*-xylene, and

Figure 2–Optimized Rtx®-VGC column dimensions (30m x 0.45mm ID) allow for correct desorb flow rates from the purge and trap, faster analyses times, and better resolution of closely eluting peaks compared to traditional 0.53mm ID columns.



30m, 0.25mm, 1.40µm Rtx®-VGC (cat.# 19415); 1:10 split at injection port; 1mm ID liner; Compounds at 100ppb in 5mL of RO water (unless otherwise noted); **Oven program:** 35°C (hold 14 min.) to 220°C @ 24°C/min. (hold 6 min.); **Carrier gas:** He @ ~1mL/min. constant; **Concentrator:** Tekmar LSC-3100 Purge and Trap; **Trap:** Vocarb™ 3000; **Purge:** 11 min. @ 40mL/min. @ ambient temperature; **Dry Purge:** 1 min. @ 40mL/min. (MCS bypassed using Silcosteel® tubing); **Desorb Preheat:** 245°C; **Desorb:** 250°C for 2 min., Flow 10mL/min.; **Bake:** 260°C for 8 min.;**Interface:** transfer line 0.32mm ID Siltek™ fused silica; **Detector:** HP 5973 MS; **Scan range:** 25 to 300 AMU.

p-xylene on many capillary columns, it is difficult to separate from the aromatic compounds found in gasoline. The Rtx®-VGC column resolves chlorobenzene from the other aromatic compounds, allowing quantitation by PID (Figure 5).

The excellent resolution achieved by the Rtx®-VGC column prevents misidentifications common with PID and FID. GC/MS methods provide positive identification of coeluting analytes, but the right column can avoid coelutions of compounds that share quantitation ions. The Rtx®-VGC column is selective, has a programmable temperature limit of 260°C, and exhibits exceptional low bleed at common operating temperatures of 230°C. It is an ideal choice for analyzing gasoline additives in GRO samples.

Figure 3–MS detection of a flash splitless 2µL injection positively identifies the oxygenates and pentanes.

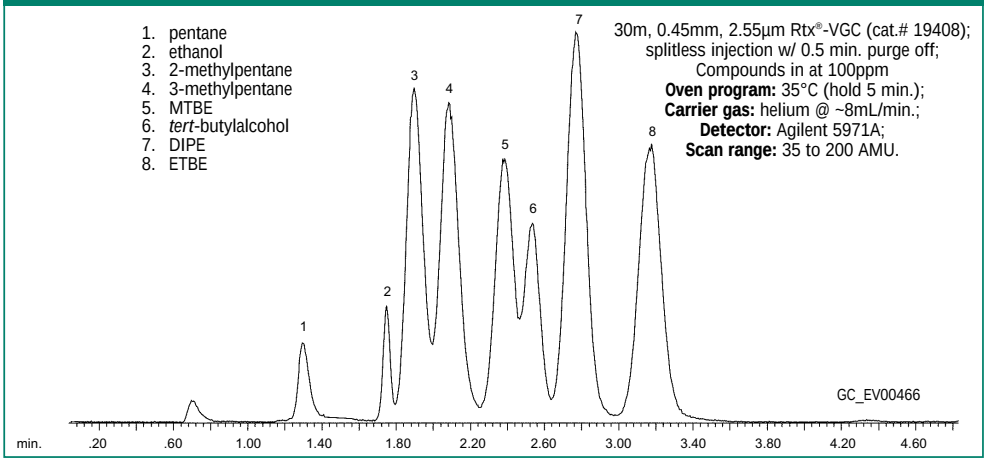


Figure 4–Using the Rtx-VGC column and a PID makes possible the identification of most oxygenates in a gasoline standard.

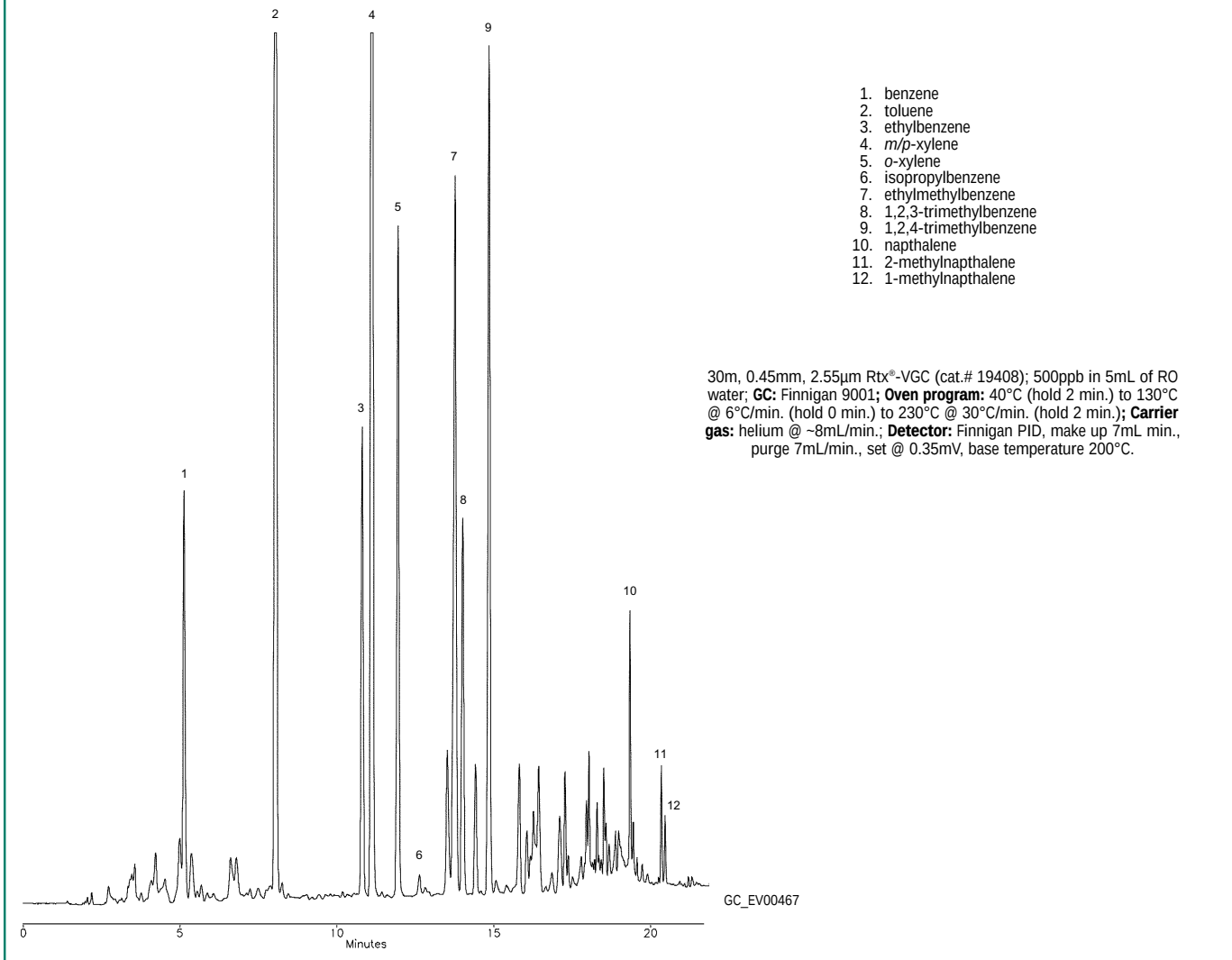
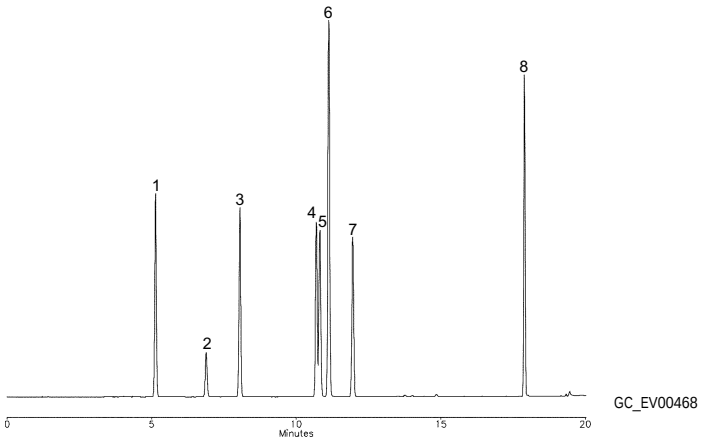


Figure 5–The Rtx®-VGC column successfully resolves chlorobenzene, allowing quantitation by PID.



1. benzene
2. a,a,a-trifluorotoluene
3. toluene
4. ethylbenzene
5. chlorobenzene
6. *m/p*-xylene
7. *o*-xylene
8. 4-bromo-1-chlorobenzene

30m, 0.45mm, 2.55µm Rtx®-VGC (cat.# 19408); 100ppb in 5mL of RO water; **GC:** Finnigan 9001; **Oven program:** 40°C (hold 2 min.) to 130°C @ 6°C/min. (hold 0 min.) to 230°C @ 30°C/min. (hold 2 min.); **Carrier gas:** helium @ ~8mL/min.; **Detector:** Finnigan PID, make up 7mL min., purge 7mL/min., set @ 0.35mV, base temperature 200°C

Rtx®-VGC Columns

(-40 to 240/260°C)

RESTEK Exclusive!

ID	df (µm)	30-Meter	60-Meter	75-Meter	105-Meter
0.25mm	1.40	19415	19416	—	—
0.32mm	1.80	19419	19420	—	—
0.45mm	2.55	19408	—	19409	—
0.53mm	3.00	19485	19488	19474	19489
ID	**df (µm)**	**20-Meter**	**40-Meter**		
0.18	1.00	49414	49415		

California Oxygenates Mix in P&T Methanol

diisopropyl ether	2,000µg/mL	*tert*-butyl alcohol	10,000µg/mL
ethyl-*tert*-butyl ether	2,000	methyl-*tert*-butyl ether	2,000
tert-amyl methyl ether	2,000		

1mL/ampul

	Each	5-pk.	10-pk.
	30465	**30465-510**	—
w/data pack	**30465-500**	**30465-520**	**30565**

Methanol Mix in DI Water

10,000µg/mL, 1mL/ampul

	Each	5-pk.	10-pk.
	30467	**30467-510**	—
w/data pack	**30467-500**	**30467-520**	**30567**

Ethanol Mix

10,000µg/mL in DI water, 1mL/ampul

	Each	5-pk.	10-pk.
	30466	**30466-510**	—
w/data pack	**30466-500**	**30466-520**	**30566**

Inlet Liners

For Agilent GCs

1mm Split Liner	*(1.0mm ID, 6.3mm OD, 78.5mm length)*
20972 (ea.)	20973 (5-pk.)

Siltek™ 1mm Uniliner®	*(1.0mm ID, 6.3mm OD, 78.5mm length)*
21052-214.1 (ea.)	21053-214.5 (5-pk.)

Unleaded Gasoline Component Standard

2,500µg/mL each in P&T methanol, 1mL/ampul

	Each	5-pk.	10-pk.
	30081	**30081-510**	—
w/data pack	**30081-500**	**30081-520**	**30181**

US EPA Method 8020A Calibration Mix

benzene	ethylbenzene
chlorobenzene	toluene
1,2-dichlorobenzene	*m*-xylene
1,3-dichlorobenzene	*o*-xylene
1,4-dichlorobenzene	*p*-xylene

2,000µg/mL each in P&T methanol, 1mL/ampul

	Each	5-pk.	10-pk.
	30222	**30222-510**	—
w/data pack	**30222-500**	**30222-520**	**30322**

aaa-trifluorotoluene

2,000µg/mL each in P&T methanol, 1mL/ampul

	Each	5-pk.	10-pk.
	30048	**30048-510**	—
w/data pack	**30048-500**	**30048-520**	**30148**

1-methylnapthalene

1,000µg/mL each in methanol, 1mL/ampul

	Each	5-pk.	10-pk.
	31283	**31283-510**	—
w/data pack	**31283-500**	**31283-520**	**31383**

PID Lamps

From Scientific Services Co.—"The Pioneers of the PID Lamp"

PID lamp model number	cat.#	PID lamp model number	cat.#
103C	20676	107-8.4	23022
108-10.0/10.6	20675	109-11.8	23023
108BTEX	23020	polishing kit	20674
108-9.6	23021		

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APPLICATIONS NOTE

Sulfurs in SilcoCan™ Canisters

Long-Term Stability of Very Low-Level (1-20ppbv) Reactive Sulfurs

tech tip

Alternative Analytical Techniques

Trace level sulfur compounds also can be analyzed using Restek's Rt-XLSulfur™ packed or micropacked columns. The specially designed packing material is optimized for low ppbv sulfur compound analysis.

For an example chromatogram, visit our website and enter GC_PC00436 in the search function.

Introduction

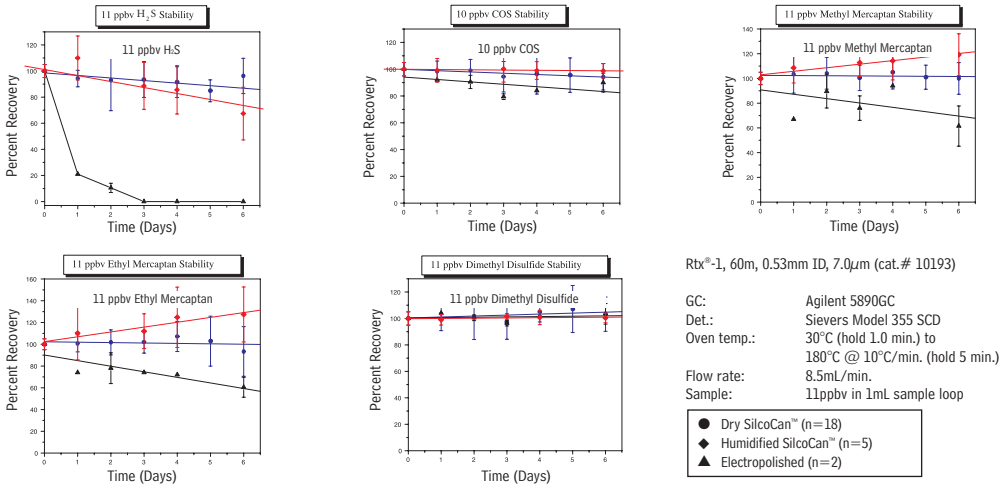
Collection and measurement of sulfur-containing volatile organic compounds (VOCs), such as hydrogen sulfide, methyl mercaptan, ethyl mercaptan, and dimethyl disulfide in the atmosphere is very difficult because of their low concentrations and high reactivity. Sulfur VOCs can react not only with each other, but also with the vessels in which they are collected. This causes low recoveries. In Tedlar® bags, the stability of low-level (100ppbv) sulfur VOCs is poor, even within 24 hours of sampling.[1] Sulfur compounds react with the metal surface in electropolished canisters, so these canisters are unsuitable for collecting and storing low-level sulfur VOCs.[2]

When you need to perform sensitive air monitoring analyses, use SilcoCan™ canisters to collect and store samples. SilcoCan™ canisters, which feature a Siltek®-treated surface, offer superior storage stability. We evaluated the stability of sulfur VOCs in SilcoCan™ canisters at very low levels (1–20ppbv) for six days, under dry or humid conditions, to demonstrate the excellent ability of SilcoCan™ canisters to store low-level sulfur VOCs (Figure 1). Hydrogen sulfide, methyl mercaptan, and ethyl mercaptan rapidly degraded in electropolished canisters.

Analytical System

High resolution capillary gas chromatography (GC) in conjunction with sensitive, selective detectors such as sulfur chemiluminescence detectors (SCD) or flame photometric detectors (FPD) offers many advantages for trace analysis of sulfur VOCs. For this study, we used an Rtx®-1 capillary column, a Siltek® treated six-port Valco® valve, and a Siltek® treated 1mL sample loop and 1/16" sample pathway. A representative chromatogram for the sulfur compounds is shown in Figure 2.

Figure 1 Stability data demonstrate ability of SilcoCan™ canisters to store low-level organic sulfur compounds in real-world conditions.



Standards: Dry standards were made by adding 2mL of a 100ppm stock sulfur standard to each precleaned and evacuated canister, then pressurizing to 30psig with ultra-pure nitrogen. The resultant concentrations are listed in Table 1. Humidified standards were made by injecting 100µL deionized water into the evacuated canisters prior to adding 2mL of stock standard. This produced 50% RH.

three **simple** words...

Plus 1™
Exceeding your expectations in everything we do.

Innovation
Turning visions into reality™.

Execution
On-time delivery of products and services.

Restek's vision is to be the company that chromatographers trust by providing the highest quality, most innovative products and services throughout the world.

We will soon reach our goal of 100% employee ownership. As owners, our success depends on your success.

Figure 2 Rtx®-1 column is ideal for trace-level sulfur analysis.

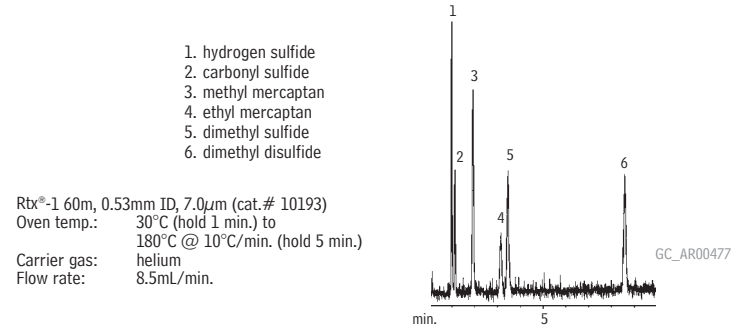


Table 1 Concentrations of sulfur compounds used in stability study.

Compound	Stock Conc. (ppmv)	Standard Conc. (ppbv)	Standard Conc. as Sulfur (ppbv)
hydrogen sulfide (H_2S)	105	11.51	10.83
carbonyl sulfide (COS)	98	10.74	5.73
methyl mercaptan (CH_3SH)	101	11.07	7.38
ethyl mercaptan (CH_3CH_2SH)	101	11.07	5.71
dimethylsulfide (CH_3SCH_3)•	99	10.85	6.81
dimethyldisulfide (CH_3SSCH_3)	100	10.96	7.46

• Internal standard

Stability

A 55ppbv reference standard (11ppbv each sulfur compound, dry standard) was analyzed three times each day for six days. Eighteen SilcoCan™ canisters and two electropolished canisters were used. The sulfur VOCs showed excellent stability in the SilcoCan™ canisters. The electropolished canisters allowed rapid degradation of hydrogen sulfide, methyl mercaptan, and ethyl mercaptan (Figure 1).

Humidity Effects

Five SilcoCan™ canisters that were used in the stability test were cleaned according to US Environmental Protection Agency (EPA) Compendium of Toxic Organic Method TO-14 and reused for the humidity study.[3] After adding 100µL of deionized H_2O to each canister, the relative humidity was 50%. Two mL of the stock sulfur standard was added to each canister and aliquots were analyzed over six days (Figure 1). The results show no difference in the performance by the SilcoCan™ canisters for storing humidified sulfur VOCs as compared to dry standards.

Conclusion

This study confirmed the stability of very low-level sulfur VOCs (1-20ppbv) in SilcoCan™ canisters. Using dry or humidified conditions, sulfur compounds exhibited virtually no loss in SilcoCan™ canisters after six days. Sampling with electropolished canisters leads to degradation of hydrogen disulfide, methyl mercaptan, and ethyl mercaptan.

Rtx®-1 Column (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	4.00	-60 to 280/300°C	30-Meter	10198
0.53mm	7.00	-60 to 240/260°C	60-Meter	10193

For other Rtx®-1 column dimensions, please request our general catalog or visit our website.

Rt-XLSulfur™ Packed/Micropacked Columns

- Optimized for low pbbv sulfur analyses.
- Eliminate the need for Teflon® tubing.
- Column and end-fittings are Sulfinert®-treated for maximum inertness.

Rt-XLSulfur™ Packed Columns

Purchase installation kit separately. Please see our catalog.

OD	ID (mm)	1-Meter*	2-Meter*
1/8"	2.0mm	80484-	80485-
3/16"*	3.2mm	80482-	80483-

*Please add configuration suffix number to cat.# when ordering. Please see our catalog.

Rt-XLSulfur™ Micropacked Columns

Purchase installation kit separately. Please see our catalog.

OD	ID (mm)	1-Meter	2-Meter
1/16"	1.0mm	19804	19805
0.95mm	0.75mm	19806	19807

References

1. Quang Tran, You-Zhi Tang; *Stability of Reduced Sulfur Compounds in Whole Air Samplers*, 1994 AWMA/EPA International Symposium of Measurement of Toxic and Related Air Pollutants.

2. Hoyt, Steven; Longacre, Vivian; and Stroupe, Michale; *Measurement of Oxygenated Hydrocarbons and Reduced Sulfur Gases by Full Scan GC/MS: EPA TO-14* in: *Sampling and Analysis of Airborne Pollutants*, Eric Winegar, and Lawrence Keith, editors. CRC Press, 1993 384pp (Restek cat. #20468).

3. Method TO-14A, *Determination of Volatile Organic Compounds (VOCs) in Ambient Air Using Specially Prepared Canisters with Subsequent Analysis by Gas Chromatography* in: *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*. US EPA, Jan 1997.

References 1 and 3 not available from Restek.

SilcoCan™ Air Monitoring Canisters

Siltek® treated - ideal for low-level reactive sulfur compounds (1-20ppb)

- Unsurpassed inertness, even for sulfur-containing or brominated compounds.
- Sizes from 1 to 15 liters support a wide range of sampling needs.
- Optional vacuum/pressure gauge for monitoring canister pressure.
- For critical applications, order a Siltek® treated valve - add suffix "-650" to the catalog number of the canister.

For ultimate inertness, we treat SilcoCan™ air monitoring canisters with our unique Siltek® passivation technology. Even highly active components, at low parts-per-billion concentrations, can be stored without loss. The valve is a high quality, metal-to-metal seal, 2/3-turn valve with metal diaphragms. Both stainless steel and Siltek®-treated valves are available, in both the 2-port and 3-port configurations.

Description	qty.	cat.#
1L Volume		
SilcoCan™ Canister, 1/4" Valve	ea.	24180
SilcoCan™ Canister, Siltek®-Treated 1/4" Valve	ea.	24180-650
SilcoCan™ Canister with Gauge, 1/4" Valve	ea.	24140
SilcoCan™ Canister with Gauge, Siltek®-Treated 1/4" Valve	ea.	24140-650
3L Volume		
SilcoCan™ Canister, 1/4" Valve	ea.	24181
SilcoCan™ Canister, Siltek®-Treated 1/4" Valve	ea.	24181-650
SilcoCan™ Canister with Gauge, 1/4" Valve	ea.	24141
SilcoCan™ Canister with Gauge, Siltek®-Treated 1/4" Valve	ea.	24141-650
6L Volume		
SilcoCan™ Canister, 1/4" Valve	ea.	24182
SilcoCan™ Canister, Siltek®-Treated 1/4" Valve	ea.	24182-650
SilcoCan™ Canister with Gauge, 1/4" Valve	ea.	24142
SilcoCan™ Canister with Gauge, Siltek®-Treated 1/4" Valve	ea.	24142-650
15L Volume		
SilcoCan™ Canister, 1/4" Valve	ea.	24183
SilcoCan™ Canister, Siltek®-Treated 1/4" Valve	ea.	24183-650
SilcoCan™ Canister with Gauge, 1/4" Valve	ea.	24143
SilcoCan™ Canister with Gauge, Siltek®-Treated 1/4" Valve	ea.	24143-650

All Restek canisters are originally equipped with high-quality Parker Hannifin diaphragm valves. Each valve is helium leak-tested to 4 x 10^{-9}cc/sec. The all-stainless steel construction eliminates contamination and the valve operates at temperatures from -100°C to 250°C. Compression outlet fitting, indicator plate to display open or closed position, 1/4" inlet and outlet.

Miniature Air Sampling Canisters

These 1000cc canisters are suitable for sampling volatile organic compounds in air according to US EPA Methods TO-14 and TO-15.

Description	Volume	qty.	cat.#
Sulfinert®-Treated Miniature Canister with Quick-Connect Stem Fitting	1000cc	ea.	24195
Sulfinert®-Treated Miniature Canister with Sulfinert®-Treated Quick-Connect Stem Fitting	1000cc	ea.	24196
Sulfinert®-Treated Miniature Canister with Metal-Seated Diaphragm Valve	1000cc	ea.	24198
Sulfinert®-Treated Miniature Canister with Sulfinert®-Treated Diaphragm Valve	1000cc	ea.	24199
Sulfinert®-Treated Miniature Canister with Nut & Ferrule	1000cc	ea.	24208

Also available: 400cc canisters. See our catalog or website.

The ultimate in controlled heating, for reliably cleaning your air canisters!

Air Canister Heating Jacket

- Heats entire canister, and valve.
- Two temperature settings, 75°C and 150°C.*
- Prevents sample condensation, for accurate sub-sampling.
- Easily fits canister up to 6 liters.
- Lightweight; comfortable to the touch when heated.
- Connect up to five Canister Heating Jackets to one 15 amp circuit.

Description	qty.	cat.#
Air Canister Heating Jacket (110 volt)	ea.	24123

*Not CE certified.

Dimensions: 2.75" diameter, 11.92" long (7 x 30.3cm)

Passive Air Sampling Kits

- Improved design eliminates leaks at the filter.
- Excellent for sampling times from 1 hour to 125 hours, or grab sampling.

Available in six sampling flow ranges, Restek's passive air sampling kit incorporates all hardware necessary to collect air samples, and is easy to assemble for field sampling.* The stainless steel kit is ideal to partner with the Restek TO-Can™ air sampling canister for TO-14A and TO-15 methods. Use the Siltek®-treated version with the Restek SilcoCan™ air sampling canister when collecting low-level volatile sulfur compounds, or other active compounds.

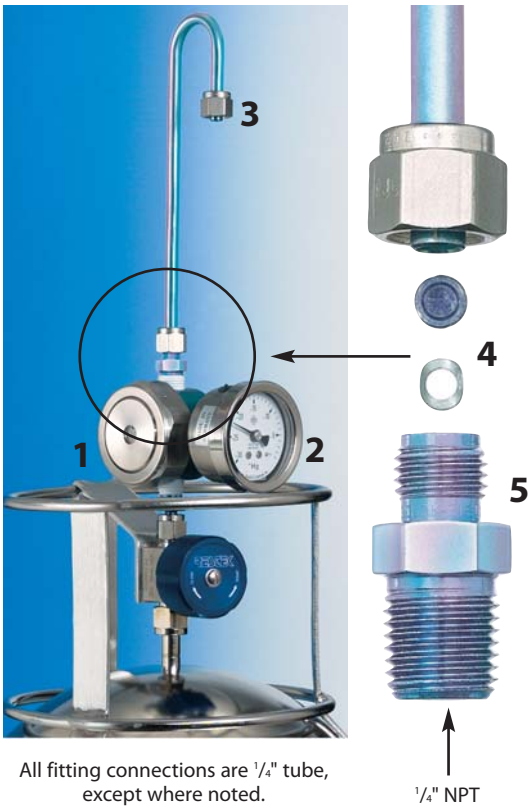
Canister Volume					Flow	Orifice	Siltek®-Treated	Stainless Steel
400cc	1 Liter	3 Liter	6 Liter	15 Liter	(sccm)	size	Complete Sampling Kits	Complete Sampling Kits
8 hour	24 hour	48 hour	125 hour	—	0.5–2	0.0008"	24217	24216
2 hour	4 hour	12 hour	24 hour	60 hour	2–4	0.0012"	24160	24165
1 hour	2 hour	6 hour	12 hour	30 hour	4–8	0.0016"	24161	24166
—	1 hour	4 hour	8 hour	20 hour	8–20	0.0020"	24162	24167
—	—	2 hour	3 hour	8 hour	20–40	0.0030"	24163	24168
—	—	—	1 hour	3 hour	40–80	0.0060"	24164	24169

*Air sampling canisters sold separately.

1. Veriflo™ SC423XL flow controller
Designed to maintain a constant mass flow as the pressure changes from 30" Hg to 5" Hg. All wetted parts of the flow controller can be Siltek®-treated.

2. Stainless steel vacuum gauge
Monitors canister pressure change during sampling.

3. 1/4-inch Siltek® sample inlet
Stainless steel nut prevents water droplets from accumulating at the edge of the tubing, where they could be pulled into the sampling train.



All fitting connections are 1/4" tube, except where noted.

4. 2-micron frit filter and washer
Replaceable. Available in stainless steel, or Siltek®-treated for optimum inertness.

5. Interchangeable critical orifice
Sapphire critical orifice controls the flow with very high precision. Available in stainless steel, or Siltek®-treated for optimum inertness.

For individual components, see our catalog or website.

free literature

For detailed information about using, cleaning, and certifying passive sampling trains in air sampling applications, request our technical guide *A Guide to Passive Air Sampling*.

Call Restek at 800-356-1688 or 814-353-1300, ext. 5, or contact your Restek representative, to request your free copy!

lit. cat.# 59977B

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Applicationsnote

Monitoring Volatile Compounds in Food Contact Packaging Using Purge and Trap GC/MS and an Rtx®-5MS Capillary Column

Introduction

Food packaging can be designed from a wide variety of materials, in a range of sizes, shapes, and colors. With the increasing popularity of convenience foods, meals often are prepared within the packaging materials. Packages might be placed in an oven, a microwave, or within a pot of boiling water. For microwavable packaging, food manufacturers prefer to use cheap, safe, and, to some extent, recyclable materials.[1] Microwave susceptor systems can be used to heat foods more rapidly, as well as to crisp and brown the foods they contact. Susceptors are made by laminating metallized polyester to paperboard. For dual-ovenable applications, ovenable paperboard and CPET (crystalline polyethylene terephthalate) can be used.

For all packaging materials, there is the potential to generate undesirable volatile compounds when used at elevated temperatures. In many instances, these compounds can migrate into or out of packaging materials. When flavor compounds migrate out of a food or beverage into the packaging, scalping occurs. This can result in a loss of flavor or a change to the flavor profile. Of more concern from a food safety perspective are compounds that have the potential to migrate out of the packaging material into the food product.

The US Food & Drug Administration (FDA) has stated, "Anyone manufacturing food contact articles for use in the home or in food service establishments should make sure that nothing from the articles imparts flavor, color, odor, toxicity, or other undesirable characteristics to food, thereby rendering the food adulterated."[2] In order to test the safety of food contact materials, packaging should be tested under simulated use conditions. Purge and trap gas chromatography/mass spectrometry (GC/MS) is a powerful tool for monitoring the volatile compounds that are generated during heating of packaging materials. This analysis technique can be applied to a wide range of packaging materials that are heated during food preparation.

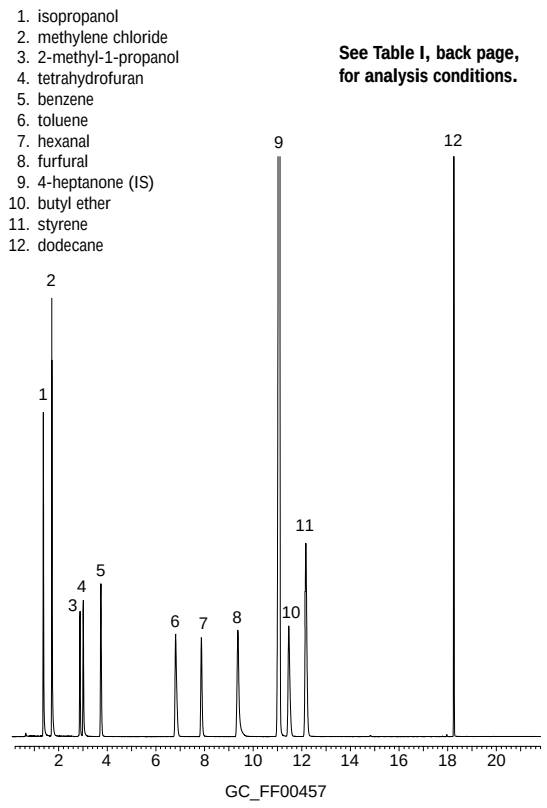
Indirect food additives are defined as substances that are used in the processing, packaging, holding, and/or transporting of food, have no functional effect on the food, but which might become a component of the food.[1] Plastic packaging in general is made of non-volatile, high molecular weight polymers, but volatile compounds often are added to improve the functional properties of the polymers. Such volatile compounds include plasticizers, antioxidants, UV blockers, and lubricants. The packaging inks and dyes used to create the label graphics can contain residual solvents, which may be toxic at certain concentrations. In addition, thermal breakdown of a polymer might occur in the inner ply of a boil-in bag or oven bag, thereby forming volatile species. Compounds such as benzene, styrene, and tetrahydrofuran (THF) have been shown to cause adverse affects on humans, and food contact materials need to be tested to ensure these volatiles are not present at significant levels.

In addition to the safety concerns, volatile compounds migrating into food materials can change the flavor profile of the food product. For example, polystyrene is used to package many aqueous-based, fatty or dry foods. Trace levels of styrene present in the polystyrene can impart a "plastic" taste to the food product.

In 21 CFR 170.39, the US FDA outlines the data needed to request an exemption from regulation as a food additive. Information needed includes the use conditions, time/temperature, food type, and whether the material is used once or multiple times. Other required information includes a detailed description of the analytical method and the method validation data, including the detection limit.

Figure 1

The Rtx®-5MS column exhibits good peak shape and high sensitivity for 12 volatile compounds that may be present in food packaging.



food & flavors

The FDA has defined two approaches to testing food contact materials: migration studies versus residual studies. In migration studies, food-simulating solvents are used to model the amount of material that migrates into the product. This is performed under worst-case, intended-use conditions. Changes in the food or ingredients used, such as a change in the fat content, can affect the migration of compounds out of the packaging material. For this reason, it is important to test the packaging under simulated use conditions to determine real-life effects on the food product.[3]

Residual studies estimate a worst-case dietary concentration level, assuming 100% migration of any volatiles generated into the food product. In residual studies, the level of each substance is measured in the finished food-contact article. If nothing is detected in the sample, then the validated detection limit can be used to estimate the dietary concentration.

While there are specific methods available for monitoring volatile compounds in defined systems, method development and validation often is the responsibility of the analyst. American Society for Testing and Materials (ASTM) Methods[4] F1308-98 and F1519-98 are qualitative and quantitative procedures (respectively) for monitoring the volatiles generated by microwave susceptor systems. Volatile extractables are defined as substances released from the susceptor and detected in the headspace. It is important to note that extractability does not necessarily mean migration to the food. ASTM F1308-98 is a quantitative procedure in which the packaging materials are heated in a sealed system within a microwave prior to drawing a headspace sample and injecting it into a GC/MS system. ASTM F1519-98 is a qualitative procedure for identifying the volatiles generated during simulated use conditions. This procedure uses a purge and trap apparatus to collect and concentrate the volatile compounds. These methods were used to develop the general procedure for testing volatiles in food contact packaging as described in this application note.

Experimental Conditions

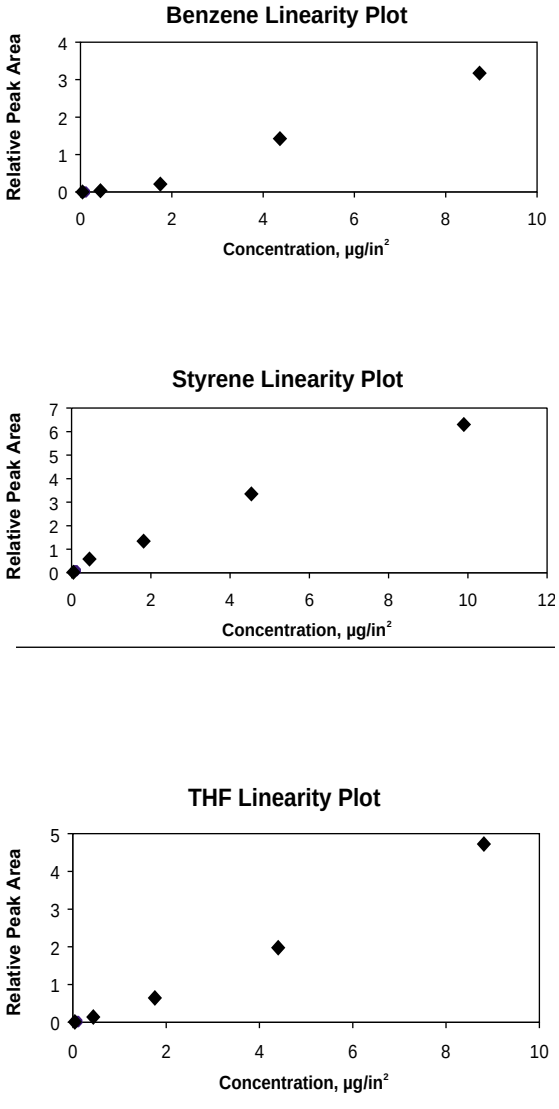
To develop a general method for monitoring volatiles in food contact packaging, we created a target list based on compounds that have been detected in processed packaging materials. The use of a mass spectral detector will enable the identification of other volatiles generated. We selected a low-bleed, Rtx®-5MS capillary column to allow analysis of the widest range of compounds. Initial separation parameters were entered into a computer modeling program, which optimizes the separation based on the column geometry, the oven temperature program, and the linear velocity (Table I, on the back page).

An internal standard (IS) solution of 4-butanone was prepared by diluting 300mL of 4-heptanone to 1 liter with purified water. The final concentration of the IS solution was 245mg/mL. The high standard solution was made by adding 50mL of each component to 475mL of the IS solution, and diluting to 500mL using the IS solution. Medium and low standard solutions were prepared using 25mL and 10mL of each component, respectively, and diluting each to 500mL using the IS solution. The chromatogram of a 12-component high standard solution is shown in Figure 1. Blank runs were performed before each standard and sample by analyzing a 100mL aliquot of the IS solution.

In order to determine the linearity of three packaging volatiles using this procedure, a series of standard solutions containing THF, benzene, styrene, and 4-heptanone (IS) were prepared and analyzed under the optimized parameters given in Table I. The standard preparation procedure described above also was used to prepare the linearity solutions. The approximate concentration range for each compound was 0-10µg/in^2. The linearity plots for three of the components are shown in Figure 2, with the relative peak area (peak area for the volatile/peak area for the IS) plotted vs. the concentration in µg/in^2. To further increase the sensitivity of this analysis, the MS can be operated in selected ion mode.

Figure 2

The Rtx®-5MS column exhibits good linearity for 3 volatile compounds that may be present in food packaging.



Tested over an approximate concentration range of 0–10 µg/in^2 for each component. R^2 = 0.992 (THF), 0.985 (benzene), and 0.994 (styrene).

Case Study of a Microwavable Bowl

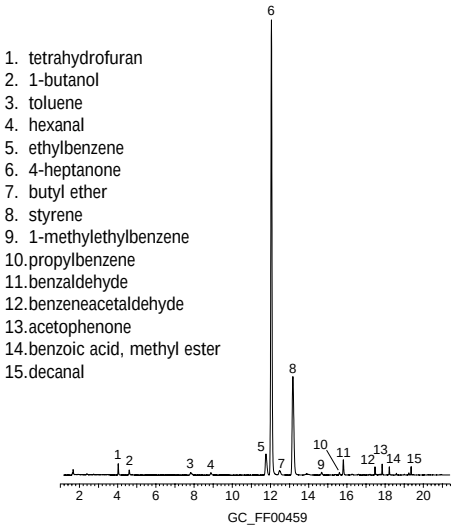
A single-serving microwavable bowl was analyzed using the protocol outlined in the experimental conditions. The goal was to determine which volatiles the packaging material generates during microwave heating of this product. Testing was performed by sampling each of the discrete materials used in the package. In this study, this resulted in four samples: an inner bowl that contains the food; a plastic lid; a printed shrink-wrapped label sampled both at the seam and away from the seam. The materials were sampled by cutting a 10mm by 65mm piece into thin strips. Each sample was placed in a Tekmar purge and trap tube, along with 100mL of IS solution. Blanks (i.e., 100mL of IS solution) were analyzed prior to each sample. The samples were preheated to 60°C and held at this temperature for 10 minutes during purging. This temperature was selected based on the approximate temperature experienced by the packaging material when heated according to label directions.

Based on this study, the highest level of volatile compounds was observed at the label seam. Residual styrene, ethyl benzene, and trace levels of solvents such as THF and toluene were detected. The label sampled away from the seam showed much lower levels of all of these components, except the styrene. The lid sample showed trace levels of ethylbenzene and styrene, while the inner bowl sample showed no significant levels of any volatile compounds. This procedure has proven to be an effective way to screen packaging materials for the presence of volatile compounds (Figure 3).

Figure 3

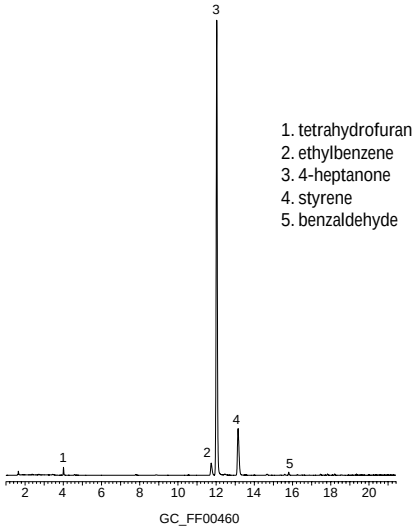
Screening microwave bowl materials for volatile compounds can be accomplished using the Rtx®-5MS column and the method outlined here.

Label overwrap sampled at the seam

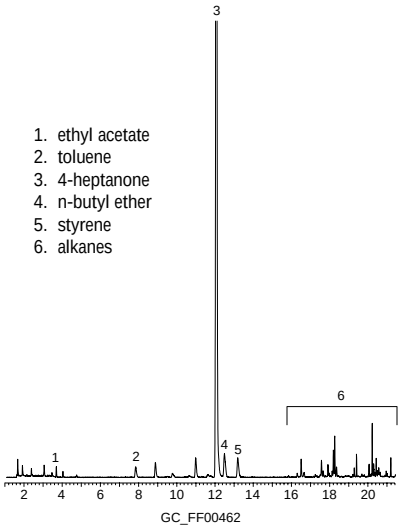


See Table I, back page, for analysis conditions.

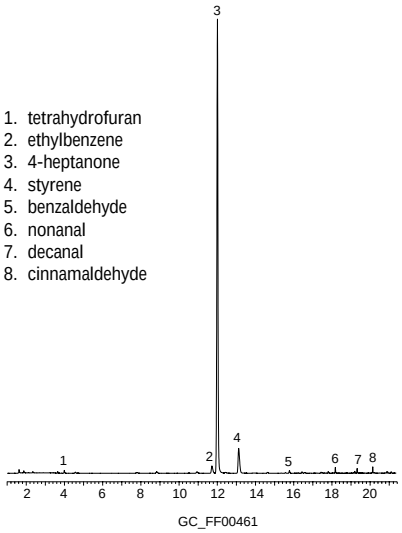
Label overwrap sampled away from the seam



Inner bowl sample



Lid sample



Conclusion

The analysis of food contact packaging is an important part of any food safety program. This is especially true with the wide variety of packaging materials available, the large range of intended use conditions, and the increasing popularity of convenience foods. With a continuing consumer emphasis on "speed to mouth," foods often are heated within the packaging material. This application note discusses an optimized procedure for monitoring common volatiles from food contact packaging using purge and trap GC/MS and an Rtx®-5MS capillary column. The Rtx®-5MS column is an excellent choice for this application. With its high stability and low bleed profile, it can be used to screen for a wide range of compounds by GC/MS. Please refer to the FDA to obtain more information on the regulatory requirements.

Table I

Optimized conditions and a low-bleed Rtx®-5MS column allow the analysis of a wide range of volatile compounds.

GC Parameters

Column: Rtx®-5MS, 30m x 0.25mm x 1.0µm (cat.# 12653)
Inj.: 250°C, 20:1 split
Carrier gas: helium, 1mL/min., constant flow
Oven: 50°C to 92°C @ 3°C/min, to 220°C @ 20°C/min. (1 min. hold)

MSD Parameters

Temp.: 280°C
Scan range: 35-260, 1 min. solvent delay
Ionization: EI @ 70eV

Purge & Trap Parameters

Concentrator: Tekmar LSC-3100 with Vocarb® 3000 (type K) trap
Transfer line: Silcosteel®-treated stainless steel tubing
Purge: 10 min. at 40mL/min., 60°C
Dry purge: 3 min. at 40mL/min.
Desorb: 2 min. at 40mL/min., 245°C

References

1. Schofield, J. Food FIPP Mag. (1989), 11(1), pp. 38-41.
2. Requirements of Laws and Regulations Enforced by the US FDA (1997), available on the US FDA website.
3. Marsili, Ray. "Techniques for Evaluating Packaging Materials" in Food Product Design (1997), editorial archives.
4. Annual Book of ASTM Standards (1998), American Society for Testing and Materials, West Conshohocken, PA.

References not available from Restek.

Product Listing

Rtx®-5MS Fused Silica Capillary Columns

ID (mm)	df µm	temp. limits	15-Meter	30-Meter	60-Meter
0.25	0.10	-60 to 330/350°C	12605	12608	12611
	0.25	-60 to 330/350°C	12620	12623	12626
	0.50	-60 to 330/350°C	12635	12638	12641
	1.00	-60 to 325/350°C	12650	12653	
0.32	0.10	-60 to 330/350°C	12606	12609	12612
	0.25	-60 to 330/350°C	12621	12624	12627
	0.50	-60 to 330/350°C	12636	12639	12642
	1.00	-60 to 325/350°C	12651	12654	
0.53	0.50	-60 to 320/340°C	12637	12640	
	1.00	-60 to 320/340°C	12652	12655	
	1.50	-60 to 310/330°C	12667	12670	

Purge-and-Trap Spargers

5mL Fritted Sparger, 1/2-inch mount: cat.# 21150
25mL Fritted Sparger, 1/2-inch mount: cat.# 21151

Moisture Control By-Pass Line

Moisture control by-pass line for Tekmar 3000: cat.# 21035

Restek's Foods, Flavors, & Fragrances products and applications catalog (Lit. cat.# 59260) features 48 pages of information and applications chromatograms. Request your FREE copy today!

#59351B

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Applicationsnote

Stx™-CLPesticides and Stx™-CLPesticides2 Columns Provide Improved System Inertness for Chlorinated Pesticides Analysis

Many laboratories performing gas chromatography (GC) analysis of chlorinated pesticides struggle with endrin breakdown caused by the compound interacting with or adsorbing to active sites throughout the analytical system, most notably in the injection port and the analytical column. Restek Siltek™ technology—used successfully to passivate injection port liners and guard columns—is now available for column technology and is used in Stx™-CLPesticides and Stx™-CLPesticides2 capillary columns. The combination of a properly deactivated injection system and inert analytical columns provides the lowest possible level of endrin breakdown.

Method Requirements

Chlorinated pesticide analysis following US Environmental Protection Agency (EPA) Methods 8081, 608, 505 and 508 recommend dual-column confirmation using electron capture detection (ECD). The compounds in Figure 1 represent some of the more common analytes. As in all analytical methods, the instrument used for quantitative analysis must be calibrated to ensure accurate results are reported. For chlorinated pesticides this usually entails a calibration curve of three to five points and check standards injected at specified time intervals during sample analysis. In addition, performance standards containing endrin are analyzed periodically to ensure system inertness. Typically, endrin breaks down to endrin aldehyde and endrin ketone when there are active sites in the sample pathway.

Endrin Breakdown

Maintaining a low breakdown level for endrin is necessary for laboratories analyzing chlorinated pesticides. Reduction of endrin breakdown generally focuses on improving the inertness of the injection port. Traditionally, deactivated injection port liners have been used for this analysis but, more recently, liners treated with Siltek™ passivation have been proven to further reduce endrin breakdown. This innovative passivation technology also was incorporated into capillary guard tubing so that the entire sample introduction pathway is inert for pesticide analysis.

Endrin Response on Capillary Columns

Through experimentation we have found that the columns typically used for pesticide analysis exhibit low response for endrin compared to other pesticides eluting in the same region even though the endrin breakdown products are not present. This indicates that endrin is being adsorbed by active sites in the sample pathway, thus effectively reducing endrin response. To minimize the on-column adsorption of endrin, we incorporated Siltek™ passivation technology into the analytical columns.

Combining Siltek™ passivation with the unique selectivity of Restek CLPesticides phases results in the Stx™-CLPesticides and Stx™-CLPesticides2 columns, and a significant improvement in endrin response (Figures 1 & 2). Using the Stx™-CLPesticides and Stx™-CLPesticides2 columns, the endrin peak response is notably higher than the analytes eluting in the same region—something not always seen in columns using traditional deactivations.

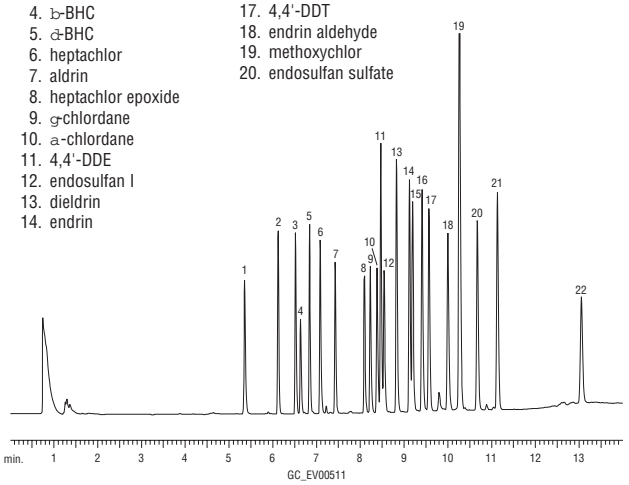
Column Installation and Optimizing Resolution

The Stx™-CLPesticides2 column is the ideal confirmational column to the Stx™-CLPesticides column. They were designed to achieve resolution of the chlorinated pesticides using the same backpressure and oven temperature program. The columns can be installed in parallel using a glass universal Press-Tight® "Y" connector or a metal MXT® "Y" connector (Figure 3). This parallel set-up reduces downtime caused by maintenance of multiple injection ports. Of course, these columns can also be installed in separate injection ports and mounted in the same GC oven.

Figure 1

The Stx®-CLPesticides column provides the ultimate in inertness for active pesticides such as endrin, DDT, and methoxychlor.

1. 2,4,5,6 -tetrachloro-*m*-xylene (ss)
2. a-BHC
3. g-BHC
4. b-BHC
5. d-BHC
6. heptachlor
7. aldrin
8. heptachlor epoxide
9. g-chlordane
10. a-chlordane
11. 4,4'-DDE
12. endosulfan I
13. dieldrin
14. endrin
15. 4,4'-DDD
16. endosulfan II
17. 4,4'-DDT
18. endrin aldehyde
19. methoxychlor
20. endosulfan sulfate
21. endrin ketone
22. decachlorobiphenyl (ss)



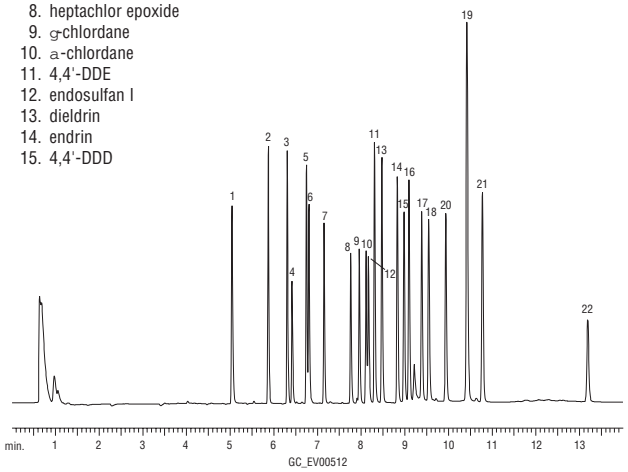
30m, 0.32mm ID, 0.50µm Stx-CLPesticides (cat# 11544)
Oven temp.: 120°C (hold 1 min.) to 300°C @ 9°c/min.; **Det.:** 310°C, Agilent ECD; **Inj.:** 1µL, pesticide standard mix A and B (cat.#32003,32004, 20/40/200ng/mL); **Inj. temp.:** 220°C (splitless, 1 min. purge off hold); **Inlet liner:** 4mm Siltek® single gooseneck (cat.# 20798-214.1); **Dead time:** 0.9 min. @ 120°C.

environmental

Figure 2

The Stx™-CLPesticides2 column is the ideal confirmation column to the Stx™-CLPesticides column for chlorinated pesticides analysis.

1. 2,4,5,6-tetrachloro-m-xylene (ss)
2. a-BHC
3. g-BHC
4. b-BHC
5. d-BHC
6. heptachlor
7. aldrin
8. heptachlor epoxide
9. g-chlordane
10. a-chlordane
11. 4,4'-DDE
12. endosulfan I
13. dieldrin
14. endrin
15. 4,4'-DDD
16. endosulfan II
17. 4,4'-DDT
18. endrin aldehyde
19. methoxychlor
20. endosulfan sulfate
21. endrin ketone
22. decachlorobiphenyl (ss)



30m, 0.32mm ID, 0.25µm Stx-CLPesticides2 (cat# 11444)
Oven temp.: 120°C (hold 1 min.) to 300°C @ 9°C/min.; **Detector:** 310°C, Agilent ECD;
Inj.: 1µL, pesticide standard mix A and B (cat.#32003,32004, 20/40/200ng/mL);
Inj. temp.: 220°C (splitless, 1 min. purge off hold); **Inlet liner:** 4mm Siltek™ single gooseneck (cat.# 20798-214.1); **Dead time:** 0.8 min. @ 120°C.

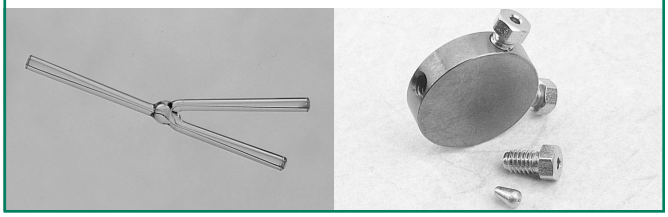
The key to maximizing resolution with these columns is setting the proper flow rate. The elution order of endosulfan I and 4,4'-DDE on the Stx™-CLPesticides column are used to indicate optimized flow rate; 4,4'-DDE should elute between a-chlordane and endosulfan I. If 4,4'-DDE and endosulfan I are not adequately resolved, decreasing the column flow will improve separation. Once resolution is acceptable for these two compounds, then the remaining compounds will be resolved in a dual-column single injection port configuration.

Conclusion

Endrin breakdown and adsorption are caused by active sites throughout the analytical system, especially the injection port and column. Using Siltek™-treated liners, guard columns, and Stx™-CLPesticides and Stx™-CLPesticides2 analytical columns for chlorinated pesticides analyses results in a chromatographic system with unsurpassed inertness, allowing for longer calibration periods.

Figure 3

Universal Press-tight® 'Y' connector and metal MXT® 'Y' connector.



Stx™-CLPesticides Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.25	-60 to 310/330°C	11540	11543
0.32mm	0.50	-60 to 310/330°C	11541	11544
0.53mm	0.50	-60 to 310/330°C	11542	11545

Siltek™ Guard Tubing

ID	5-Meter	10-Meter
0.25mm	10026	10036
0.32mm	10027	10037
0.53mm	10028	10038

Connectors

Description	Each	3-pack
Universal 'Y' Press-tight	20405	20406
MXT low dead-volume 'Y' (0.28mm)	20396	—
MXT low dead-volume 'Y' (0.53mm)	20395	—

Stx™-CLPesticides2 Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.20	-60 to 310/330°C	11440	11443
0.32mm	0.25	-60 to 310/330°C	11441	11444
0.53mm	0.42	-60 to 310/330°C	11442	11445

Stx™-CLPesticides Capillary Column Kits

Includes an Stx™-CLP column, an Stx™-CLP2 column, Siltek™ guard tubing, and a Universal 'Y' Press-Tight® Connector

0.25mm (cat.# 11190); **0.32mm** (cat.# 11191); **0.53mm** (cat.# 11192)

Siltek™ Inlet Liners

Qty.	Siltek™	Siltek™ w/Siltek™ Wool	Siltek™ w/Carbofrit™
each	-214.1	-213.1	-216.1
5-pack	-214.5	-213.5	-216.5
25-pack	-214.25	-213.25	-216.25

For Siltek™-deactivation, include the suffix number shown above to the inlet liner catalog number.

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Applicationsnote

Determining sulfur impurities in beverage-grade carbon dioxide using the Rt-XLSulfur™ micropacked column

The taste and aroma of a carbonated beverage can be affected by trace impurities from the carbonation process. Therefore, gas producers go to great lengths to purify carbon dioxide (CO_2). Carbon dioxide, a by-product of oil refining, fermentation, and power generating facilities, must be extremely pure to be suitable for a beverage additive. The beverage industry has spent much research time and dollars monitoring the impurities in CO_2.

The most common impurities in CO_2 are hydrocarbons, alcohols, permanent gases, and sulfur compounds. Sulfur impurities are the predominant problem, adding unwanted taste and odor to beverages. The most common volatile sulfur compound (VSC) impurities, listed in Table I, are targeted for monitoring by the International Society of Beverage Technologists (ISBT). Of this group, hydrogen sulfide, carbonyl sulfide, sulfur dioxide, dimethyl sulfide, and methyl mercaptan are the ones most commonly found in beverage-grade CO_2. ISBT guidelines specify Total Sulfur Content* (TSC) as 0.1ppm (v/v) maximum, excluding sulfur dioxide; the maximum level of sulfur dioxide must not exceed 1ppm (v/v) maximum.

The ability to measure reactive sulfur compounds at these levels requires a highly inert chromatographic system. The Restek Rt-XLSulfur™ micropacked column is a powerful analytical tool that can detect sulfurs in CO_2 at levels of 20ppbv, far below the ISBT guideline for TSC. This column also achieves the critical separation of hydrogen sulfide, carbonyl sulfide, and sulfur dioxide as defined in ISBT Procedure 14.0. The Rt-XLSulfur™ micropacked column contains a modified divinyl benzene polymer packed into Sulfinert™ tubing, which is a metal tubing specially deactivated for monitoring ppb levels of active sulfur compounds. Other features of the Rt-XLSulfur™ column include low bleed and thermal stability up to 300°C.

Sample introduction into the column is another critical aspect of obtaining accurate analytical results for sulfur compounds. The sample is introduced onto the column using a Valco® six-port sampling valve, fitted with a 1mL sampling loop (cat. #22845). When the valve, sample loop, and all other surfaces in the sample pathway are deactivated using the Sulfinert™ process, the analyst will see improved response compared to systems using conventional deactivations (Figure 1). The specialized inertness of the Sulfinert™ process is critical for the system to achieve detection limits of 50ppbv for sulfur dioxide and the other target sulfur impurities.

We evaluated the effectiveness of the Rt-XLSulfur™ column and Sulfinert™ sampling system by analyzing bulk CO_2 and CO_2 spiked with a sulfur standard (Figure 2). Notice how even low ppbv levels of sulfur compounds can be detected. We also sampled and measured the TSC* of two top brands of cola and a domestic beer (Figures 3 and 4). The colas show no sulfur content, ensuring that the CO_2 used for carbonation was clean. The beer shows sulfurs that naturally occur during the fermentation process.

This system is sensitive enough to monitor the levels of sulfur in CO_2 during the carbonation process, or in the headspace of the beverage after carbonation. The TSC* generated from headspace sampling of these products demonstrates the ability of the Rt-XLSulfur™ column and the Sulfinert™-deactivated GC system to easily detect sulfur compounds at the 20ppbv level. The combination of the Rt-XLSulfur™ micropacked column and a Sulfinert™-deactivated sample introduction system provides a state-of-the-art, robust sampling and analysis technique for ppbv levels of VSCs in beverage-grade CO_2.

*TSC is without SO_2.

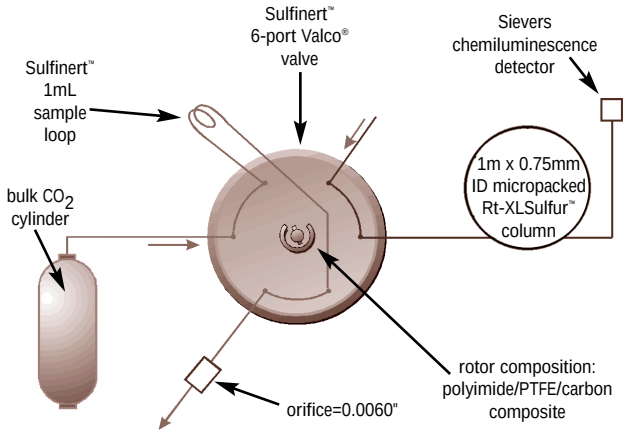
Table I

International Society of Beverage Technologists (ISBT) targets the most common VSCs.

hydrogen sulfide	isopropyl mercaptan
carbonyl sulfide	methyl ethyl sulfide
methyl mercaptan	*n*-propyl mercaptan
ethyl mercaptan	*tert*-butyl mercaptan
sulfur dioxide	*sec*-butyl mercaptan
dimethyl sulfide	diethyl sulfide
dimethyl disulfide	isobutyl mercaptan
carbon disulfide	*n*-butyl mercaptan
tert-amyl mercaptan	

Figure 1

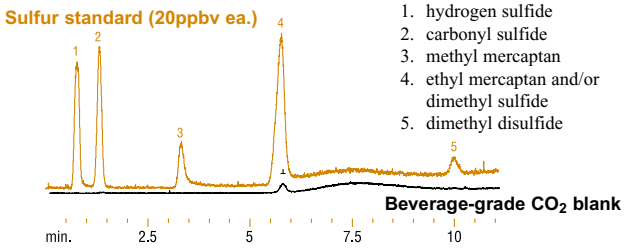
A Sulfinert™-treated sampling system designed to provide sensitive analysis of trace-level sulfur compounds.



food & flavors

Figure 2

The Rt-XLSulfur™ column and the Sulfinert™-deactivated GC system easily detect sulfur compounds at the 20ppbv level.

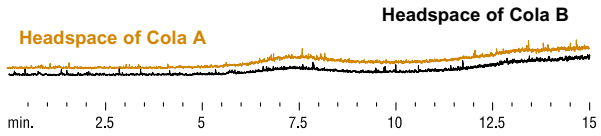


1. hydrogen sulfide
2. carbonyl sulfide
3. methyl mercaptan
4. ethyl mercaptan and/or dimethyl sulfide
5. dimethyl disulfide

1m x 0.75mm ID Rt-XLSulfur™ 100/120 mesh
Oven temp.: 60°C to 260°C @ 15°C/min. (hold 5 min.)
Det. temp.: 800°C
Carrier gas: He
Linear velocity: 10mL/min.
SCD sensitivity: Attn. x 1

Figure 3

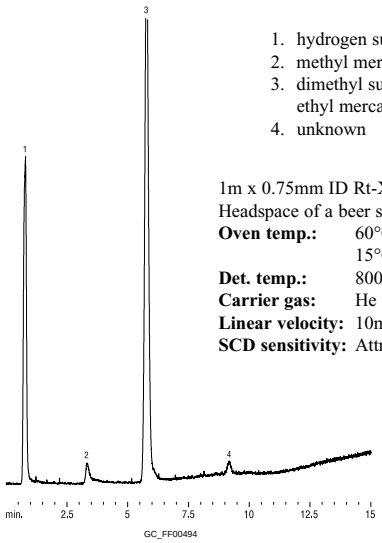
Samples of two popular colas show no sulfur compounds.



1m x 0.75mm ID Rt-XLSulfur™ 100/120 mesh
Oven temp.: 60°C to 260°C @ 15°C/min. (hold 5 min.)
Det. temp.: 800°C
Carrier gas: He
Linear velocity: 10mL/min.
SCD sensitivity: Attn. x 1

Figure 4

A sample of domestic (US) beer contains ppbv levels of sulfurs from the fermentation process.



1. hydrogen sulfide
2. methyl mercaptan
3. dimethyl sulfide and/or ethyl mercaptan
4. unknown

1m x 0.75mm ID Rt-XLSulfur™ 100/120 mesh
Headspace of a beer sample.
Oven temp.: 60°C to 260°C @ 15°C/min. (hold 5 min.)
Det. temp.: 800°C
Carrier gas: He
Linear velocity: 10mL/min.
SCD sensitivity: Attn. x 1

Rt-XLSulfur™ Packed and Micropacked Columns

OD	ID	1-meter	2-meter
1/16*	1.00	19804	19805
0.95mm*	0.75	19806	19807
1/8*	2.00	80484**	80485**
3/16	3.20	80482**	80483**

Installation Kits

Fits column IDs →	0.75mm	1mm	2mm
For valve applications	21062	21065	21067
For split applications	21063	—	—
For all Agilent GCs	21064	—	—
For direct injections	—	21066	—

Sulfinert™ Sample Loops

size	cat.#	size	cat.#	size	cat.#
5µL	22840	50µL	22844	1cc	22848
10µL	22841	100µL	22845	2cc	22849
20µL	22842	250µL	22846	5cc	22850
25µL	22843	500µL	22847		

Packed Column Configurations

Custom configurations are available. Please contact Customer Service (ext. 3) or your local Restek representative.

General Config.: **Suffix -800**

Agilent 5880, 5890, 5987: **Suffix -810**

Varian 3700, Vista Series, FID: **Suffix -820**

PE 900-3920, Sigma 1,2,3: **Suffix -830**

PE Auto System 8300, 8400, 8700 (Not On-Column): **Suffix -840**

6-Port Valco® Valve

The 6-port Valco® valve was coated with Sulfinert™ treatment on a custom basis. For custom Sulfinert™ quotes, call customer service at ext. 3, or contact your local Restek representative.

* *Installation kit must be purchased when using valve applications.*
***Please include configuration suffix number when ordering.*

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Applications note

Improved Analysis of Organophosphorus Pesticides Using Rtx®-OPPesticides and Rtx®-OPPesticides2 Columns

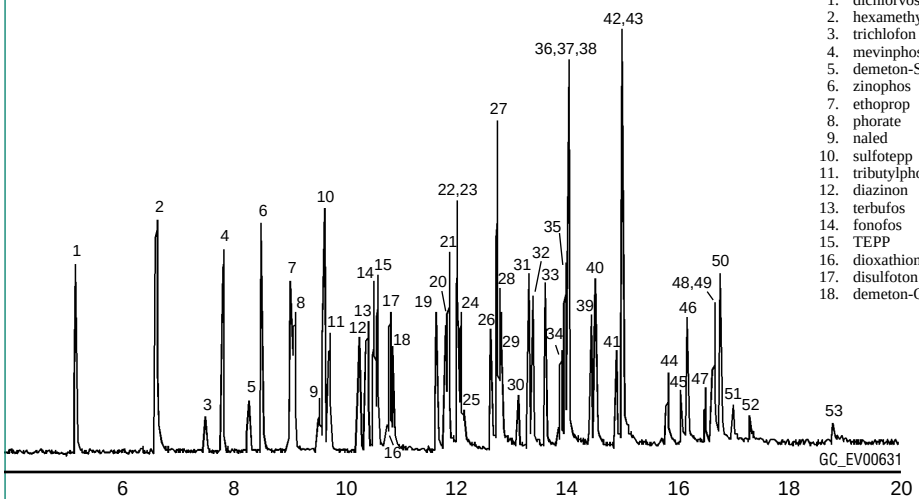
Organophosphorus pesticides (OPPs) are an important group of insect control agents used in agricultural and home settings. Although there are continuing concerns about their effects on health, their relatively low toxicity and short environmental fate have made them suitable replacements for banned organochlorine pesticides, especially in agricultural applications. Because of their widespread use, it is necessary to routinely monitor treated foods and surrounding soils and groundwater after OPP application, to ensure low residue levels.

Historically, the analysis of OPPs has presented challenges. US Environmental Protection Agency (EPA) Methods 8141 and 8141A were developed to help laboratories analyze soils, water, and solid wastes for OPPs. Method 8141A describes the many problems that can occur during capillary GC analysis. The OPP compounds represent a diverse group, many of which are photosensitive or easily degraded during routine standard preparation, storage, and analysis. In addition, individual analytes can be difficult to identify because of the large number of OPPs that might be present (e.g., a total of 49 possible analytes are listed in Method 8141A), and in the past GC/MS has not been sufficiently sensitive for routine use. Consequently, ion-specific detectors (e.g., a nitrogen phosphorus detector [NPD] or flame photometric detector [FPD] in the phosphorus mode) must be used to ensure sensitivity to detect low ppb levels of OPP compounds. This requires dual-column analysis for confirmation of analyte identities. Even the current EPA method states, "it is unlikely that all of them [OPPs] could be determined in a single analysis."[1]

Many capillary phases have been used for this dual-column analysis, but most present a large number of coelutions that make positive identifications difficult. For example, there are seven known pairs of coelutions on the 5% phenyl analytical column named in Method 8141A, and nine pairs of coelutions on the confirmation column. Until now, the Rtx®-OPPesticides column has given the best resolution of organophosphorus pesticides in the shortest time (Figure 1).

The Rtx®-OPPesticides column has advantages over other current technologies, but until now there has not been a confirmation column that has as few coelutions in as short an analysis time, to make it compatible with the Rtx®-OPPesticides column. To provide a good confirmation column to match the Rtx®-OPPesticides column, Restek chemists have developed a new polymer phase, the Rtx®-OPPesticides2 phase. In combination, these two columns will reduce the number of chromatographic coelutions and provide separations in less than 25 minutes.

Figure 1—The Rtx®-OPPesticides column provides good resolution and short analysis time for organophosphorus pesticides.



1. dichlorvos
2. hexamethylphosphoramide
3. trichlofon
4. mevinphos
5. demeton-S
6. zinophos
7. ethoprop
8. phorate
9. naled
10. sulfotepp
11. tributylphosphate (IS)
12. diazinon
13. terbufos
14. fonofos
15. TEPP
16. dioxathion
17. disulfoton
18. demeton-O
19. dichlorofenthion
20. chlorpyrifos methyl
21. dimethoate
22. dicrotophos
23. monocrotophos
24. ronnel
25. merphos
26. chlorpyrifos
27. aspon
28. fenthion
29. trichloronate
30. phosphamidon isomer (breakdown product)
31. methyl parathion
32. malathion
33. fenitrothion
34. tokuthion
35. phosphamidon
36. chlorfenvinphos
37. parathion
38. merphos oxone (merphos breakdown product)
39. stirophos
40. crotoxyphos
41. bolstar
42. carbophenthion
43. ethion
44. triphenylphosphate (IS)
45. leptophos
46. fensulfothion
47. tri-*o*-cresyl phosphate
48. phosmet
49. EPN
50. famfur
51. azinphos methyl
52. azinphos ethyl
53. coumaphos

Column: Rtx®-OPPesticides 30m, 0.32mm ID, 0.50µm (cat.# 11239)
Sample: 1.0µL of custom OP pesticides mix described in Figure 3 (GC_EV00602)
Concentration: 100ppb
Inj.: direct injection, Uniliner® DI liner (cat.# 20335)
Oven temp.: 100°C to 180°C @ 10°C/min. (hold 2 min.), to 300°C @ 18°C/min. (hold 2min.)
Inj. & Det. temp.: 250°C
Detector: FPD
Flow rate: 5cc/min., helium

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Restek's proprietary computer-assisted stationary phase development (CASPD) modeling software was used to develop the new phase. This software predicts GC polymer phase selectivity based on the retention of key analytes under controlled GC conditions. The stationary phase selectivity can be optimized, often resulting in the design of a completely new phase. In this case the new phase, Rtx®-OPPesticides2, achieves much better resolution of the OPPs than traditionally used columns, with only two coeluting pairs. This makes the new column an ideal primary column, even exceeding the performance of the Rtx®-OPPesticides column, for analyses of organophosphorus pesticides (Figure 2).

The Rtx®-OPPesticides2 column has quickly become a workhorse column for laboratories analyzing organophosphorus pesticides.

GC/MS Analysis

In some methods, such as in food analysis, low-level detection of organophosphorus pesticides is not as important as the positive identification of the pesticides. In these cases a GC/MS analysis is the best approach. The Rtx®-OPPesticides2 column is an excellent choice for GC/MS because the column exhibits low bleed and maximizes resolution of the organophosphorus pesticides. Figure 3 shows a typical GC/MS analysis.

Effect of Carrier Gas Flow

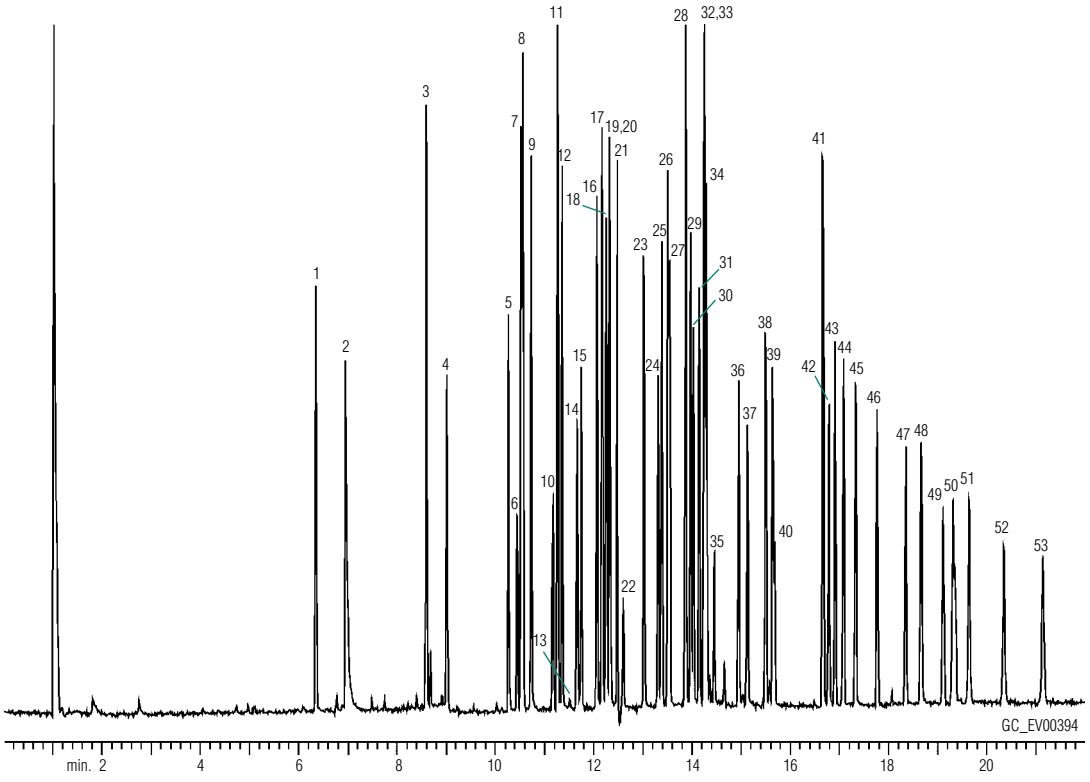
Flow conditions are extremely important to achieving the desired resolutions of OPPs. The chromatograms in Figure 4 are results from a flow rate study on the Rtx®-OPPesticides2 column. Flow can be used to enhance resolution and affect elution order. Because of the close elutions of so many of these compounds, it is critical to measure analytical velocity as either flow rate through the column or as dead time of an unretained analyte. Note that retention times and resolution (and, potentially, elution order) can shift with relatively small changes in dead time. In this analysis, we recommend using a slightly lower flow rate (longer dead time) to achieve the best overall separation.

Conclusion

Organophosphorus pesticides analyses can present many challenges to the analyst, but the Rtx®-OPPesticides2 and Rtx®-OPPesticides column pair ensures quick analysis, exceptional resolution, and confirmation for more than 48 OPP analytes and their degradation products in a single, dual-column run.

1. US EPA Method 8141A Organophosphorus Compounds by Gas Chromatography: Capillary Column Technique.

Figure 2—The Rtx®-OPPesticides2 column is an ideal primary column for fast, sensitive analyses of organophosphorus pesticides.

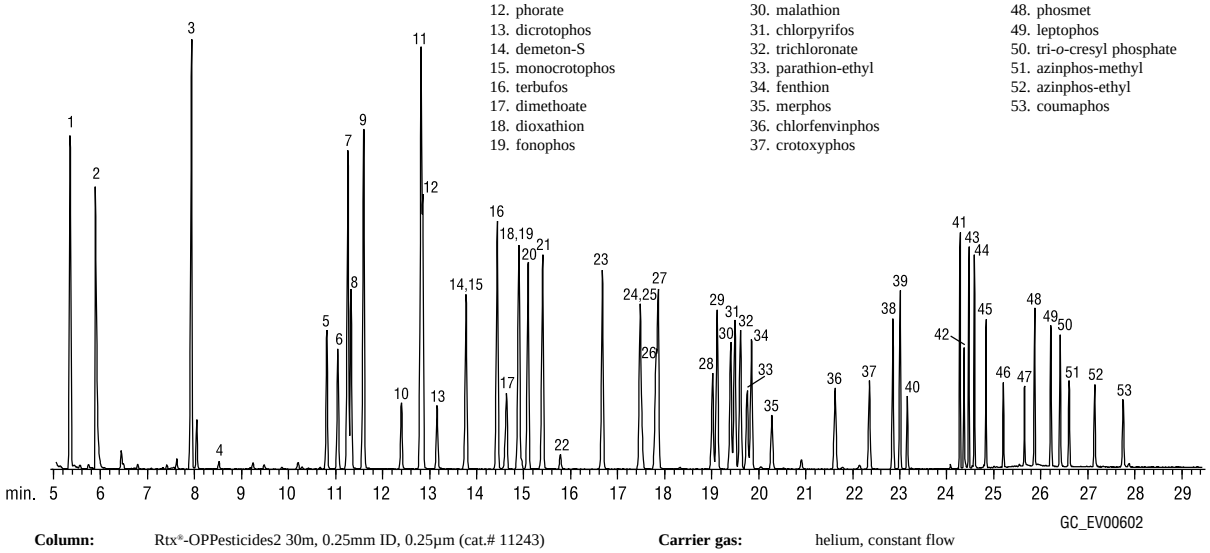


1. dichlorvos
2. hexamethylphosphoramide
3. mevinphos
4. trichlorfon
5. TEPP
6. demeton-O
7. tributylphosphate (SS)
8. thionazin
9. ethoprop
10. naled
11. sulfotepp
12. phorate
13. dicrotophos
14. monocrotophos
15. demeton-S
16. terbufos
17. dimethoate
18. diazinon
19. dioxathion
20. fonophos
21. disulfoton
22. phosphamidon isomer (breakdown product)
23. dichlorofenthion
24. phosphamidon
25. chlorpyrifos methyl
26. parathion-methyl
27. ronnel
28. aspon
29. fenitrothion
30. malathion
31. chlorpyrifos
32. trichloronate
33. parathion-ethyl
34. fenthion
35. merphos
36. chlorfenvinphos
37. crotoxyphos
38. stirofos
39. tokuthion
40. merphos oxone (merphos breakdown product)
41. ethion
42. fensulfothion
43. bolstar
44. carbophenothion
45. famphur
46. triphenylphosphate (SS)
47. EPN
48. phosmet
49. leptophos
50. tri-*o*-cresyl phosphate
51. azinphos-methyl
52. azinphos-ethyl
53. coumaphos

Column: Rtx®-OPPesticides2, 30m, 0.32mm ID, 0.32µm (cat.# 11241)
Inj.: 1µL splitless injection of 100ng/mL custom OP pesticides mix described in Figure 3 (GC_EV00602)
Oven temp.: 80°C (hold 0.5min.) to 280°C @ 12°C/min. (hold 10 min.)
Injector: 200°C
Inlet liner: 4mm Siltek™ single gooseneck (cat.# 20798)
Detector: FPD @ 250°C;
Dead time: 1.03 min. @ 80°C
Splitless hold: 1 min.

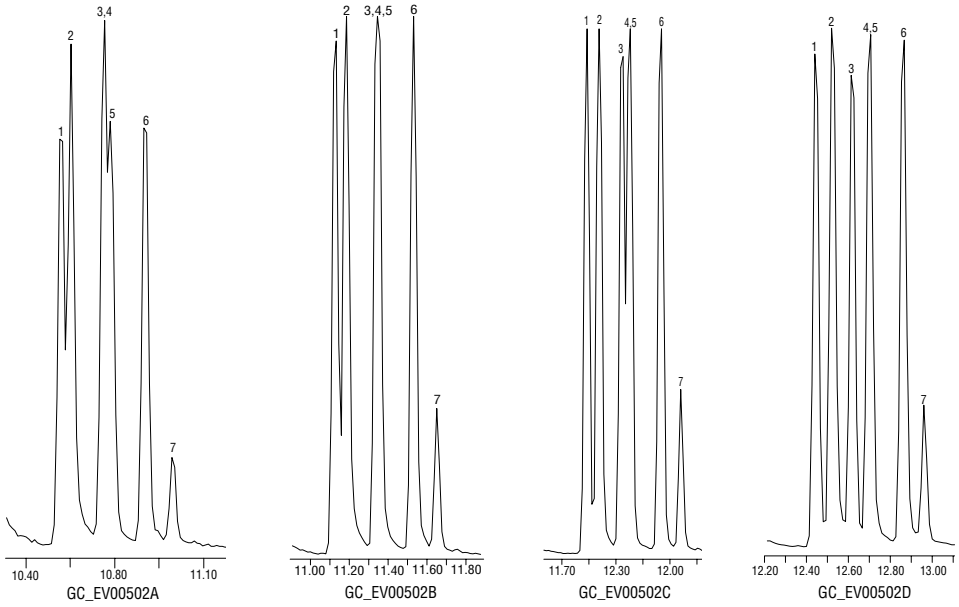
Figure 3—Low bleed and fast analysis times make the Rtx®-OPPesticides2 column an excellent choice for GC/MS analysis of organophosphorus pesticides.

1. dichlorvos
2. hexamethylphosphoramide
3. mevinphos
4. trichlorfon
5. TEPP
6. demeton-O
7. thionazin
8. tributylphosphate (IS)
9. ethoprop
10. naled
11. sulfotepp
12. phorate
13. dicrotophos
14. demeton-S
15. monocrotophos
16. terbufos
17. dimethoate
18. dioxathion
19. fonophos
20. diazinon
21. disulfoton
22. phosphamidon isomer (breakdown product)
23. dichlorofenthion
24. chlorpyrifos methyl
25. phosphamidon
26. parathion-methyl
27. ronnel
28. fenitrothion
29. aspon
30. malathion
31. chlorpyrifos
32. trichloronate
33. parathion-ethyl
34. fenthion
35. merphos
36. chlorfenvinphos
37. crotoxyphos
38. stirofos
39. tokuthion
40. merphos oxone (merphos breakdown product)
41. ethion
42. fensulfothion
43. bolstar
44. carbophenothion
45. famphur
46. triphenylphosphate (SS)
47. EPN
48. phosmet
49. leptophos
50. tri-*o*-cresyl phosphate
51. azinphos-methyl
52. azinphos-ethyl
53. coumaphos



Column: Rtx®-OPPesticides2 30m, 0.25mm ID, 0.25µm (cat.# 11243)
Sample: Custom Mix, plus:
8140/8141 OP Pesticides Calibration Mix A (cat.# 32277)
8141 OP Pesticides Calibration Mix B (cat.# 32278)
Triphenylphosphate Standard (cat.# 32281)
Tributylphosphate Standard (cat.# 32280)
1µL, 100ppm each (100ng on column)
Inj.: 1.0µL splitless (hold 0.4 min.), 4mm ID double gooseneck inlet liner (cat.# 20785)
Inj. temp.: 250°C
Carrier gas: helium, constant flow
Flow rate: 1.0mL/min.
Oven temp.: 80°C (hold 0.5 min.) to 140°C @ 20°C/min. to 210°C @ 4°C/min. (hold 1 min.) to 280°C @ 30°C (hold 5 min).
Det: MS
Transfer line temp.: 280°C
Scan range: 35-400amu
Ionization: EI

Figure 4—Adjusting the flow rate can affect elution time and resolution (and elution order) for OPPs on an Rtx®-OPPesticides2 column.



83cm/sec. dead time 0.6 min.

71cm/sec. dead time 0.7 min.

56cm/sec. dead time 0.9 min.

46cm/sec. dead time 1.1 min.

Column: Rtx®-OPPesticides2 30m, 0.32mm, 32µm (cat.#11241)
Injector: 200°C split/splitless, purge off, hold 1 min
Oven temp.: 80°C (hold 0.5 min.) to 280°C @ 12°C/min. (hold 10 min.)
Detector: FPD @ 250°C
Sample: 0.5µL 200ng/mL of custom OP pesticides mix described in Figure 3 (GC_EV00602) (partial chromatograms shown)
Other conditions as described for Figure 2 (GC_EV00394)

1. terbufos
2. dimethoate
3. diazinon
4. dioxathion
5. fonophos
6. disulfoton
7. phosphamidon isomer

Rtx®-OPPesticides (-20 to 310/330°C)

ID (mm)	df (µm)	20-meter	30-meter
0.18	0.25	56898	—
0.25	0.40	—	55239
0.32	0.50	—	11239
0.53	0.83	—	11240

Rtx®-OPPesticides2 (-20 to 310/330°C)

ID (mm)	df (µm)	20-meter	30-meter
0.18	0.20	11244	—
0.25	0.25	—	11243
0.32	0.32	—	11241
0.53	0.50	—	11242

Inlet Liners

For Agilent GCs

Uniliner® *(4.0mm ID, 6.3mm OD, 78.5mm length)*

20335 (ea.) 20336 (5-pk.)

Hole makes use with EPC possible!

Drilled Uniliner® *(4.0mm ID, 6.3mm OD, 78.5mm length)*

21054 (ea.) 21055 (5-pk.)

Open-top Uniliner® w/Wool *(4.0mm ID, 6.3mm OD, 78.5mm length)*

20843 (ea.) 20844 (5-pk.)

Gooseneck Splitless (4mm) *(4.0mm ID, 6.3mm OD, 78.5mm length)*

20798 (ea.) 20799 (5-pk.) 20800(25-pk.)

Double Gooseneck Splitless (4mm) *(4.0mm ID, 6.5mm OD, 78.5mm length)*

20784 (ea.) 20785 (5-pk.) 20786(25-pk.)

Restek will create the right solution for you!

Restek should be your first choice for custom-made reference materials. Our inventory of over 3,000 compounds ensures you of maximum convenience, maximum value, and minimum time spent blending mixtures in your lab.

For our online custom reference material request form, visit http://www.restekcorp.com/stdreq.htm

Method 8140/8141 OP Pesticide Calibration Mix A

azinphos methyl
bolstar (sulprofos)
chlorpyrifos
coumaphos
demeton, O and S
diazinon
dichlorvos
disulfoton
ethoprop
fensulfothion
fenthion
merphos
methyl parathion
mevinphos
naled
phorate
ronnel
stirofos
tokuthion (prothiofos)
trichloronate

200µg/mL each in hexane/acetone (95/5), 1mL/ampul

	Each	5-pk.	10-pk.
	32277	32277-510	
w/data pack	32277-500	32277-520	32377

Method 8141 OP Pesticide Calibration Mix B

dimethoate
EPN
malathion
monocrotophos
parathion
sulfotepp
TEPP

200µg/mL each in hexane/acetone (95/5), 1mL/ampul

	Each	5-pk.	10-pk.
	32278	32278-510	
w/data pack	32278-500	32278-520	32378

Method 8140/8141 Internal Standards & Surrogates

These solutions are prepared at 1,000µg/mL in acetone, 1mL/ampul

NPD Detector:
Internal Standard: 1-bromo-2-nitrobenzene
Surrogate: 4-chloro-3-nitrobenzotrifluoride

1-bromo-2-nitrobenzene

	Each	5-pk.	10-pk.
	32279	32279-510	
w/data pack	32279-500	32279-520	32379

4-chloro-3-nitrobenzotrifluoride

	Each	5-pk.	10-pk.
	32282	32282-510	
w/data pack	32282-500	32282-520	32382

FPD Detector:
Internal Standard: none recommended
Surrogate: tributylphosphate and triphenylphosphate

tributylphosphate

	Each	5-pk.	10-pk.
	32280	32280-510	
w/data pack	32280-500	32280-520	32380

triphenylphosphate

	Each	5-pk.	10-pk.
	32281	32281-510	
w/data pack	32281-500	32281-520	32381

RESTEK HPLC

Applicationsnote

HPLC Analysis of Trace-Level Explosives Using Pinnacle II™ C18 and Cyano Columns

Pinnacle II™ high performance liquid chromatography (HPLC) stationary phases were designed to function well under the difficult matrices encountered in environmental samples. The original Pinnacle™ columns served as benchmarks for the selectivity and efficiency of the new Pinnacle II™ columns. While striving to create new columns with characteristics similar to Pinnacle™ columns, Restek also designed the manufacturing process in such a way that they could be priced economically. Using Restek silica, we can go a step further in providing consistent quality and reproducibility.

The new Pinnacle II™ C18 and Cyano columns function as primary and confirmation columns (respectively) to efficiently separate explosives according to US Environmental Protection Agency (EPA) Method 8330A. Environmental methods frequently employ a confirmation column for two reasons. First, many environmental methods require scanning for a large number of related compounds. Because of their similarities, analysts often will encounter coelutions when using a single type of stationary phase. Second, the matrices encountered in many environmental samples can contain components that may interfere or obscure the analytes of interest. By using two columns with different selectivities, analysts can more accurately identify the analytes of interest.

Selectivity for the 14 explosives of interest listed in Method 8330A are similar on the original Pinnacle™ and the new Pinnacle II™ C18 columns (Figure 1). On these C18 columns, there are close-eluting peaks or coelutions for the following compounds: tetryl/nitrobenzene; 2-amino-4,6-dinitrotoluene/4-amino-2,6-dinitrotoluene; and 2,6- dinitrotoluene/2,4dinitrotoluene. Closer examination shows that the new Pinnacle II™ C18 column achieves slightly better resolution for several of these pairs. This may be caused by the slightly higher surface area and carbon load, and the smaller pore volume on the Pinnacle II™ column (110Å) as compared to the Pinnacle™ column (120Å). The higher carbon load of 13% for the Pinnacle II™ column versus 11% for the Pinnacle™ column translates into longer compound retention, better resolution, and column lifetime.

According to Method 8330A, these 14 compounds also need to be analyzed on a Cyano column for confirmation (Figure 2). The change from the reversed phase C18 column to the normal phase Cyano column is fairly easy. The method recommends using the same mobile phase for both columns, which allows a quick changeover from the primary analysis to the confirmation analysis. Because the mobile phase is a simple mixture of water

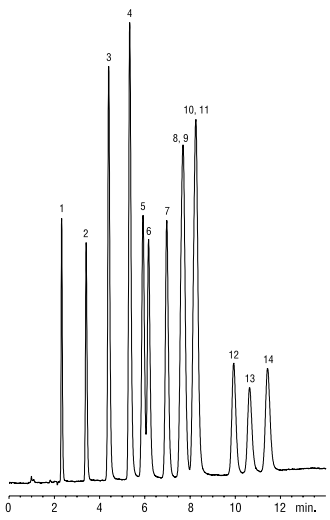
Figure 1

The new Pinnacle II™ C18 column achieves slightly better resolution for difficult to analyze pairs of explosive compounds.

Column: Pinnacle™ C18
Catalog #: 9114575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 120Å

Conditions:
Mobile phase: water:methanol (50:50, v/v)
Flow: 1.5 mL/min
Temp.: 27.0°C
Det.: UV @ 254nm

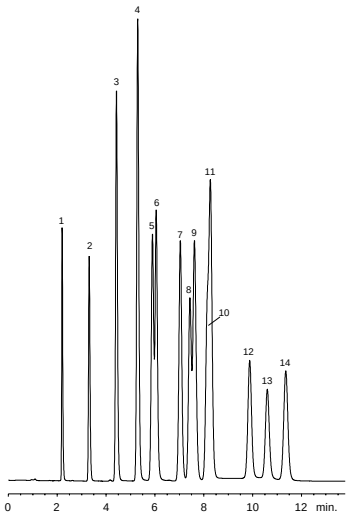
Sample:
Inj.: 3µL
Conc.: 500µg/mL each component
Solvent: acetonitrile
Restek 8330 Calibration mix #1: Cat. # 31450
Restek 8330 Calibration Mix #2 : Cat. # 31451



LC_0194

Peak List:
1. HMX
2. RDX
3. 1,3,5-trinitrobenzene
4. 1,3-dinitrobenzene
5. tetryl
6. nitrobenzene
7. 2,4,6-trinitrotoluene
8. 2-Amino-4,6-dinitrotoluene
9. 4-amino-2,6-dinitrotoluene
10. 2,6-dinitrotoluene
11. 2,4-dinitrotoluene
12. 2-nitrotoluene
13. 4-nitrotoluene
14. 3-nitrotoluene

Column: Pinnacle II C18
Catalog #: 9214575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 110Å



LC_0189

HPLC

and methanol, the process of switching from the C18 to the Cyano column is only a matter of removing the primary column and installing the confirmation column on the same HPLC system. There is relatively little downtime, only that required for the column to equilibrate.

Notice that all of the coeluting pairs form the C18 column are resolved from one another on the Cyano column. There is a cluster of compounds: 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, 1,3,5-trinitrotoluene, and 1,3 dinitrotoluene on the Cyano column, but these compounds are well resolved on the C18 column. Again, the selectivity between the original Pinnacle™ Cyano and the new Pinnacle II™ Cyano columns is similar, but the Pinnacle II™ column shows slightly better resolution.

Restek controls the raw material quality from the very beginning of the silica manufacturing process. Add our phase bonding and column packing experience to this high level of quality control, and you benefit from even better column-to-column and analysis-to-analysis reproducibility. Because of this and their economical production, Pinnacle II™ HPLC columns provide a cost-effective analytical tool for many traditional methods used in the environmental industry.

Figure 2

The Pinnacle II™ Cyano column shows slightly better resolution for explosives compounds and is an excellent confirmation column to the Pinnacle II™ C18 for this analysis.

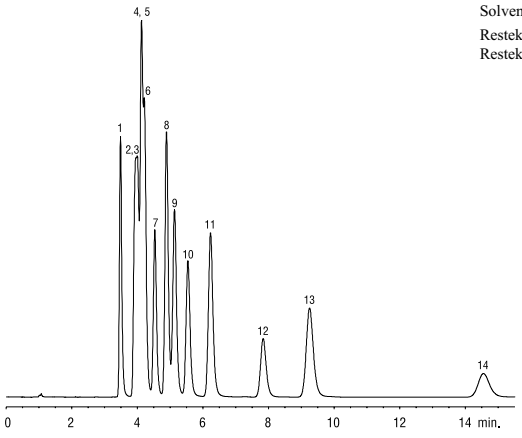
Column: **Pinnacle™ CN**
Catalog #: 9116575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 120Å

Conditions:
Mobile phase: water:methanol (50:50, v/v)
Flow: 1.5mL/min
Temp.: 27.0°C
Det.: UV @ 254nm

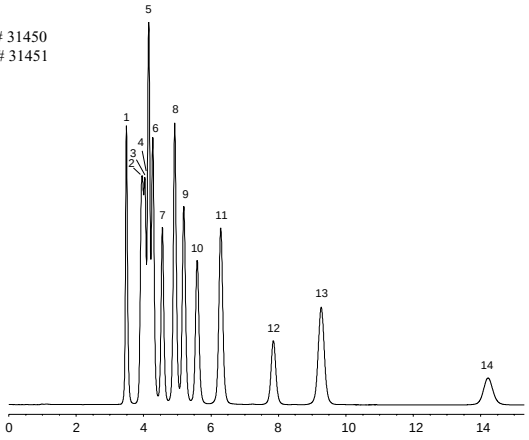
Sample:
Inj.: 3µL
Conc.: 500 µg/mL each component
Solvent: acetonitrile

Restek 8330 Calibration mix #1: Catalog # 31450
Restek 8330 Calibration Mix #2 : Catalog# 31451

Peak List:
1. nitrobenzene
2. 2-nitrotoluene
3. 4-nitrotoluene
4. 3-nitrotoluene
5. 1,3-dinitrobenzene
6. 1,3,5-trinitrobenzene
7. 2,6 dinitrotoluene
8. 2,4-dinitrotoluene
9. 2,4,6-trinitrotoluene
10. 4-amino-2,6-dinitrotoluene
11. 2-amino-4,6-dinitrotoluene
12. RDX
13. tetryl
14. HMX



LC_0193



LC_0188

Column: **Pinnacle II CN**
Catalog #: 9216575
Dimensions: 250 x 4.6mm
Particle Size: 5µm
Pore Size: 110Å

Pinnacle II™ C18 5µm Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9214551	9214552	9214553	9214555
100mm	9214511	9214512	9214513	9214515
150mm	9214561	9214562	9214563	9214565
250mm	9214571	9214572	9214573	9214575

Pinnacle II™ Cyano 5µm Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9216551	9216552	9216553	9216555
100mm	9216511	9216512	9216513	9216515
150mm	9216561	9216562	9216563	9216565
250mm	9216571	9216572	9216573	9216575

RESTEK nutraceuticals

Applicationsnote

Analyzing Nutraceutical Products by Liquid and Gas Chromatography

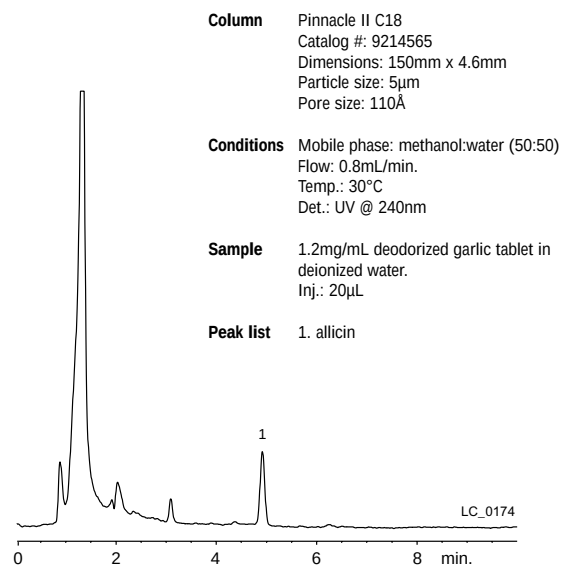
Introduction

The idea of using herbal compounds to enhance one's health has been around for thousands of years. Over the past decade, however, the nutraceutical industry has seen rapid growth as more people add flowers, leaves, roots, and fruits of botanicals to their diets in the hopes of gaining health benefits. The dietary supplement industry (including vitamins, minerals, herbals, and amino acids) was approximately $16 billion in 2000 with botanicals accounting for 25%.[1] Herbal ingredients that once were packaged primarily in pills and capsules and found in health food stores, now can be found in fruit juices, spreads, and snack foods.[2] This includes ingredients such as glucosamine, added to ease aches and pains; and kava or St. John's wort, added for calmness and a sense of well-being.

Herbal products are very complex, often containing hundreds of compounds, and it is not always clear which compounds are responsible for the beneficial properties. Marker compounds—phytochemicals that have been identified and are known to have some relationship to the reported health benefit—can be evaluated qualitatively to identify a raw material or to verify purity. To determine the concentration or strength of a material, quantitative analysis is necessary. Other testing typically performed on raw materials includes a physical exam, microscopy, and determination of ash, heavy metals, residual fumigants, and pesticide levels. Microbiological testing can be included depending on the herbal material.

Figure 1

Rapidly analyze allicin in garlic by HPLC using a Pinnacle II™ C18 column.



Dietary supplements are regulated under the Dietary Supplement Health Education Act (DSHEA) of 1994. Before DSHEA, nutraceuticals were regulated as either foods or drugs, depending on their intended purpose.[3] The Food and Drug Administration (FDA) has the authority under DSHEA to take action against unsafe products or improperly labeled products. For example, the FDA can stop the distribution of products that it finds to be toxic, unsanitary, that increase the risk of illness or injury, or that make unsubstantiated health claims. Under current regulations, however, FDA pre-market approval of dietary supplements is not required. This leaves the testing of dietary supplements to the discretion of the manufacturer.

Several organizations have recognized a need for standardizing procedures of herbal product analysis and are involved in programs that will assist the FDA in the regulation of the dietary supplement industry. The US Pharmacopoeia (USP) has launched a dietary supplement certification pilot program to address the issue of product quality. The USP program seeks to ensure that the product contains the ingredients declared on the label at the reported levels; that the product is within the required limits on contaminants; and that the general requirements for the manufacturing practices of dietary supplements are satisfied.[1] This program is meant to complement DSHEA and allows companies to add a USP certification mark on their label if the requirements are met. The pilot program includes post-market surveillance of the nutraceutical products and auditing of the manufacturing facilities. As of August 2001, the USP had over 20 official monographs for herbal materials, with many more in revision or draft form.[3]

Other organizations, such as the Institute for Nutraceutical Advancement (INA), are working to develop new methods for the quantitation of marker compounds. The goal of the INA Method Validation Program (MVP) is to submit these new methods to an organization (e.g., Association of Official Analytical Chemists [AOAC] International) for collaborative study and inclusion in their official methods program. AOAC International also has a Dietary Supplement Task Group that provides a standard set of procedures for the analysis of botanical compounds.

High performance liquid chromatography (HPLC) and gas chromatography (GC) are excellent tools for quantitative analysis of marker compounds in botanical samples. Thin layer chromatog-

raphy (TLC) also is used, primarily for the qualitative identification of herbals and for purity evaluations. HPLC is used in approximately 90% of the current methods for marker compounds and mainly relies on a reversed phase separation with UV-VIS, refractive index or light-scattering detection. GC, while not as widely applicable, is very useful for the analysis of volatile marker compounds and residual solvents. For both HPLC and GC botanical analyses, column reproducibility and robustness is very important. Restek HPLC and GC columns are able to meet the demands of analyzing these complex systems.

HPLC Analysis of Allicin in Garlic

Garlic, or Allium sativum, has the reported benefits of lowering cholesterol, reducing hypertension, and acting as an antimicrobial agent in living systems. Garlic often is added to the diet as garlic powder or garlic capsules. The active ingredients in garlic are thought to be the sulfur-containing compounds, or thiosulfinates, present in the volatile oil. Alliin, which converts to allicin (S-allyl-2-propenthiosulfinate) in the presence of the enzyme alliinase, is present at about 1% in garlic cloves.

INA Method 110.001—the analysis of allicin in garlic—is an isocratic reversed phase HPLC procedure using a C18 column, with UV detection at 240nm (Figure 1). Because allicin has limited stability in solution, samples should be analyzed soon after extracting into cold water. In addition, the allicin reference standard needs to be prepared by extracting the allicin from garlic powder by solid phase extraction (SPE) and calculating the concentration based on the UV absorbance of the standard solution.

HPLC Analysis of Hyperforin in St. John's Wort

St. John's wort, or Hypericum perforatum, is purported to ease the symptoms of mild depression, anxiety, and insomnia. The active ingredient is thought to be hyperforin, although other compounds might also contribute to the beneficial effects. St. John's wort can be added to the diet in either capsule or tablet form of the leaves, stems or flowers, or as an extract.

According to INA Method 112.001—the HPLC analysis of hyperforin and adhyperforin in St. John's wort—the samples are extracted with methanol in an ultrasonic bath. Chromatographic separation is performed on a C18 reversed phase column with acetonitrile and phosphate buffer as the mobile phase. After UV detection at 270nm, the hyperforin and adhyperforin are quantitated by comparison of the response of these compounds to the response of a standard solution of hyperforin. The analysis of a St. John's wort capsule using a Pinnacle II™ C18 column shows excellent peak shapes for the active ingredients in this herbal product (Figure 2).

HPLC Analysis of Phenolics in Echinacea

Echinacea, one of the most popular nutraceutical herbs, is thought to enhance the immune system and act as an immune stimulant against colds and flus. It can be taken in capsule or tablet form, or used in the preparation of an herbal tea. The active compounds in echinacea are thought to be caffeic acid derivatives such as caftaric acid, cichoric acid, chlorogenic acid, and echinacoside.

Figure 2

HPLC analysis of hyperforin and adhyperforin in St. John's Wort capsule using a Pinnacle II™ C18 column shows excellent peak shape for the marker compounds.

Column Pinnacle II C18
Catalog #: 9214565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions Mobile phase: acetonitrile:
0.01N phosphate buffer, pH 2.5 (85:15)
Flow: 1.2mL/min.
Temp.: 30°C, autosampler @ 15°C
Det.: UV @ 270nm

Sample St. John's Wort capsule
Inj.: 20µL
Conc.: 4.17mg/mL capsule,
contents in methanol

Peak list 1. hyperforin
2. adhyperforin

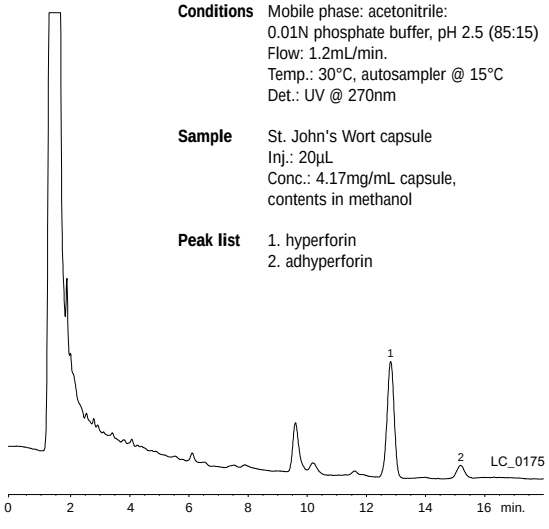


Figure 3

HPLC analysis of phenolic compounds in echinacea capsules using a Pinnacle II™ C18 column achieves resolution of four key marker compounds.

Column Pinnacle II C18
Catalog #: 9214565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 110Å

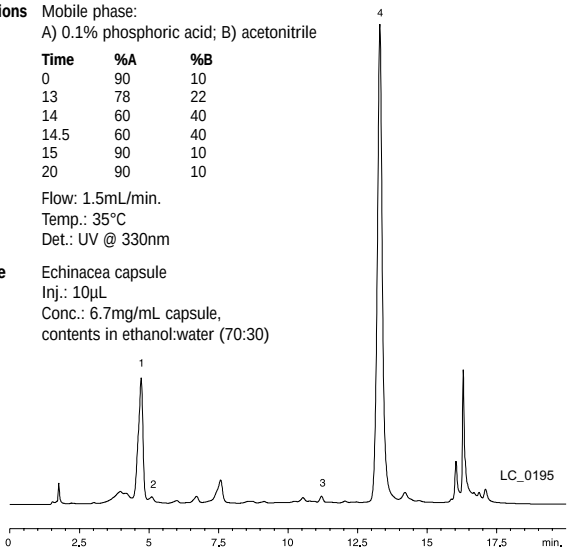
Peak list 1. caftaric acid (4.6 min.)
2. chlorogenic acid (5 min.)
3. echinacoside (11.2min.)
4. cichoric acid (13.3 min.)

Conditions Mobile phase:
A) 0.1% phosphoric acid; B) acetonitrile

Time	%A	%B
0	90	10
13	78	22
14	60	40
14.5	60	40
15	90	10
20	90	10

Flow: 1.5mL/min.
Temp.: 35°C
Det.: UV @ 330nm

Sample Echinacea capsule
Inj.: 10µL
Conc.: 6.7mg/mL capsule,
contents in ethanol:water (70:30)



According to INA Method 106.000—HPLC analysis to determine the levels of these phenolic compounds in echinacea—samples are extracted into an ethanol:water mixture with shaking. The analysis is performed on a C18 reversed phase column using a mobile phase of acetonitrile:dilute phosphoric acid. The caffeic acid derivatives are detected based on their UV absorbance at 330nm and quantitated by comparison to an external standard solution of chlorogenic acid. We used the Pinnacle II™ C18 column to analyze a capsule of echinacea and achieved resolution of four key marker compounds (Figure 3).

GC Analysis of Fatty Acids in Saw Palmetto

Saw palmetto, or Serenoa repens, has been used to treat prostrate enlargement and lower urinary tract symptoms. The partially dried, ripe fruits of this plant are typically extracted with ethanol, carbon dioxide or hexane to collect the active ingredients. Saw palmetto contains a wide range of compounds, including free fatty acids, free fatty alcohols, and monoglycerides. The free fatty acids, which make up 70-95% of the purified lipophilic extract, often are used as the marker compounds for this herbal product.

The USP monograph for saw palmetto includes a procedure for the quantitation of fatty acids by GC. To perform this analysis, the samples are pulverized to a powder, saponified, and derivatized with boron trifluoride in methanol. The test standard includes 11 fatty acid methyl esters (FAMEs) and an internal standard (IS), and the chromatographic separation is performed on a polar Carbowax® column. The analysis of free fatty acids as their methyl esters on an Rtx®-Wax capillary GC column shows excellent resolution of all of the FAMEs when analyzed according to the USP procedure (Figure 4).

Summary

An increasing number of nutraceutical ingredients and products are becoming a part of our diet. Because of this, we need accurate quantitation of the active ingredients or marker compounds in both raw materials and finished products. Restek has a wide range of HPLC and GC columns to analyze these complex systems for the nutraceutical and food and beverage industries. Restek columns offer excellent lot-to-lot reproducibility and must meet stringent quality assurance criteria before they are sent to customers. For HPLC procedures, Pinnacle II™ reversed phase columns offer excellent peak shapes for the active ingredients in a wide range of botanical compounds. The Rtx®-Wax capillary column is an excellent choice for the GC analysis of derivatized fatty acids. The Crossbond® technology used to create this column results in long column lifetimes and reproducible results.

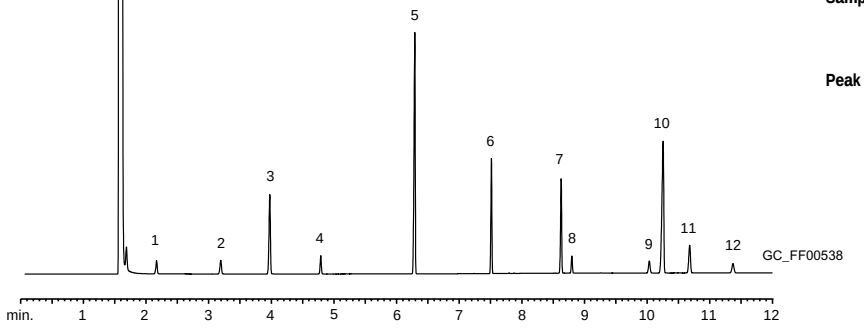
References

1. USP Quality Demonstration Program for Dietary Supplements, Draft 2.0 (2000), The US Pharmacopoeia, Rockville, MD.
2. Barnes, Julian G. and Winter, Greg. The New York Times, May 27, 2001.
3. The US Pharmacopoeia, Rockville, MD.

References not available from Restek.

Figure 4

An Rtx®-Wax capillary GC column achieves excellent resolution of the FAMEs in a saw palmetto standard.



Column Rtx®-Wax
Catalog #: 12423
Dimensions: 30m, 0.25mm, 0.25µm

Conditions Oven temp.: 120°C (hold 3 min.) to 220°C at 20°C/min. (hold 12 min.)
Inj./det. temp.: 250°C/300°C
Carrier gas: helium
Linear velocity: 1mL/min. 34cm/sec.
Split ratio: 100:1

Sample Saw palmetto standard
Inj.: 1µL
Conc.: see peak list

Peak list
1. methyl caproate (C6:0), 0.4mg/mL
2. methyl caprylate (C8:0), 0.4mg/mL
3. methyl nonanoate (C9:0), 2.0mg/mL
4. methyl caprate (C10:0), 0.4mg/mL
5. methyl laurate (C12:0), 5.0mg/mL
6. methyl myristate (C14:0), 2.0mg/mL
7. methyl palmitate (C16:0), 2.0mg/mL
8. methyl palmitoleate (C16:1), 0.4mg/mL
9. methyl stearate (C18:0), 0.4mg/mL
10. methyl oleate (C18:1), 5.0mg/mL
11. methyl linoleate (C18:2), 1.0mg/mL
12. methyl linolenate (C18:3), 0.4mg/mL

Pinnacle II™ 5µm C18 HPLC Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9214551	9214552	9214553	9214555
100mm	9214511	9214512	9214513	9214515
150mm	9214561	9214562	9214563	9214565
250mm	9214571	9214572	9214573	9214575

Pinnacle II™ 5µm C8 HPLC Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9213551	9213552	9213553	9213555
100mm	9213511	9213512	9213513	9213515
150mm	9213561	9213562	9213563	9213565
250mm	9213571	9213572	9213573	9213575

Pinnacle II™ 5µm Cyano HPLC Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9216551	9216552	9216553	9216555
100mm	9216511	9216512	9216513	9216515
150mm	9216561	9216562	9216563	9216565
250mm	9216571	9216572	9216573	9216575

Pinnacle II™ 5µm Phenyl HPLC Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9215551	9215552	9215553	9215555
100mm	9215511	9215512	9215513	9215515
150mm	9215561	9215562	9215563	9215565
250mm	9215571	9215572	9215573	9215575

Trident™ Direct Guard Column System*

Description	qty.	cat.#
High pressure filter	each	25082
1cm guard cartridge holder with filter	each	25084
2cm guard cartridge holder with filter	each	25086
PEEK® connection tip for Waters®-style end fittings	each	25088
Replacement cap frits: 4mm, 2.0µm	5-pack	25022
Replacement cap frits: 4mm, 0.5µm	5-pack	25023
Replacement cap frits: 2mm, 2.0µm	5-pack	25057

**The standard PEEK® tip in Trident™ Direct systems is compatible with Parker, Upchurch®, Valco®, and other CPI-style fittings. To use Trident™ Direct systems with Waters®-style end fittings, the tip must be replaced with cat.# 25088.*

Other Literature:

Food, Flavor, & Fragrances Catalog (lit. cat. #59260)

HPLC Catalog (lit. cat. #59241)

Pinnacle™ II New Product Flyer (lit. cat. #59281)

Selection Guide for Polar WAX GC Columns (lit. cat. #59890)

The Institute for Nutraceutical Advancement (INA) Validates GC Methods for Saw Palmetto Using Rtx®-5 and Stabilwax® Columns Applications Note (lit. cat. #59136)

Analyzing the Heat Levels of Hot Sauces Using an Ultra C18 HPLC Column Applications Note (lit. cat. #59199)

Monitoring Volatiles in Food Contact Packaging by Purge & Trap GC/MS Applications Note (lit. cat. #59348)

Analyzing Free Fatty Acids Using a Stabilwax® DA Column Applications Note (lit. cat. #59155B)

Rtx®-Wax Fused Silica Capillary Columns

ID	df	15-meter	30-meter	60-meter
0.25mm	0.10	12405	12408	—
	0.25	12420	12423	12426
	0.50	12435	12438	12441
0.32mm	0.10	12406	12409	—
	0.25	12421	12424	12427
	0.50	12436	12439	12442
	1.00	12451	12454	12457
0.53mm	0.25	12422	12425	—
	0.50	12437	12440	12443
	1.00	12452	12455	12458
ID	**df**	**10-meter**	**20-meter**	
0.10mm	0.10	41601	41602	
	0.20	41603	41604	

Resprep™ SPE Cartridge—Reversed Bonded Phase

Description	1mL 100mg 100-pk	3mL 200mg 50-pk	3mL 500mg 50-pk	6mL 500mg 30-pk	6mL 1000mg 30-pk	20mL 5g 20-pk	60mL 10g 16-pk
C18	26030	26031	24050	24052	24051	26034	26035
C8	26036	26037	26038	26039	26040	—	—

RESTEK
Petrochemical

Applications note

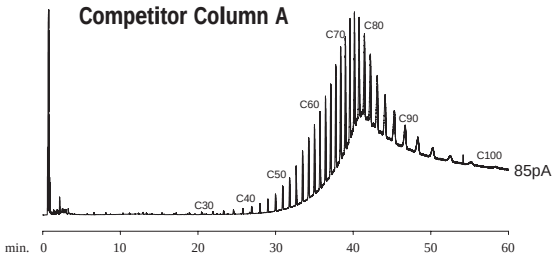
Fast, High-Temperature Sim Dist Analyses Using MXT®-1 HT Sim Dist Capillary GC Column and the GC Racer

ASTM Method D-6352 is a gas chromatography (GC) method developed for the determination of petroleum distillates with a boiling point range of 174˚C to 700˚C. Often referred to as "high-temperature simulated distillation," this method requires a capillary column capable of withstanding GC oven temperatures up to 430˚C. This presents many challenges for analysts because most capillary columns are manufactured using polyimide-coated fused silica tubing. At temperatures above 380˚C, even the best polyimide coating becomes brittle, which leads to very short column lifetimes. In addition, the methyl silicone stationary phase recommended in the method also must survive these high temperatures.

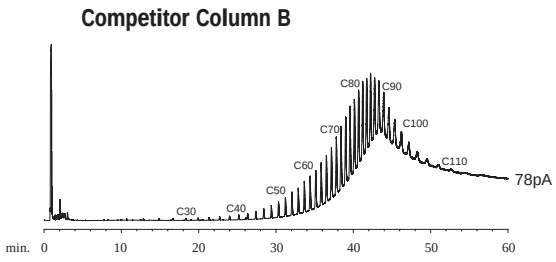
The MXT®-1 HT Sim Dist column is a major improvement in column technology for high-temperature simulated distillation. By combining a new, proprietary polymer synthesis technology, Siltek™ deactivation, and rugged Silcosteel® tubing, we developed a capillary column that meets all the criteria of ASTM Method D-6352. This MXT®-1HT Sim Dist column is available as in a 5m, 0.53mm ID, 0.1µm film dimension to conform to the requirements of this method. It exceeds the resolution, peak shape, and bleed criteria for hydrocarbons ranging up to C-110. Because the MXT®-1HT Sim Dist column is coated with a 100% dimethyl polysiloxane polymer, it will give the correct retention time/boiling point curve. The MXT®-1HT Sim Dist column exhibits low bleed and excellent inertness, and the rugged Silcosteel® tubing will hold up to temperatures in excess of 430°C.

To demonstrate the lower bleed and improved peak shape of this innovative column, a Polywax® 1000 reference material was analyzed using a MXT®-1HT Sim Dist column and two other columns that are commonly used for this application **(Figure 1)**. The MXT®-1 HT Sim Dist column exhibits lower bleed and improved peak symmetry compared to competitor columns.

Figure 1
The MXT-1HT Sim Dist column offers lower bleed and improved peak symmetry compared to other high temperature columns.



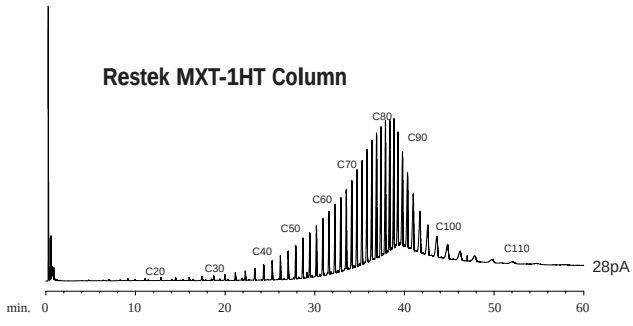
GC_PC00541



GC_PC00542

Run Conditions: C44-C100 Hydrocarbons

Column:	MXT-1HT Sim Dist
Serial #:	339749B
Description:	5m, 0.53mmID, 0.10µm
Sample:	Polywax® 1000
Part #:	36227
Solvent:	Carbon Disulfide
Sample Size:	0.2uL
Instrument:	HP6890 GC
Injector:	on-column (track oven)
Carrier Gas:	Helium(constant pressure)
Head Pressure:	1.0psi
Linear Velocity:	60cm/sec.
Flow Rate:	7.8mL/min.
Detector:	FID/430°C
Make up Gas Flow:	40cc/min.
Temp. Program:	40°C to 430°C@10°C/min. (hold 30 min.)



GC_PC00530

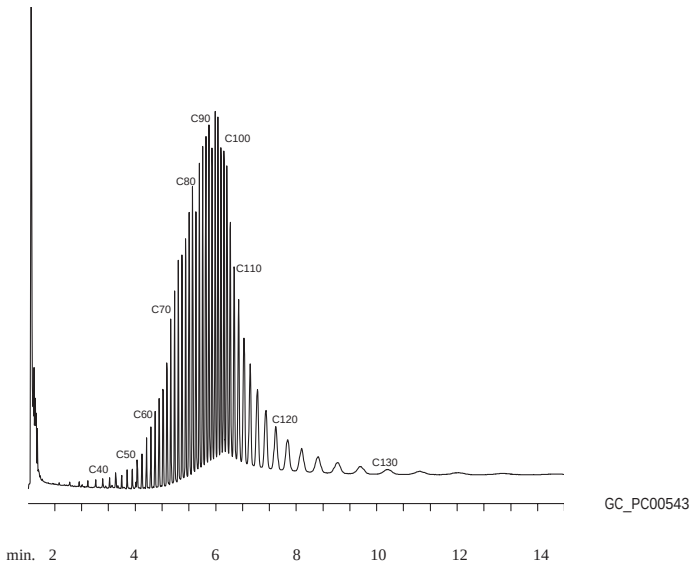
Petrochemical

To maintain the low bleed and high performance of the MXT®-1HT Sim Dist column, it is critical to prevent oxygen from entering the column. This can be achieved by checking your entire system for leaks before and after each thermal cycle. We also recommend the use of graphite ferrules; Vespel® or Vespel®/graphite ferrules will not withstand the high temperatures required for this analysis as they may crack.

As part of cost reduction efforts, many laboratories try to reduce individual analysis time in the interest of increasing overall throughput. High-temperature simulated distillation analyses can take as long as an hour, especially when samples contain hydrocarbons up to C110. An effective technique to reduce analysis time is to use rapid temperature programming. Unfortunately, most GC systems have temperature-programming limitations of 20˚C to 25˚C/min. Some GCs can heat quickly at low temperatures, but cannot maintain the fast rate at higher temperatures, like those needed for Sim Dist. To overcome these limitations, Restek offers the GC Racer, an attachment to your Agilent 5890A GC and 5890 Series II, that increases the rate of temperature programming. Using the GC Racer, the analysis of the Polywax® 1000 reference material can be reduced from over 50 minutes to less than 15 minutes by temperature programming at 60˚C/min. **(Figure 2)**!

The Restek MXT®-1HT Sim Dist column is the ideal choice for high-temperature simulated distillation. It meets all the criteria of ASTM Method D-6352 while providing low bleed, excellent peak shape and resolution. Combining the MXT®-1HT Sim Dist column with the GC Racer attachment significantly reduces overall analysis time and greatly increases sample throughput.

Figure 2
Significantly reduce simulated distillation analysis time using the MXT®-1HT Sim Dist column and the GC Racer attachment.



Column:	MXT-1HT Sim Dist
Description:	5m, 0.53mmID, 0.10um
Part #:	70100
Sample:	Polywax 1000
Solvent:	Carbon Disulfide
Sample Size:	0.2ul
Sample:	Polywax 1000
Solvent:	Carbon Disulfide
Sample Size:	0.2ul
Temp. Program:	40°C 60°C/min. to 430°C (30min.)

Product Listing

MXT®-1HT Sim Dist (metal column)

ID	df (µm)	temp. limits	5-Meter
0.53mm	0.10	-60 to 430°C	70100

Polywax Standards

Decription	qty.	cat#
Polywax 1000	1 gram	36227

GC Racer™ GC Temperature Programmer

Decription	qty.	cat#
For Agilent 5890 Series II (only) GC	ea.	23024
For Agilent 5890A (only) GC	ea.	23025

For more information on the GC Racer attachment contact Restek's Technical Service Department or your local representative.

RESTEK HPLC

Applicationsnote

Analysis of Preservatives Using HPLC

Background

Preservatives are chemical compounds that are used in a wide range of applications to maintain overall product quality.[1] For example, preservatives are used in foods, beverages, pharmaceuticals, and personal care products. Some preservatives act as antimicrobial agents, some act as antioxidants, and some can perform both functions. To some extent, the ability of the chemical to act as a preservative depends on the environment, so factors such as the type of product, water content, pH, and storage conditions all need to be considered when selecting a preservative.

Of the chemical compounds commonly used as preservatives, many of them can be effectively analyzed by high performance liquid chromatography (HPLC).[2] Chromatographic techniques such as HPLC separate preservative compounds from the rest of the sample matrix, providing more accurate results compared to traditional techniques such as spectrophotometry. Because preservatives include a number of different compound types, there are a variety of HPLC stationary phases, mobile phases, and detectors that can be used.

Microbial Growth Inhibitors

Microbes such as molds, yeasts, and bacteria need to have certain conditions (e.g., water, pH, temperature) in order to flourish. Chemical preservatives can be used to kill or prevent the growth of these microbes by either changing their environment or reacting directly with the microbes.[3] Selecting the best preservative for a given product can be an important part of product development. In addition, other chemical compounds known as synergists can increase the effectiveness of some preservatives. Examples of synergists include: citric acid, isopropyl citrate, phosphoric acid, ascorbic acid, ascorbyl palmitate, tartaric acid, and lecithin.

Antimicrobial compounds include organic acids, benzoate and sorbate salts, sulfur dioxide and sulfites, nitrites, propionates, and parabens. Some antimicrobials act on yeasts, molds, and bacteria, while some specifically target certain classes of microbes. Sulfites can inhibit bacteria, but are not effective against yeasts. For this reason, they often are used as preservatives in wines. Nitrites can inhibit botulism (bacterial spores) in meats. Sorbates and benzoates are specific inhibitors of bacteria, while propionates act on molds and rope bacteria, but not on yeasts. Therefore propionates can be used in yeasted bread products. The parabens have both antimicrobial activity and antioxidant activity.

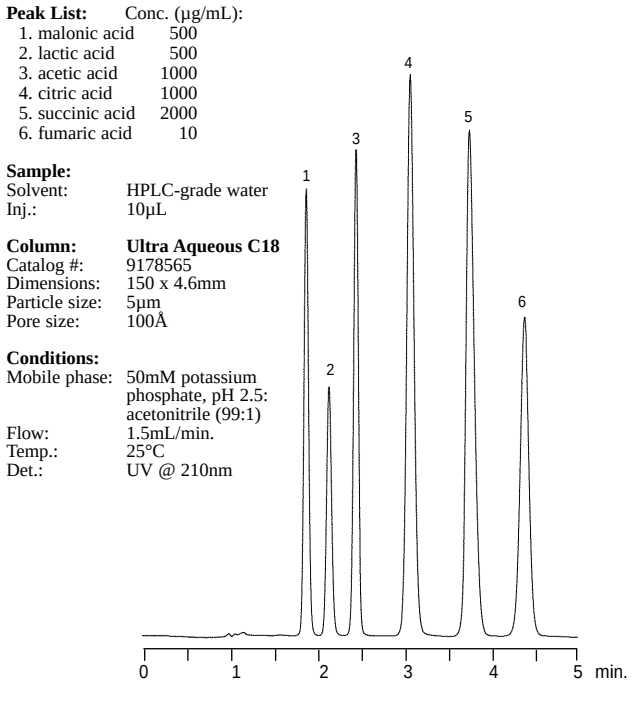
Organic acids, such as acetic acid and citric acid, can be used to control the pH of a product. For example, in food products these acidulants can lower the pH out of the optimum pH range for bacteria, yeast, and/or molds. Organic acids such as malic acid and citric acid can be found naturally in fruits; oxalic acid can be found in spinach and rhubarb; and tartaric acid can be found in grapes.

Using HPLC, concentrations of these preservatives can be monitored. However, analyzing polar organic acids can be difficult on conventional reversed phase columns, even when using highly aqueous mobile phases. The Ultra Aqueous C18 column provides enhanced retention and selectivity for challenging applications such as this. The novel bonding chemistry used for this phase allows the alkyl groups to remain extended, even in highly aqueous mobile phase, preventing the chain folding that occurs with conventional C18 phases. Therefore, stable and reproducible retention is possible even with 100% aqueous mobile phases. Notice the excellent retention for a series of organic acids using the Ultra Aqueous C18 column and UV detection (Figure 1).

Benzoate and sorbate salts also can be used as preservatives in a range of consumer products. These salts interact with the bacteria itself, limiting the viability of the microorganism. These compounds can be analyzed in their acid form (i.e. as benzoic acid and

Figure 1

Analysis of a series of organic acids typically found in items such as foods, beverages, and personal care products, using an Ultra Aqueous C18 column shows excellent retention of organic acids, even with a highly aqueous mobile phase.



HPLC

sorbic acid) by reversed phase HPLC using a Pinnacle II™ Phenyl column and acidified water:methanol as the mobile phase (Figure 2). By monitoring the UV absorbance at 245nm, sensitive detection of both benzoic and sorbic acids can be achieved. For optimum sensitivity, benzoic acid can be monitored at 228nm and sorbic acid at 259nm.

Parabens, such as propyl paraben, can be used as antimicrobial agents and as antioxidants. As antimicrobials, they act on yeasts and molds. Although they tend to be somewhat higher in cost, they still are used in a range of applications. The maximum allowable concentration in most products is 0.1%. A series of parabens analyzed using a Pinnacle II™ C8 column is shown in Figure 3. The separation was effected using an acidified mobile phase to suppress ionization of the analytes. Because these compounds are strong UV absorbers, very sensitive detection can be achieved by monitoring the UV at 254nm.

Antioxidants

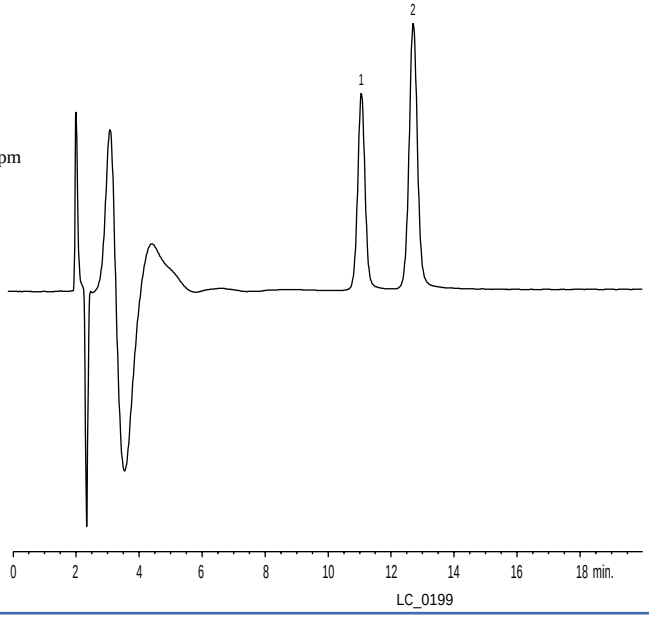
Products containing fats and oils are prone to lipid oxidation, which can promote off-flavors, off-odors, and color changes as well as limiting shelf life. To inhibit lipid oxidation, antioxidants

Figure 2

The Pinnacle II™ phenyl column is an excellent choice to analyze medium polarity compounds such as sorbic and benzoic acids.

Peak List:
1. sorbic acid
2. benzoic acid

Sample:
Inj.: 10µL
Conc.: each 100ppm
Solvent: methanol



Column: **Pinnacle II™** Phenyl
Catalog #: 9215565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 110Å

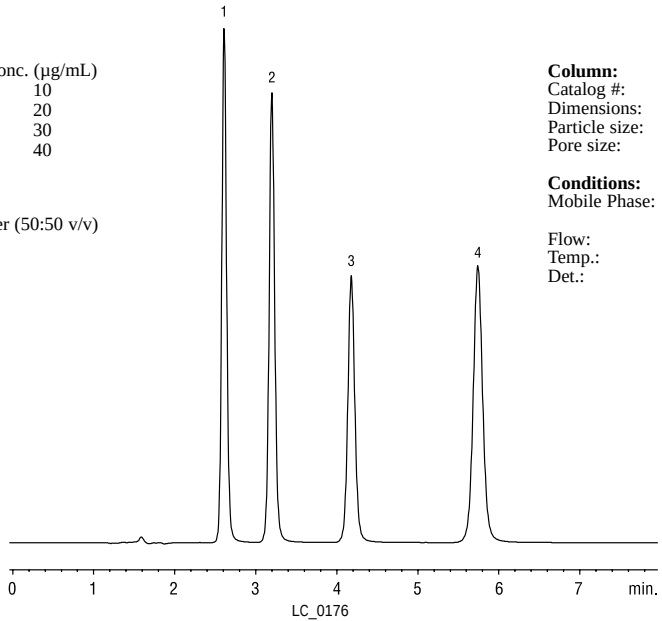
Conditions:
Mobile Phase: 1% acetic acid:methanol (80:20)
Flow: 1.0 mL/min
Temp.: 25°C
Det.: UV @ 230nm

Figure 3

A series of parabens can be efficiently separated on a Pinnacle II™ C8 column, allowing for fast and accurate quantitation.

Peak List:	conc. (µg/mL)
1. methyl paraben	10
2. ethyl paraben	20
3. propyl paraben	30
4. butyl paraben	40

Sample:
Inj.: 5µL
Solvent: methanol:water (50:50 v/v)



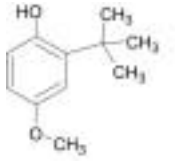
Column: **Pinnacle II™ C8**
Catalog #: 9213565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile Phase: 0.1% acetic acid in water: acetonitrile (50:50, v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

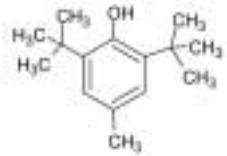
Figure 4

Structures of three commonly-used phenolic antioxidants.

BHA



BHT



PG

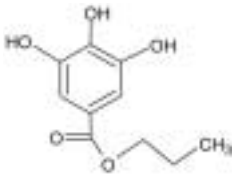


Figure 5

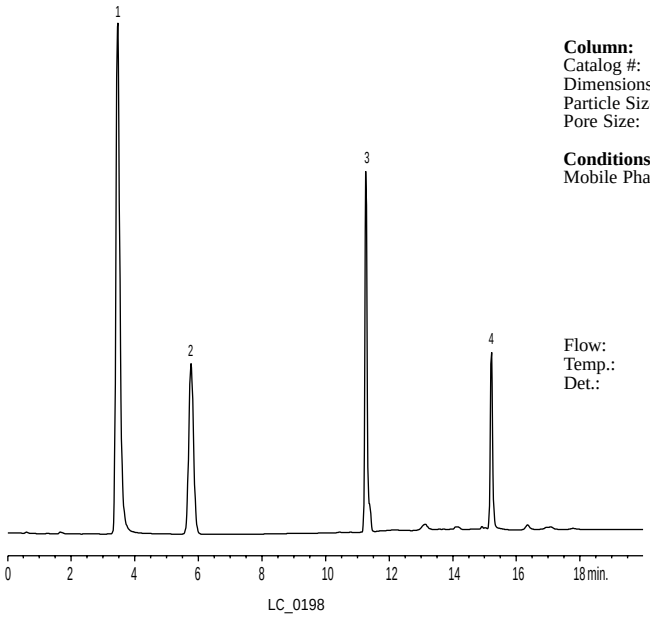
Phenolic antioxidants can be quantitated easily using a Pinnacle II™ C18 column and UV detection at 280nm.

Peak List: **conc.:** (ppm)

1. propyl gallate	168
2. TBHQ	182
3. 2-BHA + 3-BHA	197
4. BHT	193

Sample:

Inj.:	10µL
Conc.:	see peak list
Solvent:	methanol



Column: **Pinnacle II™ C18**

Catalog #:	9214565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	110Å

Conditions:

Mobile Phase: A = 1% acetic acid
B = methanol

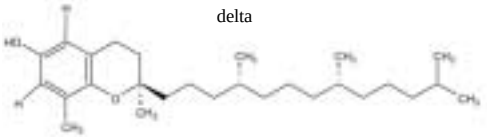
Time (min.)	A (%)	B (%)
0	50	50
4	50	50
10	10	90
25	10	90
26	50	50

Flow:	1.0mL/min
Temp.:	30°C
Det.:	UV @ 280nm

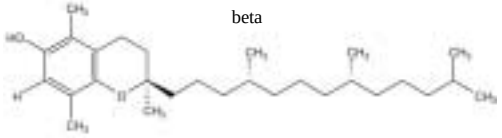
Figure 6

Structures of tocopherols—these compounds readily oxidize when exposed to light or oxygen. (the activity is as follows: $\delta > \gamma > \beta > \alpha$)

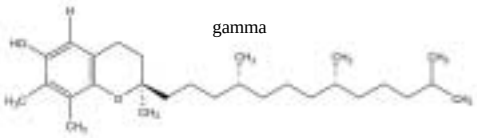
delta



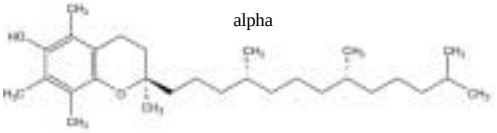
beta



gamma



alpha



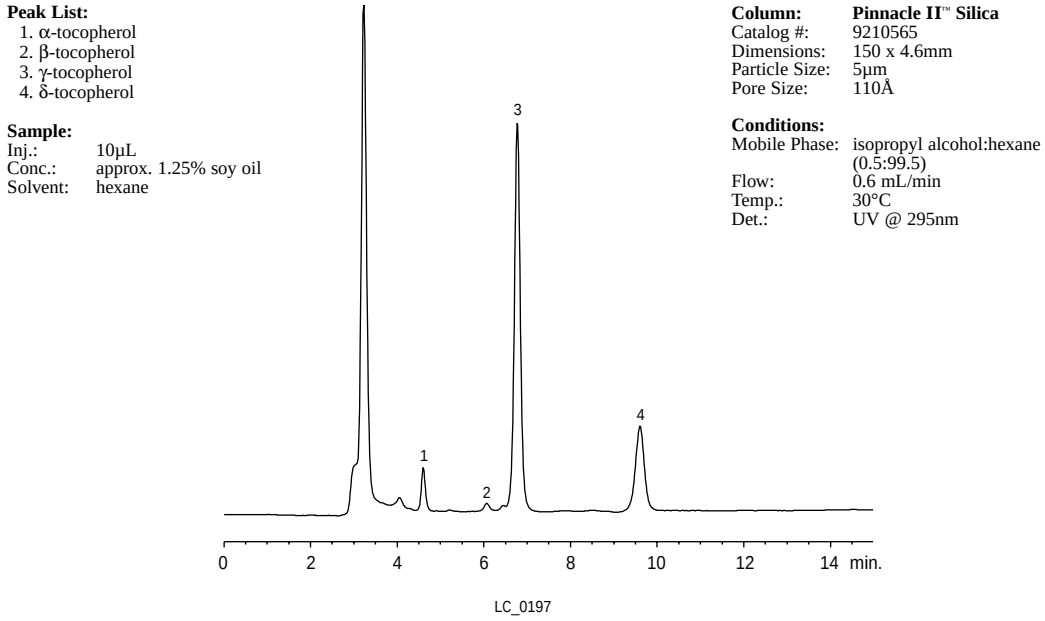
can be added to the product. Phenolic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ). These four, plus the tocopherols, act as the primary antioxidants found in foods and beverages produced in the US. The structures of BHA, BHT, and PG—the three most common phenolic antioxidants—are shown in Figure 4. Phenolic antioxidants, such as BHT, are regulated by the FDA, and can be added to many products at levels up to 200ppm based on the fat content.

Phenolic antioxidants can be analyzed by reversed phase HPLC using a Pinnacle II™ C18 column and an acidified mobile phase. As with the previous methods, an acidic mobile phase is used to suppress ionization of the analytes. The HPLC separation of BHA, BHT, PG, and TBHQ using UV detection at 280nm shows how effectively these compounds can be separated using the Pinnacle II™ C18 column (Figure 5).

"Natural" antioxidants, such as tocopherols and tocotrienols, are used to inhibit lipid oxidation and to promote general health in the consumer. These compounds are found naturally in products such as fats and oils. When used as additives, however, they are regulated. Antioxidants such as tocopherols can be challenging to analyze, because they readily oxidize when exposed to light or oxygen. The structures of four different tocopherols are shown in Figure 6. The analysis of these tocopherols by normal phase

Figure 7

A Pinnacle II™ silica column effectively separates the positional isomers of tocopherol by normal phase HPLC.



HPLC using a Pinnacle II™ silica column shows how effectively these positional isomers can be separated (Figure 7). These compounds can be quantified using either fluorescence or UV detection.

Summary

Preservatives are added to a wide range of products to help maintain the overall quality of the product as well as to extend its shelf life. The level of each preservative compound present needs to be monitored for several reasons. First, because many of these products are consumed, the preservative content needs to be measured to ensure a safe level. Second, the stated shelf-life is dependent upon a certain concentration of preservatives and incorrect concentrations can lead to premature spoilage of the product.

HPLC is a powerful tool for analyzing preservatives in a wide range of consumer products. One of its benefits is that many times only minimal sample preparation is required. Chromatographic techniques allow analysts to separate preservatives from other compounds in the sample matrix, improving the overall quality of the results. The Pinnacle II™ line of HPLC columns is an excellent choice for the analysis of preservative compounds such as parabens, benzoate and sorbate salts, phenolic antioxidants, and tocopherols. Pinnacle II™ columns are available in a range of stationary phases, including C18, C8, phenyl, amino, and silica. For analyzing organic acids, the Ultra Aqueous C18 column is the perfect choice, offering superior retention and reproducibility for polar compounds, even when using highly aqueous mobile phases.

References

1. Fennema, Owen R. Food Chemistry (1996), Marcel Dekker, New York.
2. Nollet (ed.), Food Analysis by HPLC (2000), 2nd edition, Marcel Dekker, New York.
3. Foulke, Judith E. "A Fresh Look at Food Preservatives" in FDA Consumer (October 1993), US. Food & Drug Administration.

Ultra Aqueous C18 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9178531	9178532	9178533	9178535
50mm	9178551	9178552	9178553	9178555
100mm	9178511	9178512	9178513	9178515
150mm	9178561	9178562	9178563	9178565
200mm	9178521	9178522	9178523	9178525
250mm	9178571	9178572	9178573	9178575

Ultra Aqueous C18 Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	917850212	3-pk.
10 x 4.0mm	917850210	3-pk.
20 x 2.1mm	917850222	2-pk.
20 x 4.0mm	917850220	2-pk.

Pinnacle II™ C18 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9214531	9214532	9214533	9214535
50mm	9214551	9214552	9214553	9214555
100mm	9214511	9214512	9214513	9214515
150mm	9214561	9214562	9214563	9214565
200mm	9214521	9214522	9214523	9214525
250mm	9214571	9214572	9214573	9214575

Pinnacle II™ C18 Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	921450212	3-pk.
10 x 4.0mm	921450210	3-pk.
20 x 2.1mm	921450222	2-pk.
20 x 4.0mm	921450220	2-pk.

Pinnacle II™ C8 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9213531	9213532	9213533	9213535
50mm	9213551	9213552	9213553	9213555
100mm	9213511	9213512	9213513	9213515
150mm	9213561	9213562	9213563	9213565
200mm	9213521	9213522	9213523	9213525
250mm	9213571	9213572	9213573	9213575

Pinnacle II™ C8 Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	921350212	3-pk.
10 x 4.0mm	921350210	3-pk.
20 x 2.1mm	921350222	2-pk.
20 x 4.0mm	921350220	2-pk.

Pinnacle II™ Silica 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9210531	9210532	9210533	9210535
50mm	9210551	9210552	9210553	9210555
100mm	9210511	9210512	9210513	9210515
150mm	9210561	9210562	9210563	9210565
200mm	9210521	9210522	9210523	9210525
250mm	9210571	9210572	9210573	9210575

Pinnacle II™ Silica Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	921050212	3-pk.
10 x 4.0mm	921050210	3-pk.
20 x 2.1mm	921050222	2-pk.
20 x 4.0mm	921050220	2-pk.

Pinnacle II™ Phenyl 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9215531	9215532	9215533	9215535
50mm	9215551	9215552	9215553	9215555
100mm	9215511	9215512	9215513	9215515
150mm	9215561	9215562	9215563	9215565
200mm	9215521	9215522	9215523	9215525
250mm	9215571	9215572	9215573	9215575

Pinnacle II™ Phenyl Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	921550212	3-pk.
10 x 4.0mm	921550210	3-pk.
20 x 2.1mm	921550222	2-pk.
20 x 4.0mm	921550220	2-pk.

Trident™ Direct Guard Column System

Unlike "one size fits all" guard systems, the Trident™ Direct system gives you the power to select the right level of protection for your analysis. The system offers three levels of protection and guard cartridges in four dimensions, with a variety of bonded phases to match your analytical column. The economical, leak-free cartridge design provides an unprecedented combination of convenience, economy, and reliability. The foundation of the Trident™ Direct system is a reusable direct connect holder that easily attaches to any HPLC column using CPI- or Waters®-style end fittings.* The system is available in the following configurations to match different protection level needs: in-line filter, in-line filter with holder for 1cm guard cartridge, and in-line filter with holder for 2cm guard cartridge. The guard cartridges are available in 2.1 and 4.0mm ID and are interchangeable with the appropriate length holder.

Trident™ Direct provides three levels of protection

Trident™ Direct high-pressure filter
Protection against particulate matter.
cat.# 25082

Trident™ Direct 1cm guard cartridge holder with filter
Moderate protection against particulate matter and irreversibly adsorbed compounds.
cat.# 25084

Trident™ Direct 2cm guard cartridge holder with filter
Maximum protection against particulate matter and irreversibly adsorbed compounds.
cat.# 25086

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Applicationsnote

Analysis of Narcotics and Narcotic / Acetaminophen Admixtures: What to do When Compendium Methods Don't Work

At some point in their careers, especially if performing raw materials or generic testing analyses for pharmaceuticals, analytical chemists are referred to compendium methodologies, most often to the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), or the British Pharmacopoeia (BP), but occasionally to other volumes. Often the methods described in these compendia provide less than the desired robustness in separation and reproducibility, or the results may be marginal—barely passing system suitability requirements. Sometimes a particular delivery system formulation absolutely will not work with a generic method, due to interference from other ingredients in the sample. Modifications must be made to the problem methodology, and the results compared statistically to the original. To improve analysis efficiency and reduce laboratory supply costs associated with revalidating and testing a method, it may be desirable to create and validate a single analytical method for a wide range of similar drug products.

Many narcotics are very similar in structure, often varying by only a single substitution. Morphine, codeine, hydrocodone, and oxycodone are quite similar, for example (Figure 1). Some of these closely related compounds—all but morphine, in fact—might be blended with other analgesics, such as acetaminophen (APAP). However, USP 25 describes more than 7 different methods to test these raw materials and admixtures, and some of these older raw material methods do not use HPLC as a primary test for purity.

One of the chromatographic applications in USP 25 is for the analysis of oxycodone raw material. After reading the mobile phase section, which lists sulfonic acids, triethylamine, water, phosphoric acid and methanol as the constituents, we saw potential problems with the method, including:

1) The use of methanol in this analysis can lead to high background absorption and loss of linear range, because the analytical wavelength is 206nm, and the UV cutoff point for methanol is 235nm. In extreme cases this also can reduce sensitivity, because the lamp is a finite energy source—the more energy the background absorbs, the less is available to the sample.

2) An ion-pairing agent (heptane sulfonic acid) is introduced into the mobile phase without a proper buffer to maintain pH at a known level. This situation can lead to widened peaks, tailing peaks, and retention time drift. The goal of ion pairing is to create a "neutral" species.

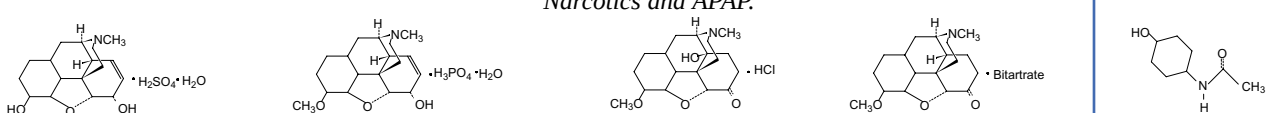
3) TEA modifier is included in the method. When basic compounds are analyzed on older-type HPLC columns, TEA often is added as competing base, to reduce the tailing caused by acidic silanol activity. If the analytical species are neutral, or have been "neutralized" by the addition of an ion-pairing agent, the addition of TEA should have no beneficial effect. Since TEA is a base, adding it to a mobile phase containing sulfonic acids will instantly cause an acid/base neutralization, producing a salt and water and reducing the effective concentration of the acidic ion-pairing agent. This reaction could lead to the formation of undesirable side products in the mobile phase that also will absorb in the low UV range, creating noisy baselines. Furthermore, TEA is volatile, and its composition might change over time if the mobile phase is sparged.

With these concerns in mind, we tested the USP method, using our Ultra and Pinnacle II™ C-8 columns (Application 1).

Initial analyses were performed according to USP 25. The Ultra C8 column gave the better performance at the specified detection wave-

Figure 1

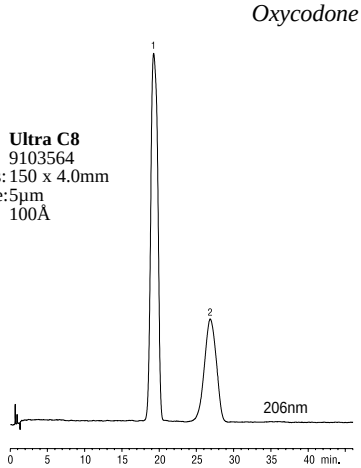
Narcotics and APAP.



Application 1

Oxycodone HCl raw material monograph performed per USP 25.

Column: **Ultra C8**
Catalog #: 9103564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 100Å

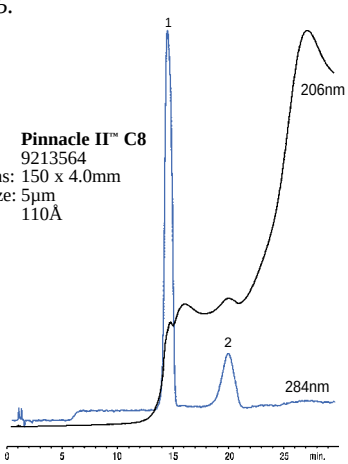


Peak List: **Conc.** (µg/mL)
1. codeine phosphate 13
2. oxycodone HCl 9

Sample:
Inj.: 10µL
Sample: resolution solution
Solvent: mobile phase

Conditions:
Mobile phase: A: 5mM hexane sulfonic acid with 2mL TEA and 3mL of phosphoric acid per liter. pH adjusted to 2.5
B: methanol
(90A:10B, v/v)
Flow: 1.5mL/min
Temp.: 40°C
Det.: UV @ 206 and 284nm

Column: **Pinnacle II™ C8**
Catalog #: 9213564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 110Å



Pharmaceutical

length, 206nm. The Pinnacle II™ C8 column performed well at 284nm, another wavelength commonly used for analyzing narcotics. At 206nm the Pinnacle II™ C8 column exhibited significant noise and took excessive time to equilibrate, due to the competing mobile phase constituents. A longer equilibration might have solved the problem. Both columns meet the criteria—with the exception of the wavelength change with the Pinnacle II™ column—for USP 25 system suitability.

Overall both columns behaved extremely well in performing the USP 25 oxycodone HCl raw material method. However many aspects of the method appear redundant and might actually be compromising the separation. In addition, some of the reagents, such as TEA, might not be necessary for modern columns. The fewer reagents a mobile phase contains, the smaller the control that should be needed to affect a robust and practical separation. After performing the USP 25 method as written, we made some tests to determine actual needs to achieve the system suitability requirements as specified.

The first step in simplifying a convoluted analysis is to apply the KISS principle (Keep it Simple, Scientist!). With peak shape, separation, and proper analytical technique in mind, we attempted to eliminate some of the perceived problems. We realized that by using 284 nm as the detection wavelength we might not see some impurities, but in real life the material should be tested against some known source for potency. Note that with the additional reagents removed, both columns provided good results at the 206nm wavelength.

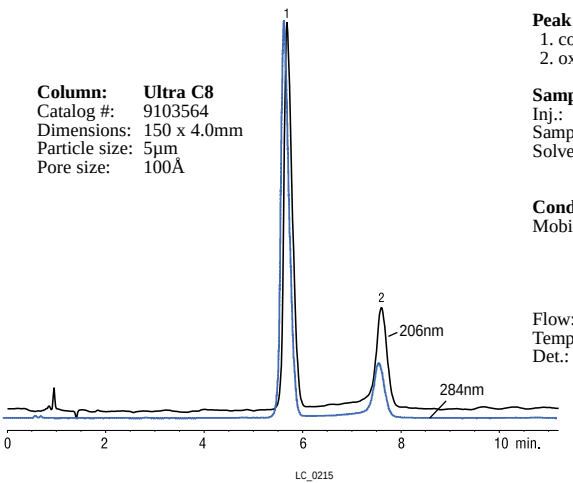
Next we removed the ion pairing agent and the TEA. We elected to keep a 20 mmolar phosphate buffer system to maintain a pH of 2.5. Then we reduced the temperature to 27°C. This reduced fluctuations in retention time caused by changes in air temperature (i.e., air conditioning), and prevented the increase in mass transfer and solubility in the mobile phase from masking other potential problems. The temperature change also helped promote column longevity; phosphate buffers tend to dissolve silica more readily at higher temperatures.

These changes led to a slight increase in tailing for all compounds on both columns, but the difference was acceptable, especially because the run time for the analysis was reduced by a factor of 3 and resolution was improved by 59% to 79%(Application 2). The system passed the system suitability requirements set forth in the USP monograph.

In the next experiment, we re-introduced the ion pair reagent hexane

Application 2

Ion pair agents and TEA removed—run time decreases 3x and resolution improves.

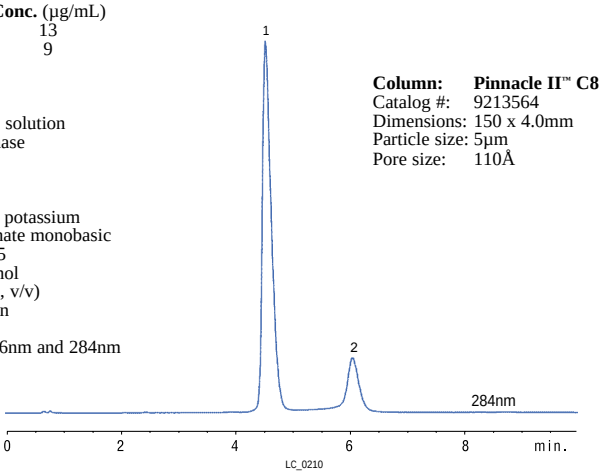


Column: **Ultra C8**
Catalog #: 9103564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 100Å

Peak List: **Conc.** (µg/mL)
1. codeine phosphate 13
2. oxycodone HCl 9

Sample:
Inj.: 10µL
Sample: resolution solution
Solvent: mobile phase

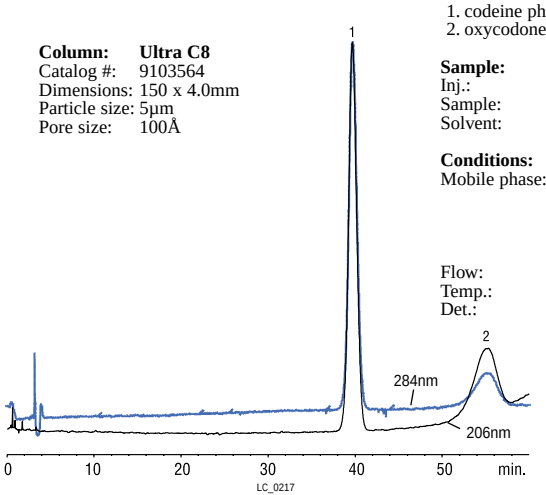
Conditions:
Mobile phase: A: 20mM potassium phosphate monobasic pH=2.5
B: methanol
(90A:10B, v/v)
Flow: 1.5mL/min
Temp.: 27°C
Det.: UV @ 206nm and 284nm



Column: **Pinnacle II™ C8**
Catalog #: 9213564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 110Å

Application 3

Hexane sulfonic acid plus a controlled buffer system doubles analysis time.

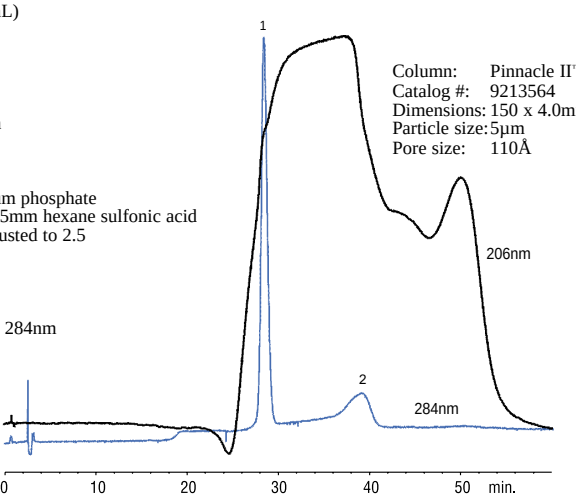


Column: **Ultra C8**
Catalog #: 9103564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 100Å

Peak List: **Conc.** (µg/mL)
1. codeine phosphate 13
2. oxycodone HCl 9

Sample:
Inj.: 10µL
Sample: resolution solution
Solvent: mobile phase

Conditions:
Mobile phase: A: 20mM potassium phosphate monobasic and 5mm hexane sulfonic acid in water pH adjusted to 2.5
B: methanol
(90A:10B, v/v)
Flow: 1.5mL/min
Temp.: 27°C
Det.: UV @ 206nm and 284nm



Column: Pinnacle II™ C8
Catalog #: 9213564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 110Å

sulfonic acid into the system under the control of the pH 2.5 phosphate buffer system. The run time doubled, demonstrating that TEA did affect the concentration of the ion-pairing agent. Reducing the concentration of ion pairing agent, or using a shorter chain length ion-pairing agent, might have been a better alternative to adding TEA. The system still passed the system suitability requirements listed by the USP, but the chromatogram was much noisier—and equilibration problems returned (Application 3).

In addition to oxycodone HCl raw material, we chromatographed hydrocodone bitartrate raw material, morphine sulfate, and an admixture of hydrocodone bitartrate and acetaminophen. All methods followed USP 25 requirements and all chromatograms passed system suitability requirements (Application 4).

After reviewing the monographs for admixtures containing structurally related narcotics and acetaminophen, we created a single separation for morphine sulfate, acetaminophen, codeine phosphate, oxycodone HCl, and hydrocodone bitartrate. The goal was to create an adequate separation while keeping the method as simple as possible. For this purpose we chose an Ultra C18 column and set detection to 235nm. All components, including a small unknown peak, were separated to baseline (Application 5).

Next, we increased the amount of buffer to 90% (a 5% increase), to determine the effect on the separation. The separation was very sensitive to the amount of buffer present; this simple 5% increase doubled the analysis time. Resolution doubled between most components, with the greatest change between acetaminophen and codeine. The unknown peak disappeared, however, and probably co-eluted with morphine (Application 6).

To further improve resolution for codeine and the other later eluting components, we adjusted the mobile phase ratio to 85:15, buffer:organic solvent, using a 90:10 mixture of acetonitrile and methanol as the organic solvent. Resolution improved, relative to the original mobile phase composition, analysis again was under 10 minutes, and the co-eluting unknown peak returned (Application 7).

In an effort to further improve peak shape, we heated the column to 35°C. The higher temperature reduced the analysis time by one minute, at the sacrifice of resolution between analytes, and with further distortion of the leading baseline for oxycodone and hydrocodone (Application 8). For this analysis, the conditions used to produce (Application 7) provided the most desirable results.

In summary, the goal of any method should be to achieve the most stable and robust separation possible. Too often methods are made more complicated than they need be, perhaps from lack of chromatographic experience or, possibly, to make analysis unnecessarily difficult. Even troubleshooting such methods adds to production costs. When preparing to follow a compendium method always

Application 4

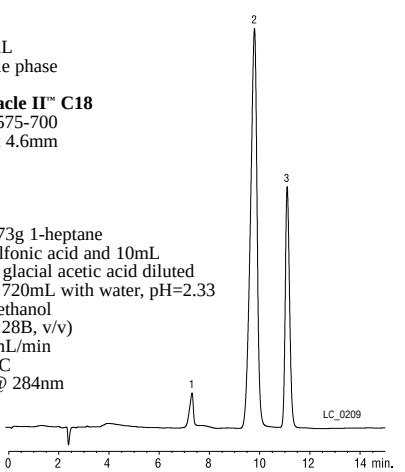
Analyses of morphine sulfate and a hydrocodone bitartrate / acetaminophen admixture according to USP 25

Peak List:	Conc. (mg/mL)	Ret. Time (min.)	RRT	Tailing	Resolution
1. unknown		7.1			
2. phenol	0.15	9.8	0.88		
3. morphine	0.24	11.0	1.00	1.17	3.2

Sample:
Inj.: 20.0µL
Solvent: mobile phase

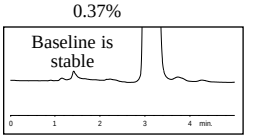
Column: Pinnacle II™ C18
Catalog #: 9214575-700
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile phase: A: 0.73g 1-heptane sulfonic acid and 10mL of glacial acetic acid diluted to 720mL with water, pH=2.33
B: methanol
(72A:28B, v/v)
Flow: 1.00mL/min
Temp.: 26.5°C
Det.: UV @ 284nm



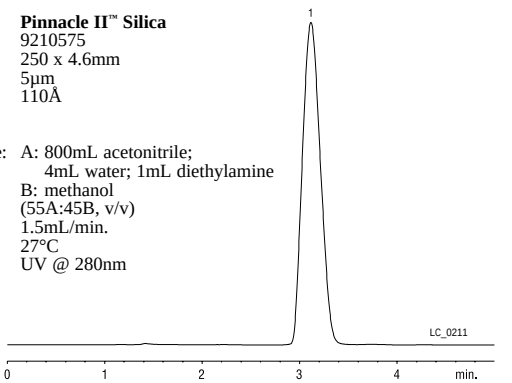
Peak List:	Ret. Time (min.)	%Inj. RSD (n=5)
1. hydrocodone bitartrate	3.1	0.37%

Sample:
Inj vol: 20µL
Conc.: 1 mg/mL
Sample: assay standard
Solvent: mobile phase



Column: Pinnacle II™ Silica
Catalog #: 9210575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile phase: A: 800mL acetonitrile; 4mL water; 1mL diethylamine
B: methanol
(55A:45B, v/v)
Flow: 1.5mL/min.
Temp.: 27°C
Det.: UV @ 280nm

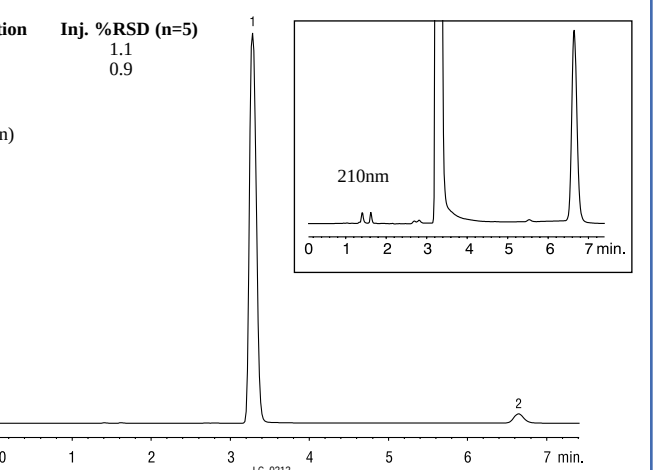


Peak List:	Conc. (mg/mL)	Ret. Time (min.)	RRT	Tailing	Resolution	Inj. %RSD (n=5)
1. acetaminophen	0.76	3.304	1.0	1.1	—	1.1
2. hydrocodone bitartrate	0.0076	6.664	2.0	1.1	17.0	0.9

Sample:
Inj.: 25µL
Sample: 500mg acetaminophen with 5mg hydrocodone bitartrate (standard solution)
Solvent: mobile phase

Column: Ultra C18
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: 6.8 g potassium monobasic with 0.2 mL of TEA per liter
B: acetonitrile
(85A:15B, v/v)
Flow: 1.5mL/min.
Temp.: 27°C
Det.: UV @ 210nm (hydrocodone bitartrate) and 295nm (acetaminophen). 210nm channel shown



attempt to determine the reason a reagent would be added to a mobile phase, but any change or modification should have an established scientific purpose. By creating more universal methods for analyses of structurally related compounds, it should be possible to reduce inventory costs for supplies, increase laboratory analysis efficiency, and reduce personnel training time.

Some analysts may have neither the time nor the experience to troubleshoot a problem methodology. If you encounter problems when analyzing your samples according to an established method, our experienced Technical Service chemists will be glad to help. Contact them at 800-356-1688, ext. 4 or 814-353-1300, ext. 4, or contact your Restek representative.

Application 5

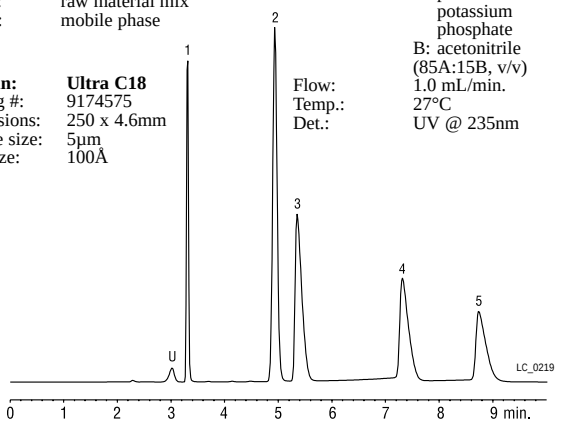
Separation of Narcotic Analgesics and Acetaminophen on Ultra C18.

Peak List:	Conc. (µg/mL)	Ret. Time (min.)	Tailing	Resolution
U. unknown	unknown	3.0	NA	NA
1. morphine sulfate	204	3.3	0.97	2.3
2. acetaminophen	92	5.0	1.1	14.9
3. codeine phosphate	216	5.3	1.8	2.1
4. oxycodone HCl	206	7.3	1.9	6.9
5. hydrocodone bitartrate	218	8.8	1.9	4.1

Sample:
Inj.: 10µL
Sample: raw material mix
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile Phase: A: pH 2.8 10mm potassium phosphate
B: acetonitrile (85A:15B, v/v)
Flow: 1.0 mL/min.
Temp.: 27°C
Det.: UV @ 235nm



Application 6

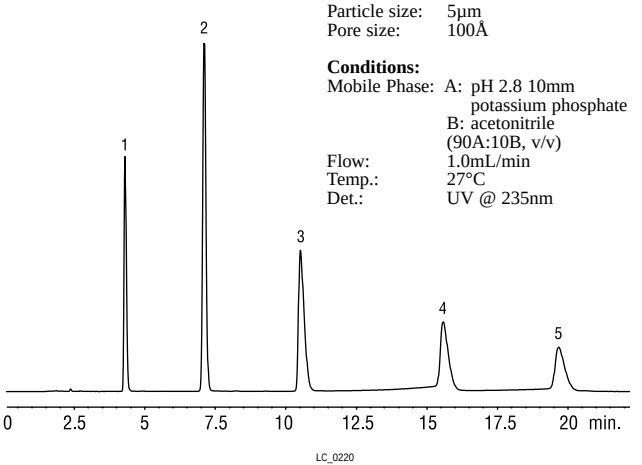
A small (5%) increase in the aqueous buffer doubles resolution and retention.

Peak List:	Conc. (µg/mL)	Ret. Time (min.)	Tailing	Resolution
1. morphine sulfate	204	3.3	1.2	NA
2. acetaminophen	92	5.0	1.1	15.0
3. codeine phosphate	216	5.3	1.7	11.3
4. oxycodone HCl	206	7.3	1.5	11.0
5. hydrocodone bitartrate	218	8.8	1.5	7.0

Sample:
Inj.: 4.0µL
Sample: raw material mix
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile Phase: A: pH 2.8 10mm potassium phosphate
B: acetonitrile (90A:10B, v/v)
Flow: 1.0mL/min
Temp.: 27°C
Det.: UV @ 235nm



Application 7

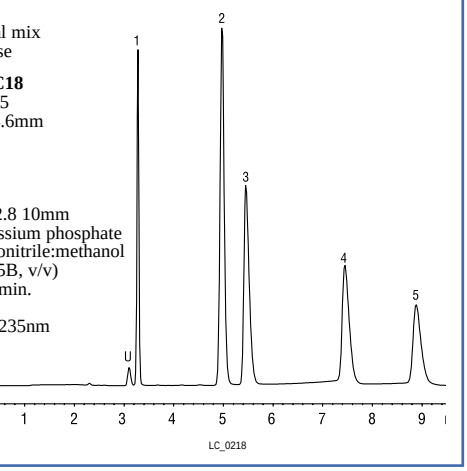
Further alteration of the organic phase improves resolution without increasing analysis time.

Peak List:	Conc. (µg/mL)	Ret. Time (min.)	Tailing	Resolution
U. unknown	unknown	3.1	NA	NA
1. morphine sulfate	204	3.3	1.0	1.8
2. acetaminophen	92	5.0	1.1	14.1
3. codeine phosphate	216	5.5	1.4	2.6
4. oxycodone HCl	206	7.5	1.4	8.5
5. hydrocodone bitartrate	218	8.9	1.4	5.0

Sample:
Inj.: 4.0µL
Sample: raw material mix
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: pH 2.8 10mm potassium phosphate
B: acetonitrile:methanol (85A:15B, v/v)
Flow: 1.0mL/min.
Temp.: 27°C
Det.: UV @ 235nm



Application 8

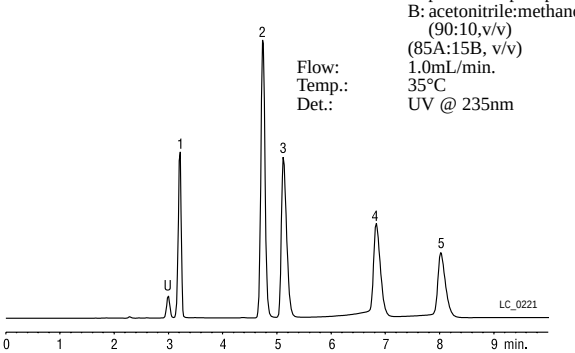
Increasing column temperature has detrimental effects on the chromatography of narcotic / acetaminophen admixtures.

Peak List:	Conc. (µg/mL)	Ret. Time (min.)	Tailing	Res.
U. unknown	unknown	3.0	NA	NA
1. morphine sulfate	204	3.2	0.90	1.9
2. acetaminophen	92	4.8	1.1	12.2
3. codeine phosphate	216	5.1	1.4	2.3
4. oxycodone HCl	206	6.9	1.4	8.2
5. hydrocodone bitartrate	218	8.0	1.4	4.8

Sample:
Inj.: 4.0µL
Sample: raw material mix
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: pH 2.8 10mm potassium phosphate
B: acetonitrile:methanol, (90:10,v/v)
(85A:15B, v/v)
Flow: 1.0mL/min.
Temp.: 35°C
Det.: UV @ 235nm



Pinnacle II® C8 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9213531	9213532	9213533	9213535
50mm	9213551	9213552	9213553	9213555
100mm	9213511	9213512	9213513	9213515
150mm	9213561	9213562	9213563	9213565
200mm	9213521	9213522	9213523	9213525
250mm	9213571	9213572	9213573	9213575

Pinnacle II® C8 5µm Columns with Trident® Inlet Fitting

Length	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9213532-700	9213533-700	9213535-700
50mm	9213552-700	9213553-700	9213555-700
100mm	9213512-700	9213513-700	9213515-700
150mm	9213562-700	9213563-700	9213565-700
200mm	9213522-700	9213523-700	9213525-700
250mm	9213572-700	9213573-700	9213575-700

Pinnacle II® C18 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9214531	9214532	9214533	9214535
50mm	9214551	9214552	9214553	9214555
100mm	9214511	9214512	9214513	9214515
150mm	9214561	9214562	9214563	9214565
200mm	9214521	9214522	9214523	9214525
250mm	9214571	9214572	9214573	9214575

Pinnacle II® C18 5µm Columns with Trident® Inlet Fitting

Length	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9214532-700	9214533-700	9214535-700
50mm	9214552-700	9214553-700	9214555-700
100mm	9214512-700	9214513-700	9214515-700
150mm	9214562-700	9214563-700	9214565-700
200mm	9214522-700	9214523-700	9214525-700
250mm	9214572-700	9214573-700	9214575-700

Pinnacle II® Silica 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9210531	9210532	9210533	9210535
50mm	9210551	9210552	9210553	9210555
100mm	9210511	9210512	9210513	9210515
150mm	9210561	9210562	9210563	9210565
200mm	9210521	9210522	9210523	9210525
250mm	9210571	9210572	9210573	9210575

Pinnacle II® Silica 5µm Columns with Trident® Inlet Fitting

Length	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9210532-700	9210533-700	9210535-700
50mm	9210552-700	9210553-700	9210555-700
100mm	9210512-700	9210513-700	9210515-700
150mm	9210562-700	9210563-700	9210565-700
200mm	9210522-700	9210523-700	9210525-700
250mm	9210572-700	9210573-700	9210575-700

Ultra C8 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9103531	9103532	9103533	9103535
50mm	9103551	9103552	9103553	9103555
100mm	9103511	9103512	9103513	9103515
150mm	9103561	9103562	9103563	9103565
200mm	9103521	9103522	9103523	9103525
250mm	9103571	9103572	9103573	9103575

Ultra C18 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9174531	9174532	9174533	9174535
50mm	9174551	9174552	9174553	9174555
100mm	9174511	9174512	9174513	9174515
150mm	9174561	9174562	9174563	9174565
200mm	9174521	9174522	9174523	9174525
250mm	9174571	9174572	9174573	9174575

Resprep® SPE Syringe Filters

- Solvent-resistant polypropylene housing.
- Better flow characteristics because of a glass fiber prefilter.
- Available in the most popular filter sizes and membrane porosities.
- Available in non-leaching nylon or PTFE.

Filter Diameter	Porosity	qty.	Nylon	PTFE
13mm	0.20µm	100-pk.	26066	26068
13mm	0.45µm	100-pk.	26067	26069
25mm	0.20µm	50-pk.	26070	26072
25mm	0.45µm	50-pk.	26071	26073
25mm	1.00µm	50-pk.	-	26074

Trident® Direct Guard Column System

Unlike "one size fits all" guard systems, the Trident™ Direct system gives you the power to select the right level of protection for your analysis. The system offers three levels of protection and guard cartridges in four dimensions, with a variety of bonded phases to match your analytical column. The economical, leak-free cartridge design provides an unprecedented combination of convenience, economy, and reliability. The foundation of the Trident™ Direct system is a reusable direct connect holder that easily attaches to any HPLC column using CPI- or Waters®-style end fittings.* The system is available in the following configurations to match different protection level needs: in-line filter, in-line filter with holder for 1cm guard cartridge, and in-line filter with holder for 2cm guard cartridge. The guard cartridges are available in 2.1 and 4.0mm ID and are interchangeable within the appropriate length holder.

*Replace tip when using Waters®-style fittings–see our catalog.

Trident® Direct provides three levels of protection

Trident™ Direct high-pressure filter
Protection against particulate matter.
cat.# 25082

Trident™ Direct 1cm guard cartridge holder with filter
Moderate protection against particulate matter and irreversibly adsorbed compounds.
cat.# 25084

Trident™ Direct 2cm guard cartridge holder with filter
Maximum protection against particulate matter and irreversibly adsorbed compounds.
cat.# 25086

RESTEK ISO 9001 REGISTERED

RESTEK environmental

Applications note

Fast Analyses of Aroclor® PCBs, Using the Zip Scientific GC Racer* Temperature Programming System

Introduction

Analytical chemists using traditional gas chromatographs (GC) have control of four fixed parameters (column length and internal diameter, phase type and film thickness) and three variable parameters (carrier gas composition and velocity, and temperature programming) that affect analytical run times. The maximum oven temperature ramp rate has been limited, however, based on chromatograph design. Now, the temperature-programming rate can be effectively increased, significantly shortening analysis time for Aroclor® PCBs and thereby reducing instrument operating time and increasing sample throughput.

Aroclor® PCBs

Aroclor® mixtures are mixtures of polychlorinated biphenyl (PCB) congeners, each phenyl ring of which contains 1–5 chlorine atoms. Since each PCB can contain up to 10 chlorine atoms, in any combination of positions, there are 209 PCB congeners. Environmental samples are commonly screened for seven Aroclor® mixtures that are or were widely used, but there are other Aroclor® mixtures and PCB mixtures are also manufactured under other tradenames.

The seven commonly analyzed Aroclor® mixtures are Aroclor® 1016, 1221, 1232, 1242, 1248, 1254, and 1260. Other Aroclor® mixtures include "technical" Aroclor® 1254, Aroclor® 1262, and Aroclor® 1268. The Aroclors® contain mixes of congeners based on distillation fractions. The last two digits of the Aroclor® number indicate the content of chlorine, by weight (e.g., Aroclor® 1232 is 32% chlorine by weight). The exception is Aroclor® 1016, which is similar to Aroclor® 1242. The range of PCB congeners differs among Aroclor® mixtures (e.g., Aroclor® 1242 is made up mainly of congeners ranging from approximately dichlorobiphenyls to tetrachlorobiphenyls; late eluting Aroclor® 1260 is made up mostly of hexachlorobiphenyls to octachlorobiphenyls) and, since the mixtures were made to weight percent chlorine, not to specific composition standards, the exact mixture of congeners varies among various manufacture lots. All Aroclor® mixtures have some PCB congeners in common, so retention times among Aroclor® mixtures overlap, but each Aroclor® mixture has an identifiable fingerprint pattern.

Reducing Analysis Time

Because the chromatographic patterns of the various Aroclor® mixtures are easily differentiated, the chromatographer could accelerate the GC analysis, and thereby accomplish more analyses during a set period of time. To reduce analysis time without changing the column, the chromatographer has three options: change carrier gas (from helium to hydrogen), increase the velocity at which the carrier gas passes through the column, or increase the oven temperature program rate.

Changing the carrier gas might not be a practical option. To increase the velocity of the carrier gas the backpressure at the column inlet must be increased. Generally, 30m x 0.32mm columns are operated at a pressure of 5-10psig, but the pressure can be increased to 25 psig without a loss of column efficiency that would affect Aroclor® quantification.

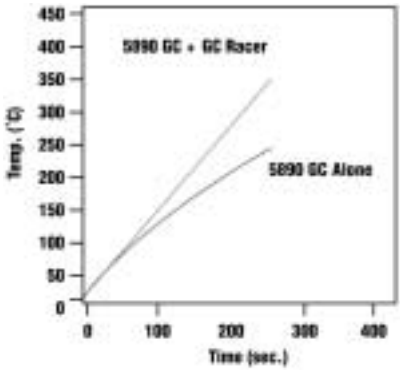
The third option, increasing the oven temperature program rate, has been a problem until now: oven temperature ramp rates are limited. Most older chromatograph models have a maximum reliable programming rate of 20°C/minute; newer models allow programming rates of up to 70°C/minute, but only to relatively low maximum temperatures.

Now, by using the Zip Scientific GC Racer auxiliary heating unit, temperature program rates of up to 70°C/min. can be maintained to temperatures up to 350°C—conditions that allow analyses of Aroclor® PCB mixtures to be accelerated.

The GC Racer consists of a program controller and a resistive heating element that is placed on the floor of the GC oven (Figure 1). The heating element is connected to the controller which, in turn, is plugged into the main PC board of the GC. When the GC Racer programmer detects that the factory heating elements are not keeping up with the programmed heating rate, the GC Racer heater is brought into the circuit to augment the heat being supplied to the oven. Oven temperature with and without supplementation from a GC Racer has been empirically tested with an Agilent 5890 chromatograph; results are shown in Figure 1.

Faster Aroclor® Analyses

Figure 1—The GC Racer allows a temperature program rate of up to 70°C/min. to be maintained up to 350°C.



GC: Agilent 5890; **Service:** 120V/15 amp; **Start Temp:** 20°C; set oven to 400°C and monitor oven temp.

The GC Racer consists of a control unit and a resistive heating element placed on the floor of the GC oven.

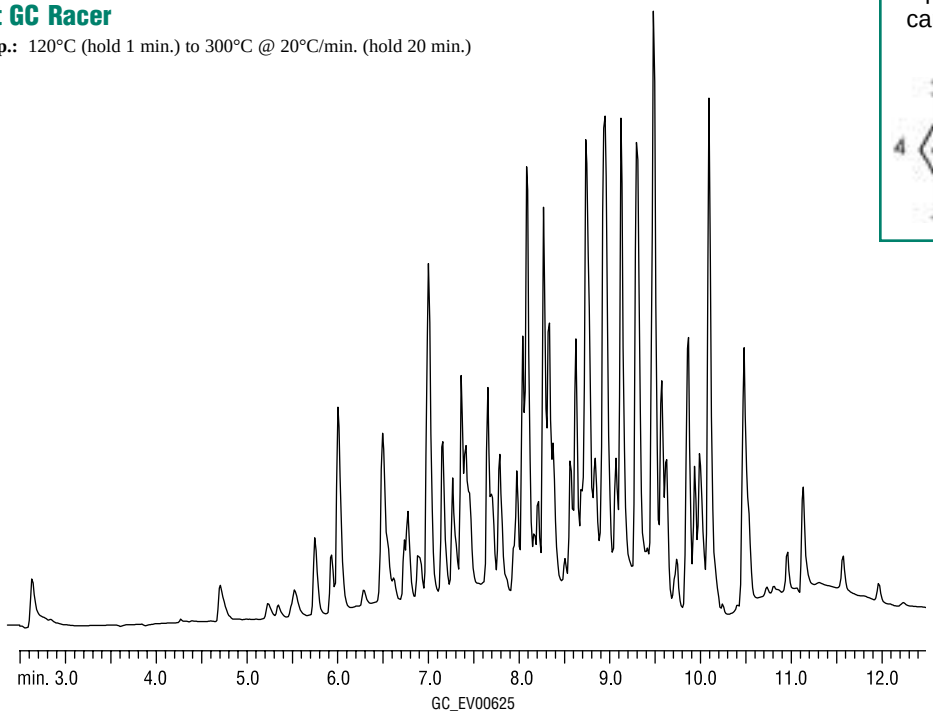
**Patent pending.*

environmental

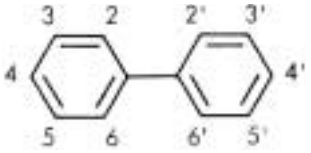
Figure 2—Analysis of mixed Aroclor® standards is significantly faster with the GC Racer temperature programmer.

Aroclor® 1221, 1242, 1254, 1260 Mix without GC Racer

Oven temp.: 120°C (hold 1 min.) to 300°C @ 20°C/min. (hold 20 min.)



Structure of polychlorinated biphenyls. Each molecule can have 1 to 10 chlorine atoms.



More than 40% reduction in analysis time with fast temperature programming!

Aroclor® 1221, 1242, 1254, 1260 Mix with GC Racer

Oven temp.: 120°C (hold 0.5 min.) to 300°C @ 60°C/min. (hold 6.5 min.)
Zip Scientific GC Racer for Agilent 5890 Series II (cat.# 23024)

Column:	Rtx®-CLPesticides2 30m, 0.32mm ID, 0.25µm (cat.# 11324)
Sample:	400ng/mL Aroclor® 1221, 1242, 1254, 1260 mix in hexane
Inj.:	1.0µL direct, Uniliner® inlet liner (cat.# 20335)
Inj. temp.:	210°C
Carrier gas:	helium, constant pressure
Linear velocity:	46cm/sec. @ 120°C
Det.:	ECD @ 310°C
Guard Column:	Siltek™-Deactivated 5m, 0.32 ID (cat.# 10027)
Connector:	Universal Angled Press-Tight® (cat.# 20446)

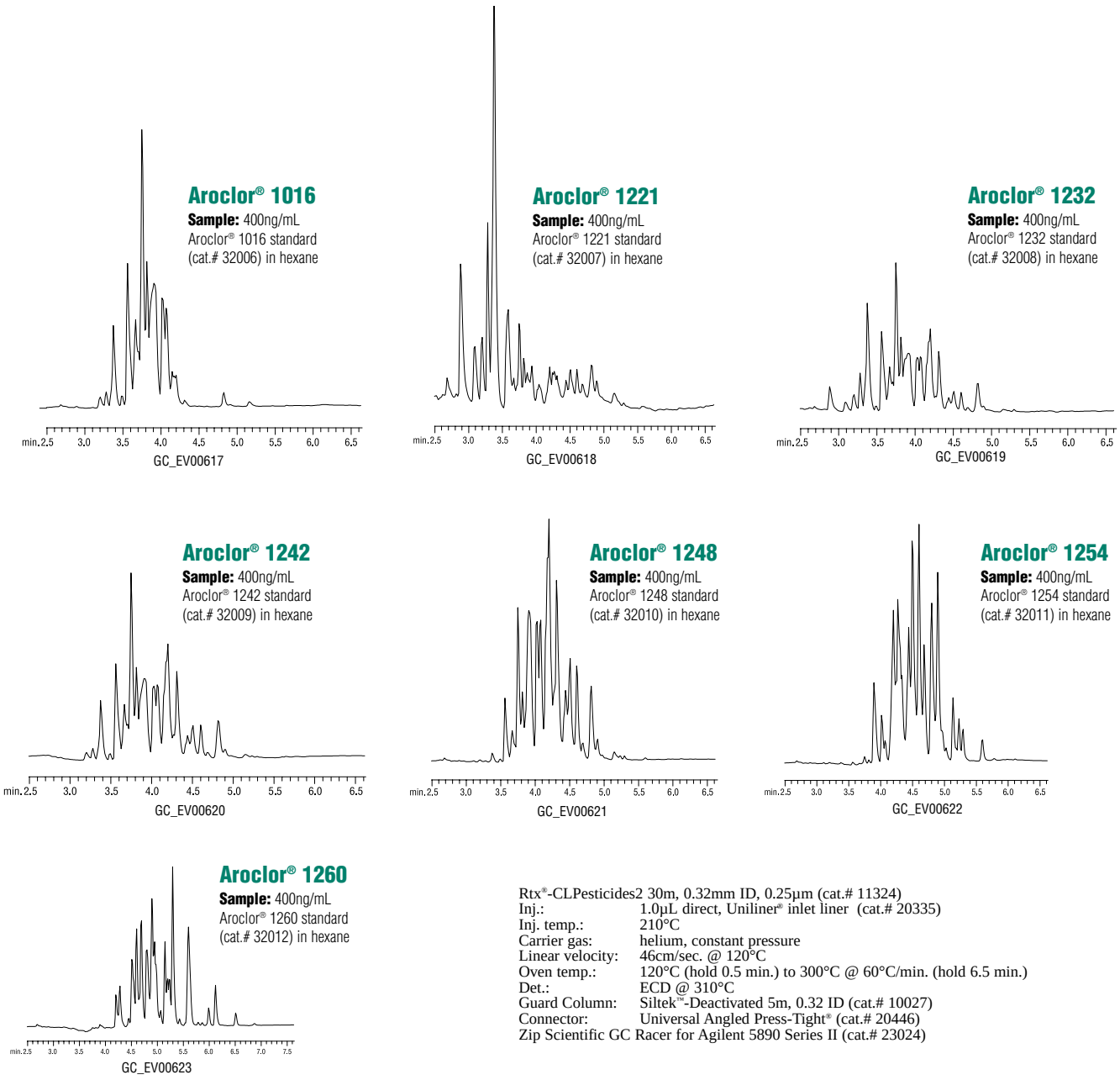
Using a GC Racer, the oven temperature program for an Agilent 5890 chromatograph can be tripled, from 20°C/min. to 60°C/min. With all other chromatographic parameters the same, analysis time will be reduced significantly. Figure 2 shows chromatograms of a mixture of Aroclor® standards analyzed at both programming rates. Time savings that accrue from the five-minute shorter analysis are significant. Using a common GC cool-down time of two minutes, total cycle times at the two programming rates are 14 minutes and 9 minutes. In an 8-hour period 34 cycles can be made at a 14-minute cycle time, versus 53 cycles with the GC Racer—**a 56% increase in sample throughput!**

Typical chromatographic patterns for the seven common Aroclor® mixtures analyzed under fast temperature programming are shown in Figure 3.

Conclusion

The Zip Scientific GC Racer system is a highly effective, easily installed tool that enhances the performance of a gas chromatograph. By decreasing run time and increasing sample throughput, the higher analysis speeds that can be achieved can lead to significant long-term savings of time and money. PCB analyses are shown here, but this example application is a good model for analyses of long-chain hydrocarbons, high molecular weight compounds, low-volatility molecules, and other late eluting analytes that require long analysis times at slow temperature program rates.

Figure 3—Typical chromatographic patterns for Aroclor® PCBs analyzed under fast temperature programming.



Rtx®-CLPesticides2 30m, 0.32mm ID, 0.25µm (cat.# 11324)
Inj.: 1.0µL direct, Uniliner® inlet liner (cat.# 20335)
Inj. temp.: 210°C
Carrier gas: helium, constant pressure
Linear velocity: 46cm/sec. @ 120°C
Oven temp.: 120°C (hold 0.5 min.) to 300°C @ 60°C/min. (hold 6.5 min.)
Det.: ECD @ 310°C
Guard Column: Siltek™-Deactivated 5m, 0.32 ID (cat.# 10027)
Connector: Universal Angled Press-Tight® (cat.# 20446)
Zip Scientific GC Racer for Agilent 5890 Series II (cat.# 23024)

GC Racer by Zip Scientific*

Operate Your Agilent 5890 as fast as a 6890!

CE pending

Description		qty.	cat.#
GC Racer for Agilent 5890 Series II (only) GC	120 volt	ea.	23024
GC Racer for Agilent 5890A (only) GC	120 volt	ea.	23025
GC Racer for Agilent 6890 (only) GC	120 volt	ea.	23028

**Patent pending.*

PCB Kit #1

32006: Aroclor® 1016
32007: Aroclor® 1221
32008: Aroclor® 1232
32009: Aroclor® 1242
32010: Aroclor® 1248
32011: Aroclor® 1254
32012: Aroclor® 1260

1,000µg/mL each in hexane, 1mL/ampul

Kit	Kit w/Data Pack
32089	32089-500

PCB Kit #2

32064: Aroclor® 1016
32065: Aroclor® 1221
32066: Aroclor® 1232
32067: Aroclor® 1242
32068: Aroclor® 1248
32069: Aroclor® 1254
32070: Aroclor® 1260

200µg/mL each in isooctane, 1mL/ampul

Kit	Kit w/Data Pack
32090	32090-500

PCB Kit #3

32007: Aroclor® 1221
32008: Aroclor® 1232
32009: Aroclor® 1242
32010: Aroclor® 1248
32011: Aroclor® 1254
32039: Aroclor® 1016/1260

1,000µg/mL each in hexane, 1mL/ampul

Kit	Kit w/Data Pack
32400	32400-500

PCB Kit #4

32065: Aroclor® 1221
32066: Aroclor® 1232
32067: Aroclor® 1242
32068: Aroclor® 1248
32069: Aroclor® 1254
32299: Aroclor® 1016/1260

200µg/mL each in isooctane, 1mL/ampul

Kit	Kit w/Data Pack
32401	32401-500

Rtx®-CLPesticides2 Columns (Fused Silica)

ID	df (µm)	temp. limits	15-Meter	20-Meter	30-Meter	60-Meter
0.10mm	0.10	-60 to 310/330°C		43302		
0.18mm	0.14	-60 to 310/330°C		42302		
0.25mm	0.20	-60 to 320/340°C	11320		11323	11326
0.32mm	0.25	-60 to 320/340°C	11321		11324	
0.53mm	0.42	-60 to 300/320°C	11337		11340	

Rtx®-5 Columns (Fused Silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits*	15-Meter	30-Meter	60-Meter
0.25mm	0.25	-60 to 330/350°C	10220	10223	10226
	0.50	-60 to 330/350°C	10235	10238	10241
	1.00	-60 to 320/340°C	10250	10253	10256
0.32mm	0.25	-60 to 330/350°C	10221	10224	10227
	0.50	-60 to 330/350°C	10236	10239	10242
	1.00	-60 to 330/350°C	10251	10254	10257
0.53mm	0.25	-60 to 320/340°C	10222	10225	10228
	0.50	-60 to 310/330°C	10237	10240	10243
	1.00	-60 to 310/330°C	10252	10255	10258
	1.50	-60 to 310/330°C	10267	10270	10273

**The maximum temperatures listed are for 15- and 30-meter lengths. Longer lengths may have a slightly reduced maximum temperature.*

Restek offers GC columns in virtually any bore, film thickness, and length. Also, we stock thousands of inlet liners for all major GC models for immediate shipment! For a complete product listing, request our annual Chromatography Products Guide.

Uniliner® Inlet Liner For Agilent GCs

(for 0.32/0.53mm ID columns)

(4.0mm ID, 6.3mm OD, 78.5mm length)	
20335 (ea.)	20336 (5-pk.)

Aroclor® Solutions

Compound Packed 1mL/ampul	Solvent	µg/mL	Individual	Individual w/data pack	5-pk.	5-pk. w/data pack	10-pk. w/data pack
Aroclor® 1016	hexane	1,000	32006	32006-500	32006-510	32006-520	32106
Aroclor® 1221	hexane	1,000	32007	32007-500	32007-510	32007-520	32107
Aroclor® 1232	hexane	1,000	32008	32008-500	32008-510	32008-520	32108
Aroclor® 1242	hexane	1,000	32009	32009-500	32009-510	32009-520	32109
Aroclor® 1248	hexane	1,000	32010	32010-500	32010-510	32010-520	32110
Aroclor® 1254	hexane	1,000	32011	32011-500	32011-510	32011-520	32111
Aroclor® 1260	hexane	1,000	32012	32012-500	32012-510	32012-520	32112
Aroclor® 1016/1260	hexane	1,000	32039	32039-500	32039-510	32039-520	32139
Aroclor® 1262	hexane	1,000	32409	32409-500	32409-510	32409-520	32509
Aroclor® 1268	hexane	1,000	32410	32410-500	32410-510	32410-520	32510

Compound Packed 1mL/ampul	Solvent	µg/mL	Individual	Individual w/data pack	5-pk.	5-pk. w/data pack	10-pk. w/data pack
Aroclor® 1016	isooctane	200	32064	32064-500	32064-510	32064-520	32164
Aroclor® 1221	isooctane	200	32065	32065-500	32065-510	32065-520	32165
Aroclor® 1232	isooctane	200	32066	32066-500	32066-510	32066-520	32166
Aroclor® 1242	isooctane	200	32067	32067-500	32067-510	32067-520	32167
Aroclor® 1248	isooctane	200	32068	32068-500	32068-510	32068-520	32168
Aroclor® 1254	isooctane	200	32069	32069-500	32069-510	32069-520	32169
Aroclor® 1260	isooctane	200	32070	32070-500	32070-510	32070-520	32170
Aroclor® 1016/1260	isooctane	200	32299	32299-500	32299-510	32299-520	32399

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Applicationsnote

A High-Temperature Polydimethylsiloxane-Phase Column for ASTM D-6352-98 Simulated Distillation Analyses: MXT® 1HT Sim Dist

- Stable to 430ºC.
- Low bleed.
- Long lifetime at high temperatures.
- Symmetrical hydrocarbon peaks.
- Consistent resolution and retention times.
- Boiling point elution of hydrocarbons.
- Polarity equivalent to existing liquid phases.

Simulated distillation per ASTM D-6352-98 is used for determining the boiling point range distribution of petroleum distillate fractions with initial boiling points (BP) > 174ºC and final boiling points < 700ºC at atmospheric pressure. High temperature SimDist presents many challenges. The stationary phase must meet rigid resolution and retention time requirements, yet be stable at high temperatures. Further, the polyimide protective coating on the outer surface of most capillary columns has a maximum working temperature of about 380ºC. Above this temperature the polyimide rapidly deteriorates. When repeatedly programmed to temperatures above 400ºC, or allowed to cool below 50ºC, the aluminum sheath on most aluminum-clad fused silica columns separates from the underlying fused silica surface. The tubing becomes extremely brittle, and column lifetime is significantly shortened.

To conform to the critical criteria set forth by ASTM, Restek chemists have developed the MXT® 1HT Sim Dist simulated distillation column. The MXT® 1HT polymer is a 100% polydimethylsiloxane (PDMS) material that is thermally stable to 430ºC, requires minimal conditioning, and is 100% crosslinked. The MXT® 1HT phase is coated onto highly deactivated stainless steel tubing that has the inertness of fused silica without the temperature limitations. The MXT® 1HT Sim Dist column has a lifetime of at least 400 injections under typical SimDist conditions.

To demonstrate the robustness of MXT® 1HT Sim Dist columns, we made a series of 400 injections of Polywax® 1000 (cold on-column, CS2 solvent, 1µL each) onto a randomly chosen column, and monitored critical performance characteristics over the course of these injections: resolution, retention times, stationary phase bleed. Figure 1 illustrates the Polywax® 1000 analysis after 400 injections. The hydrocarbon components still are well resolved and are easily quantified. Figure 2 plots the reproducibility of C50/C52 resolution and Figure 3 shows retention time reproducibility. After 400 injections, both of these critical characteristics still meet simulated distillation specifications. Figure 4 plots the consistently low bleed at 430ºC over the series of 400 injections.

The stainless steel tubing used to make MXT® 1HT Sim Dist columns incorporates state-of-the-art Sulfinert™ deactivation. The deactivation layer is incorporated into the framework of atoms on the tubing surface, and therefore will not fracture or flake off, even if the column is flexed or bent. MXT® 1HT Sim Dist columns do not exhibit higher selectivity toward aromatics than toward normal hydrocarbons, thus they provide true boiling point values.

Figure 1
Polywax® 1000 hydrocarbons well resolved on an MXT® 1HT Sim Dist column after 400 injections.

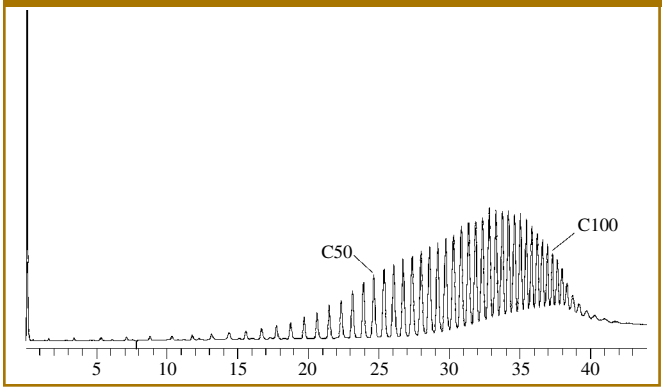


Figure 2
C50/C52 resolution is stable over a series of 400 injections on an MXT® 1HT Sim Dist column.

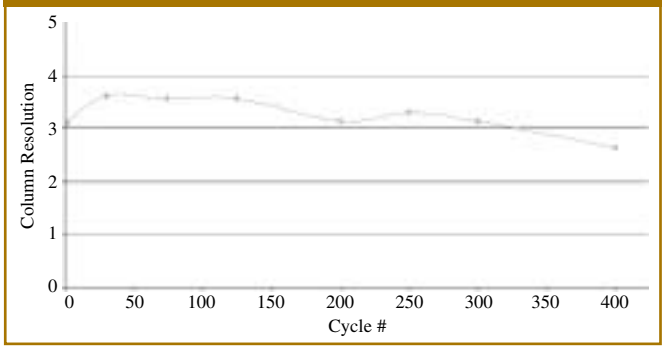
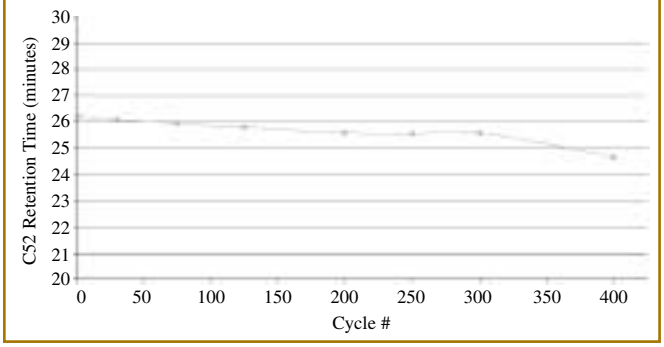


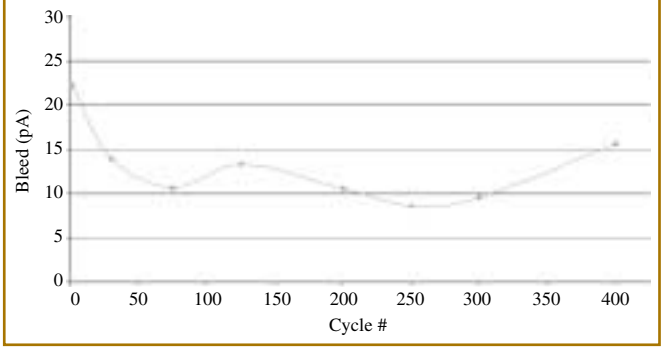
Figure 3
C52 retention shows little change after 400 injections on an MXT® 1HT Sim Dist column.



petroleum & petrochemicals

Figure 4

An MXT® 1HT column produces less than 20pA bleed over a series of 400 injections.



These excellent performance characteristics make MXT® 1HT Sim Dist columns the columns of choice for ASTM D-6352-98 SimDist analyses. Note that the demanding temperature conditions of simulated distillation analyses make GC system integrity a prime concern. It is imperative that the GC system be oxygen-free, to prevent phase degradation and maintain the highest level of chromatographic performance. We strongly recommend using oxygen-free carrier gas and routinely leak-testing your system with an electronic leak detection device, such as our Leak Detective™ II electronic leak detector (cat.# 20413), to ensure protection from oxygen.

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Product Listing

MXT®-1HT Sim Dist (metal column)

ID	df (µm)	temp. limits	5-Meter
0.53mm	0.10	-60 to 430°C	70100

Polywax Standards

Description	qty.	cat.#
Polywax® 500	1 gram	36224
Polywax® 655	1 gram	36225
Polywax® 850	1 gram	36226
Polywax® 1000	1 gram	36227

Leak Detective™ II Leak Detector CE

The compact, affordable tool every analyst should have!

Description	qty.	cat.#
Leak Detective™ II Leak Detector	ea.	20413

Never use liquid leak detectors on a capillary system because liquids can be drawn into the system.

Caution: NOT designed for determining leaks of combustible gases. A combustible gas detector should be used for determining combustible gas leaks in possibly hazardous conditions.

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Applicationsnote

Acrylamide Analysis by Gas Chromatography

Introduction

How much acrylamide is in that French fry? Is this potato chip safe to eat? Since the release of a report by Sweden's National Food Administration in April 2002, consumers have had something else to think about when choosing what to eat. Acrylamide is a toxic and potentially cancer-causing chemical, although the toxicological effects on humans are still under investigation. The topic of acrylamide in foods, especially in fried and baked goods, has generated a significant amount of interest in 2002. The United Kingdom's Food Standards Agency, the Center for Science in the Public Interest (CSPI), and the US Food & Drug Administration (FDA) are among the groups that have begun testing for acrylamide in food products.

Researchers are postulating that acrylamide is formed in relatively high concentrations when carbohydrate-rich foods such as potatoes, rice, and cereals are cooked at high heat.[1,2] This seems to be particularly true when the products are fried. Raw or boiled starchy foods do not seem to form detectable amounts of acrylamide. Of the products tested, the highest levels of acrylamide were found in potato chips and French fries, on the order of 400 - 1200 ppb. By comparison, the World Health Organization (WHO) has specified a maximum concentration of 0.5 µg/L (0.5 ppb) acrylamide in drinking water.[1]

The FDA has published a draft method for the analysis of acrylamide in foods by LC/MS/MS.[3] The procedure calls for a reversed phase C18 column and a highly aqueous mobile phase (0.1% acetic acid, 0.5% methanol). Because many of the sample matrices can be quite complex, solid phase extraction is used to remove interferences prior to the chromatographic analysis. Positive ion electrospray is used for the mass spectral interface, with quantification based on comparison to a ^{13}C isotopically labeled internal standard. The method has been validated for a limited number of matrices, such as potato chips and French fries, and public and private researchers are in the process of validating the LC/MS/MS approach for other food products.

Gas chromatography (GC) has been used to quantify acrylamide in a variety of industrial and environmental applications. With increasing interest in acrylamide analysis, we investigated the feasibility of using GC to screen for this compound in food samples. GC is a low-cost, efficient way to detect semivolatile compounds, and as an analytical tool is available in many food laboratories. In this note, we describe a GC approach to analyzing acrylamide, and discuss sample pretreatment using solid phase extraction.

Methodology & Results

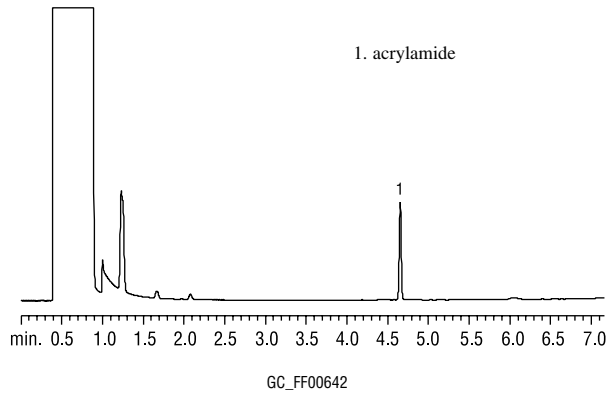
We used the following GC conditions in analyzing both acrylamide standards and food samples:

Column: Stabilwax® - 15m, 0.53 ID, 0.50µm film (cat.# 10637)
Inj.: 1.0µL, 0.5min. hold
Liner: 2mm splitless with wool (cat.# 20830)
Injector temp.: 260°C
Carrier gas: helium, constant pressure
Linear velocity: 62cm/sec. @ 100°C
Oven temp.: 100°C (hold 0.5min.) to 200°C @15°C/min.
Detector: FID @260°C

The chromatogram produced by injecting 1µL of a 25µg/mL (25 ppm) acrylamide standard is shown in Figure 1. Figure 2 is the linearity plot for standard solutions over a range of 20 - 5000 ppb.

The sample preparation method we followed was based on the draft U.S. Food & Drug Administration method *Detection and Quantitation of Acrylamide in Foods* dated June 20, 2002.[3]

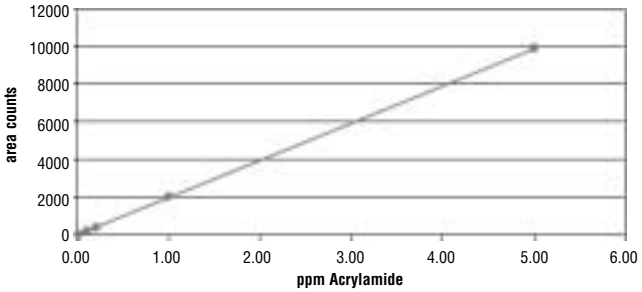
Figure 1
A Stabilwax® column is an excellent choice for acrylamide analysis by capillary GC.



Stabilwax® 15m, 0.53 ID, 0.50µm (cat.# 10637)

Sample:	25µg/mL acrylamide standard in water
Inj.:	1.0µL, 0.5min hold
Liner:	2mm splitless with wool (cat.# 20830)
Inj. temp.:	260°C
Carrier gas:	helium, constant pressure
Linear velocity:	62cm/sec. @ 100°C
Oven temp.:	100°C (hold 0.5 min.) to 200°C @ 15°C/min.
Det.:	FID @ 260°C

Figure 2
Acrylamide standard solutions were tested over a concentration range of 0.02 - 5 ppm (20 - 5000 µg/L) in water. A plot of peak counts vs. concentration shows a wide linear range for the GC assay, with R^2 = 0.99996.



Chromatographic conditions listed in text.

foods & flavors

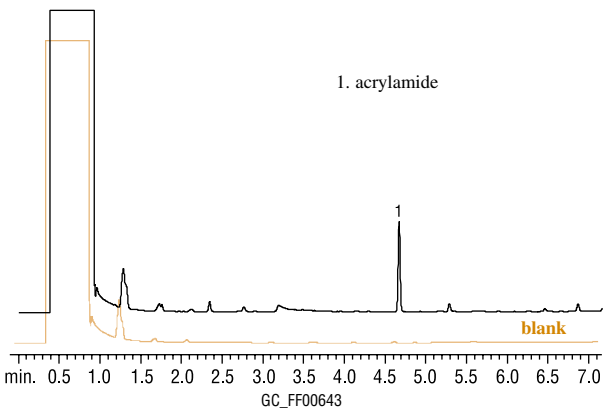
The procedure we used in our analysis of potato chips was as follows:

1. Analytically weigh 1g crushed potato chips.
2. Combine chip sample with 10mL 0.1% formic acid solution and mix on a wrist action shaker for 20 minutes.
3. Refrigerate extract for easier removal of oily top layer.
4. Filter supernatant through a 0.45µm nylon syringe filter (cat.#26071); remove and store for cleanup and analysis.
5. Condition CarboPrep™ 200 SPE tube, 6mL, 500mg (cat.#26087):
   a. 2mL acetone
   b. 2mL 0.1% formic acid
6. Apply 2mL of filtered, extracted chip solution to SPE tube. Allow sample solution to pass through tube with only gravity flow.
7. Wash SPE tube:
   a. 0.5 - 1.0mL water; pass through tube quickly.
   b. Use vacuum for up to 1 minute to dry excess water from tube.
8. Elute with 2mL of acetone, using gravity only. Eluate is ready for GC-FID analysis.

The chromatogram we obtained from the analysis of a reduced fat potato chip extract is shown in Figure 3. The chip sample was spiked with 100µg (50µg/mL) of acrylamide for this analysis. The amount of acrylamide in the reduced fat chip sample alone was below the quantitation limit of this procedure.

Figure 3

Acrylamide can be detected in reduced fat potato chips, using a Stabilwax® capillary GC column.



Stabilwax® 15m, 0.53 ID, 0.50µm (cat.# 10637)
Sample: 1g of potato chip spiked with 100µg acrylamide
Inj.: 1.0µL, 0.5min hold
Liner: 2mm splitless with wool (cat.# 20830)
Inj. temp.: 260°C
Carrier gas: helium, constant pressure
Linear velocity: 62cm/sec. @ 100°C
Oven temp.: 100°C (hold 0.5 min.) to 200°C @ 15°C/min.
Det.: FID @ 260°C

Discussion

Gas chromatography offers a rapid, cost-effective approach to screening for acrylamide in food samples such as potato chips. The Stabilwax® capillary column exhibits excellent selectivity for acrylamide, even when analyzing complex matrices, such as food samples. Detection limits on the order of 0.01µg/mL (10 ppb) in solution can be achieved. CarboPrep™ 200 sample preparation cartridges provide excellent flow properties for rapid cleanup of samples, using either vacuum pressure or gravity. The chromatographic grade, graphitized carbon packing material demonstrates reproducible recovery. This strong adsorbent has a wide range of selectivity, resulting in high capacity, even for analytes not usually well retained by reversed phase C18 adsorbents. For additional sensitivity, extracted acrylamide can be brominated, then quantified using an electron capture detector (ECD).[4]

References

1. Hileman, Bette, *C&E News*, July, 2002.
2. Schildhouse, Jill, *Food Product Design*, July, 2002.
3. http://www.cfsan.fda.gov/~dms/acrylami.html
4. US EPA Method 8032A

Product Listing

Ordering Information | Stabilwax® Columns (Fused Silica)

(Crossbond® Carbowax®—provides oxidation resistance) Stable to 250°C

ID	df (µm)	temp. limits	15-Meter	30-Meter	30-Meter 6/pk.	60-Meter
0.25mm	0.10	40 to 250°C	10605	10608		10611
	0.25	40 to 250°C	10620	10623		10626
	0.50	40 to 250°C	10635	10638		10641
0.32mm	0.10	40 to 250°C	10606	10609		10612
	0.25	40 to 250°C	10621	10624		10627
	0.50	40 to 250°C	10636	10639		10642
	1.00	40 to 240/250°C	10651	10654	10654-600	10657
0.53mm	0.10	40 to 250°C	10607	10610		10613
	0.25	40 to 250°C	10622	10625		10628
	0.50	40 to 250°C	10637	10640		10643
	1.00	40 to 240/250°C	10652	10655	10655-600	10658
	1.50	40 to 230/240°C	10666	10669		10672
	2.00	40 to 220/230°C	10667	10670		

CarboPrep™ SPE Cartridges

SPE Cartridge	Tube Volume, Bed Weight	Surface Area	qty.	cat#
CarboPrep™ 200	3mL, 250mg	200 m^2/gm	50-pk.	26088
CarboPrep™ 200	6mL, 500mg	200 m^2/gm	30-pk.	26087
CarboPrep™ 90	3mL, 250mg	90 m^2/gm	50-pk.	26091
CarboPrep™ 90	6mL, 500mg	90 m^2/gm	30-pk.	26092

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Applications note

Higher Responses for Chlorinated Pesticides, Using a Drilled Uniliner® GC Inlet Liner and Rtx®-CLPesticides Columns

Inlet reactivity is the primary drawback to using hot flash injection when analyzing chlorinated pesticides by GC. Residues of heavier and non-volatile materials often build up on the inner surface of the injection port, leaving a reactive surface that can cause compounds such as endrin and DDT to break down. An inlet liner that makes a press-fit connection with the analytical column prevents analytes from coming in contact with a major portion of this reactive surface—the bottom of the injection port. The physical connection between the column and the liner also improves the accuracy of the analysis, by minimizing injection port discrimination.

Used together, a Drilled Uniliner® inlet liner (Figure 1), an Rtx®-CLPesticides column, and an Rtx®-CLPesticides2 column ensure excellent responses in analyses of chlorinated pesticides. Markedly better results are apparent from comparisons of calibration data obtained by using a Drilled Uniliner® inlet liner and an Rtx®-CLPesticides/Rtx®-CLPesticides2 column pair to data obtained by using a conventional splitless inlet liner, or by using a conventional splitless inlet liner packed with fused silica wool.

Figure 1—The drilled hole in a Uniliner® injection port liner makes direct injection possible with EPC systems by equalizing pressure in the injection port.

Table 1—RSD values for chlorinated pesticides are well within acceptable limits (20%) when the inlet is fitted with a Drilled Uniliner® inlet liner.

Analyte	% RSD Rtx®-CLPesticides Column	%RSD Rtx®-CLPesticides2 Column
TCX*	2.3	2.0
α-BHC	7.8	9.5
γ-BHC	6.3	5.1
β-BHC	1.5	2.5
δ-BHC	8.5	3.3
heptachlor	3.2	11.6
aldrin	2.2	5.6
heptachlor epoxide	4.4	1.2
γ-chlordane	2.5	1.4
α -chlordane	3.8	2.9
4,4'-DDE	2.6	5.0
endosulfan I	3.2	2.8
dieldrin	1.0	2.1
endrin	1.7	3.5
4,4'-DDD	4.9	3.9
endosulfan II	3.5	2.8
4,4'-DDT	2.1	1.5
endrin aldehyde	4.8	4.8
methoxychlor	12.5	14.2
endosulfan sulfate	3.4	2.5
endrin ketone	1.9	3.3
DCB**	6.7	6.5

*2,4,5,6-tetrachloro-m-xylene
**decachlorobiphenyl
Columns and conditions listed in Figure 2.

Laboratories following US EPA Method 8081 and 8000 must document the quantification results they generate, to ensure reliability, precision, and accuracy. Beginning with a five-point calibration curve, a calibration factor must be calculated for each analyte. The relative standard deviation (RSD) for each analyte should be no more than 20%. In evaluations made using a Drilled Uniliner® inlet liner, RSD values were between 1.0 and 12.5% for an Rtx®-CLPesticides column and between 1.2 and 14.2% for an Rtx®-CLPesticides2 column (Table 1).

Analysts following Method 8081 and 8000 also must regularly analyze and quantify a calibration standard. In the analysis, the concentration of each analyte in the calibration standard should be within ±15% of the "true" value. The accuracy of data obtained from a system that included a Drilled Uniliner® inlet liner was tested with a pesticide standard mix at the 20/40/200ng/mL concentration level (Figure 2). The percent difference from the "true" value for each analyte ranged from 0 to a maximum of only 6.2%, well within the acceptable limit. Table 2 summarizes the data.

Because some analytes readily break down as the injection port inlet becomes contaminated (e.g., endrin and DDT in this analysis), a performance evaluation mix must be analyzed and breakdown for each analyte calculated. Breakdown should not exceed 15%. The Drilled Uniliner® inlet liner reduced endrin and DDT breakdown, relative to the splitless or splitless/wool-packed liners (Table 3), because the latter liners allow the analytes to contact more of the reactive surface in the inlet. Wool packing in the splitless liner makes this problem worse; it greatly increases the surface area within the liner and introduces additional activity.

In addition to reducing variability and increasing the accuracy of calibration data, a Drilled Uniliner® inlet liner increases overall response for individual analytes, enhancing minimum detection levels compared to splitless or splitless/wool-packed inlet liners. This is most apparent from the area counts for the last eluting peak, decachlorobiphenyl, which were greater by 18–39%, relative to area counts for injections made on the splitless liners (Table 3). Finally, a Drilled Uniliner® inlet liner ensures greater sensitivity, because less of the injected sample remains in the inlet when the inlet is swept clean to prepare it for the next sample.

By eliminating the bottom of the injector from the sample pathway, a Drilled Uniliner® inlet liner makes the pathway more inert. This reduces breakdown of labile analytes, such as endrin and DDT, and increases accuracy and precision. For analysts using hot flash injection techniques in analyses of chlorinated pesticides, or other labile analytes, these results clearly show that a Drilled Uniliner® inlet liner is the liner of choice.

Table 2—Measured analyte concentrations are very near true values, using a Drilled Uniliner® inlet liner.

Analyte	True Value	Rtx®-CLPesticides Column Measured Value	% Difference*	Rtx®-CLPesticides2 Column Measured Value	% Difference*
TCX	20.0	20.4	2.0	20.4	2.0
α-BHC	20.0	20.0	0	20.5	2.5
γ-BHC	20.0	20.2	1.0	20.4	2.0
β-BHC	20.0	20.3	1.5	20.5	2.5
δ-BHC	20.0	20.0	0	20.7	3.5
heptachlor	20.0	20.3	1.5	19.0	5.0
aldrin	20.0	20.2	1.0	20.3	1.5
heptachlor epoxide	20.0	20.5	2.5	20.4	2.0
γ-chlordane	20.0	20.3	1.5	20.4	2.0
α-chlordane	20.0	20.4	2.0	20.6	3.0
4,4'-DDE	40.0	40.6	1.5	40.4	1.0
endosulfan I	20.0	20.2	1.0	20.7	3.5
dieldrin	40.0	40.7	1.8	41.5	3.8
endrin	40.0	40.6	1.5	42.4	6.0
4,4'-DDD	40.0	41.3	3.2	42.5	6.2
endosulfan II	40.0	40.6	1.5	42.2	5.5
4,4'-DDT	40.0	40.4	1.0	41.1	2.8
endrin aldehyde	40.0	41.0	2.5	40.9	2.2
methoxychlor	200.0	203.1	1.5	206.2	3.1
endosulfan sulfate	40.0	40.8	2.0	40.3	0.8
endrin ketone	40.0	41.2	3.0	41.6	4.0
DCB	20.0	20.7	3.5	20.6	3.0

*Allowed maximum = 15%. Columns and conditions listed in Figure 2.

Table 3—Lowest analyte breakdown, and highest responses, are obtained by using a Drilled Uniliner® inlet liner.

% Breakdown*

Analyte	Column	Drilled Uniliner®	4mm splitless	4mm splitless with wool
Endrin	Rtx®-CLPesticides	4.4	4.7	9.8
	Rtx®-CLPesticides2	4.9	6.9	8.3
DDT	Rtx®-CLPesticides	0.2	0.3	2.6
	Rtx®-CLPesticides2	0.3	0.9	3.1

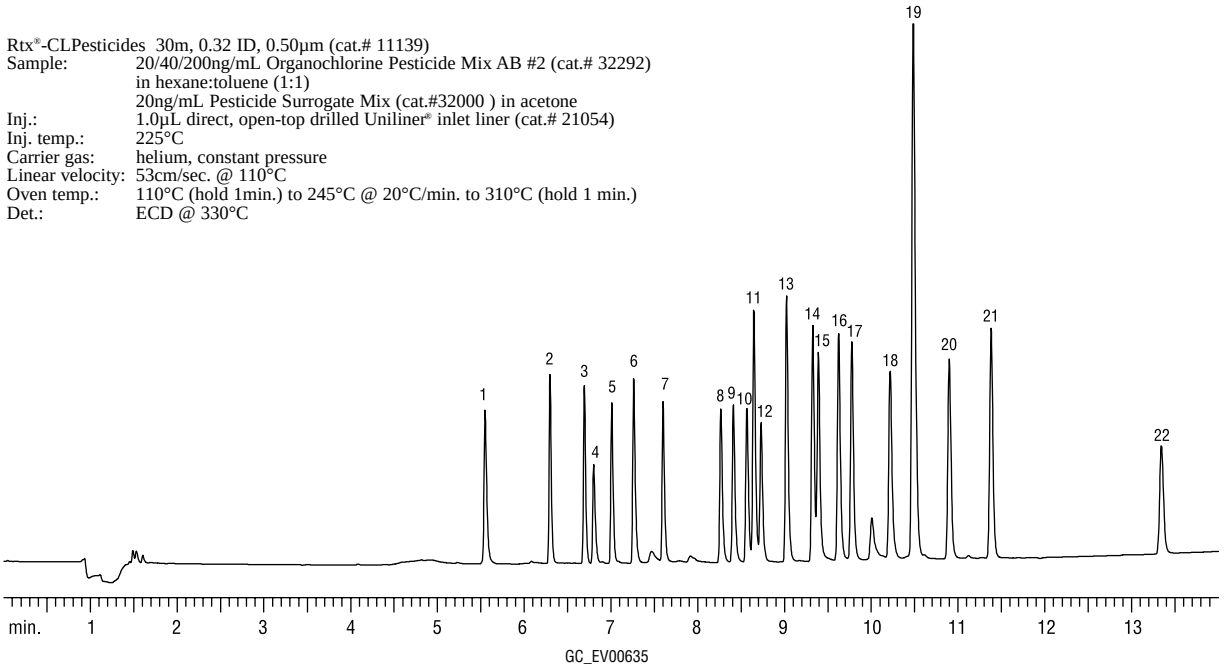
Area Response**

Analyte	Column	Drilled Uniliner®	4mm splitless with wool	4mm splitless
Tetrachloro-*m*-xylene (TCX)	Rtx®-CLPesticides	147	111	106
	Rtx®-CLPesticides2	191	167	162
Decachlorobiphenyl (DCB)	Rtx®-CLPesticides	150	119	108
	Rtx®-CLPesticides2	209	177	166

*Allowed maximum = 15%.
**Mean response (n=2); value in table x 10^3 = response units.
Columns and conditions listed in Figure 2, except:
Inj.: direct, open-top Drilled Uniliner® inlet liner (cat.# 21054)
splitless (hold 1 min.), 4mm single gooseneck inlet liner (cat.# 20798)
splitless (hold 1 min.), 4mm single gooseneck inlet liner w/ fused silica wool (cat.# 22405)

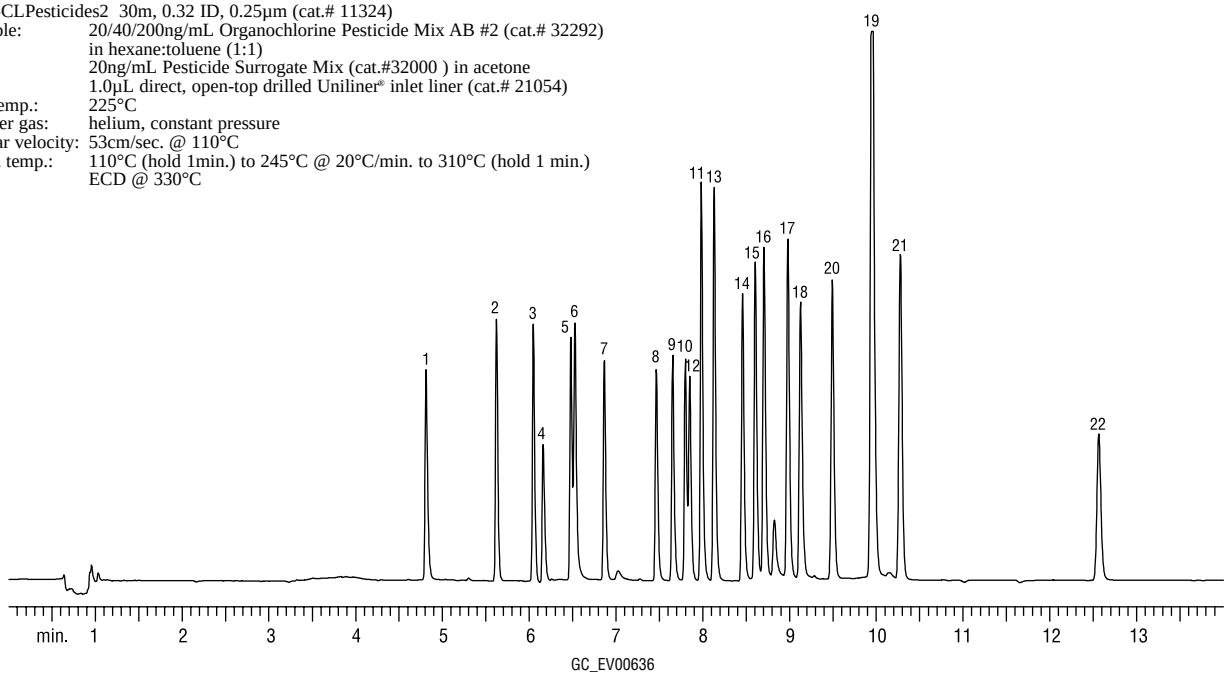
Figure 2—Chlorinated pesticides show excellent response, using the Drilled Uniliner® inlet liner and Rtx®-CLPesticides columns—even at the 20/40/200ng/mL concentration level.

Rtx®-CLPesticides 30m, 0.32 ID, 0.50µm (cat.# 11139)
Sample: 20/40/200ng/mL Organochlorine Pesticide Mix AB #2 (cat.# 32292) in hexane:toluene (1:1)
20ng/mL Pesticide Surrogate Mix (cat.#32000) in acetone
Inj.: 1.0µL direct, open-top drilled Uniliner® inlet liner (cat.# 21054)
Inj. temp.: 225°C
Carrier gas: helium, constant pressure
Linear velocity: 53cm/sec. @ 110°C
Oven temp.: 110°C (hold 1min.) to 245°C @ 20°C/min. to 310°C (hold 1 min.)
Det.: ECD @ 330°C



1. 2,4,5,6-tetrachloro-*m*-xylene (TCX)
2. α-BHC
3. γ-BHC
4. β-BHC
5. δ-BHC
6. heptachlor
7. aldrin
8. heptachlor epoxide
9. γ-chlordane
10. α-chlordane
11. 4,4'-DDE
12. endosulfan I
13. dieldrin
14. endrin
15. 4,4'-DDD
16. endosulfan II
17. 4,4'-DDT
18. endrin aldehyde
19. methoxychlor
20. endosulfan sulfate
21. endrin ketone
22. decachlorobiphenyl (DCB)

Rtx®-CLPesticides2 30m, 0.32 ID, 0.25µm (cat.# 11324)
Sample: 20/40/200ng/mL Organochlorine Pesticide Mix AB #2 (cat.# 32292) in hexane:toluene (1:1)
20ng/mL Pesticide Surrogate Mix (cat.#32000) in acetone
Inj.: 1.0µL direct, open-top drilled Uniliner® inlet liner (cat.# 21054)
Inj. temp.: 225°C
Carrier gas: helium, constant pressure
Linear velocity: 53cm/sec. @ 110°C
Oven temp.: 110°C (hold 1min.) to 245°C @ 20°C/min. to 310°C (hold 1 min.)
Det.: ECD @ 330°C



Ordering Information | Rtx®-CLPesticides Columns (Fused Silica)

ID	df (µm)	temp. limits	10-Meter	15-Meter	20-Meter	30-Meter
0.10mm	0.10	-60 to 310/330°C	43101			
0.18mm	0.18	-60 to 310/330°C	42101		42102	
0.25mm	0.25	-60 to 320/340°C		11120		11123
0.32mm	0.50	-60 to 320/340°C		11136		11139
0.53mm	0.50	-60 to 300/320°C		11137		11140

Ordering Information | Rtx®-CLPesticides2 Columns (Fused Silica)

ID	df (µm)	temp. limits	10-Meter	15-Meter	20-Meter	30-Meter	60-Meter
0.10mm	0.10	-60 to 310/330°C	43301		43302		
0.18mm	0.14	-60 to 310/330°C	42301		42302		
0.25mm	0.20	-60 to 320/340°C		11320		11323	11326
0.32mm	0.25	-60 to 320/340°C		11321		11324	
0.53mm	0.42	-60 to 300/320°C		11337		11340	

Ordering Information | Rtx®-CLPesticides Kits

0.25mm ID Rtx®-CLPesticides Kit cat.# 11199 (kit)

Includes:	cat.#
30m, 0.25mm ID, 0.25µm Rtx®-CLPesticides Column	11123
30m, 0.25mm ID, 0.20µm Rtx®-CLPesticides2 Column	11323
Siltek™ Universal Angled "Y" Press-Tight® Connector	20487
5m, 0.25mm ID Siltek™ Guard Column	10026

0.32mm ID Rtx®-CLPesticides Kit cat.# 11198 (kit)

Includes:	cat.#
30m, 0.32mm ID, 0.50µm Rtx®-CLPesticides Column	11139
30m, 0.32mm ID, 0.25µm Rtx®-CLPesticides2 Column	11324
Siltek™ Universal Angled "Y" Press-Tight® Connector	20487
5m, 0.32mm ID Siltek™ Guard Column	10027

0.53mm ID Rtx®-CLPesticides Kit cat.# 11197 (kit)

Includes:	cat.#
30m, 0.53mm ID, 0.50µm Rtx®-CLPesticides Column	11140
30m, 0.53mm ID, 0.42µm Rtx®-CLPesticides2 Column	11340
Siltek™ Universal Angled "Y" Press-Tight® Connector	20487
5m, 0.53mm ID Siltek™ Guard Column	10028

Columns are not pre-connected in these kits.

DI Liners for Agilent 5890 & 6890 GCs (For 0.25/0.32/0.53mm ID Columns)	ID*/OD & Length (mm)	cat.# ea.	cat.# 5-pk.
 Drilled Uniliner®	4.0 ID 6.3 OD x 78.5	21054	21055
 Siltek™ Drilled Uniliner®	4.0 ID 6.3 OD x 78.5	21054-214.1	21055-214.5
 Siltek™ 1mm Drilled Uniliner®	1.0 ID 6.3 OD x 78.5	21390-214.1	21391-214.5

**Nominal ID at syringe needle expulsion point.*

Allows splitless injections as well as direct injections with EPC-equipped GCs!

Pesticide Surrogate Mix

04.1, 3/90, 4/89, and 2/88 SOW

decachlorobiphenyl
2,4,5,6-tetrachloro-*m*-xylene

200µg/mL each in acetone, 1mL/ampul

	Each	5-pk.	10-pk.
	32000	32000-510	—
w/data pack	32000-500	32000-520	32100

Pesticide Performance Evaluation Mix w/Surrogates (8 components)

04.1 and 3/90 SOW

α-BHC	1µg/mL
β-BHC	1
γ-BHC (lindane)	1
4,4'-DDT	10
decachlorobiphenyl	2
endrin	5
methoxychlor	25
2,4,5,6-tetrachloro-*m*-xylene	2

In hexane, 1mL/ampul

	Each	5-pk.	10-pk.
	32074	32074-510	—
w/data pack	32074-500	32074-520	32174

Organochlorine Pesticide Mix AB #2

(20 components)

aldrin	8µg/mL	dieldrin	16
α-BHC	8	endosulfan I	8
β-BHC	8	endosulfan II	16
δ-BHC	8	endosulfan sulfate	16
γ-BHC (lindane)	8	endrin	16
α-chlordane	8	endrin aldehyde	16
γ-chlordane	8	endrin ketone	16
4,4'-DDD	16	heptachlor	8
4,4'-DDE	16	heptachlor epoxide (B)	8
4,4'-DDT	16	methoxychlor	80

In hexane:toluene (1:1), 1mL/ampul

	Each	5-pk.	10-pk.
	32292	32292-510	—
w/data pack	32292-500	32292-520	32392

Restek will create the right solution for you!

Restek should be your first choice for custom-made reference materials. Our inventory of over 3,000 compounds ensures you of maximum convenience, maximum value, and minimum time spent blending mixtures in your lab.

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RESTEK pharmaceutical

Applications note

HPLC Stationary Phase Selection for the Analysis of Steroids

Steroids are an important class of pharmaceuticals. Of the top 200 prescribed drugs in 1998, 29 are classified as steroids.[1] Their uses range from contraception and hormone therapy to treatment of bronchial asthma. Steroids of differing sub-classes frequently are used in combinations to generate a desired therapeutic effect.

High performance liquid chromatography (HPLC) analysis of steroids can be performed with many types of stationary phases. Restek's Ultra C18, Ultra C4, Ultra Cyano, Allure™ C18, and Allure™ Basix columns can provide the selectivity and retention characteristics needed to develop rapid and robust analytical methods for a wide range of steroids. Therefore, the choice of column phase essentially is dictated by the sample matrix and sensitivity concerns.

[1] *RxList, www.rxlist.com*

Hydrophobic Interaction

C18 phases may provide excellent separation of steroid components but can produce added retention for certain hydrophobic steroids or lipophylic matrix components, resulting in longer analysis times. The Ultra C4 stationary phase provides excellent stability and selectivity for steroids. Its reduced hydrophobic nature can significantly reduce retention time while maintaining the desired selectivity. Reducing the retention time of an analyte increases its signal-to-noise response for better detection. Retention and resolution of two estrogens and a progestrogen using Ultra C4 and Ultra C18 columns with the same mobile phase conditions show that both columns offer similar resolution of these compounds. The retention of other more hydrophobic components should be significantly reduced on the Ultra C4 (Figure 1).

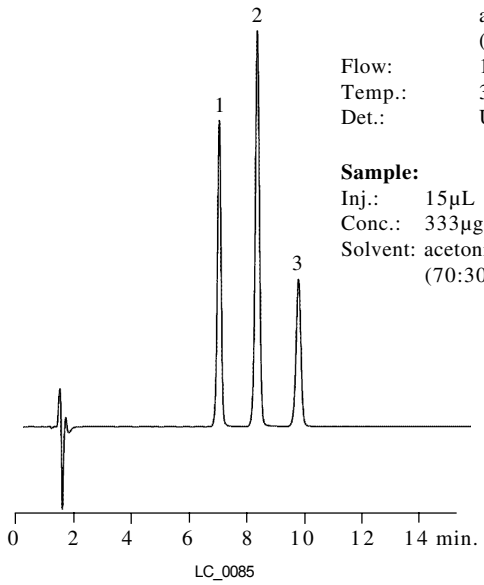
Figure 1

The Ultra C4 column's reduced hydrophobic tendencies provide excellent resolution and reduced analysis time for two estrogens and a progestogen when compared to the Ultra C18 phase.

Peak List:
1. ß-estradiol
2. norethindrone
3. ethynyl estradiol

Column: **Ultra C4**
Catalog #: 9102365
Dimensions: 150 x 4.6mm
Particle Size: 3µm
Pore Size: 100Å
Conditions:
Mobile Phase: 10mM ammonium formate, pH 3.0: acetonitrile (60:40, v/v)
Flow: 1.2mL/min.
Temp.: 30°C
Det.: UV @ 230nm

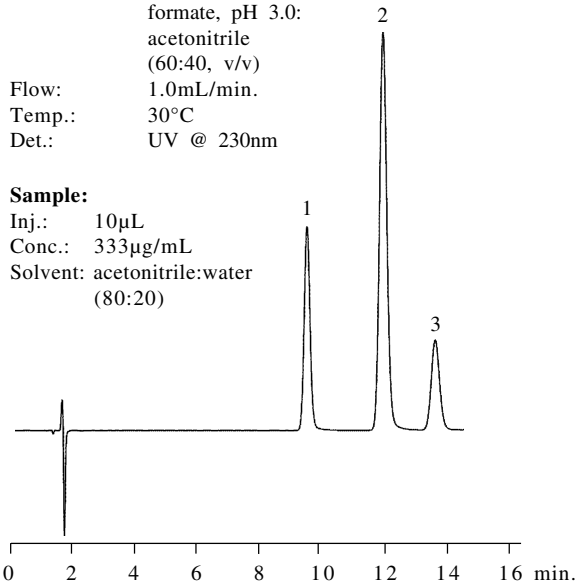
Sample:
Inj.: 15µL
Conc.: 333µg/mL
Solvent: acetonitrile:water (70:30)



LC_0085

Column: **Ultra C18**
Catalog #: 9174565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å
Conditions:
Mobile Phase: 10mM ammonium formate, pH 3.0: acetonitrile (60:40, v/v)
Flow: 1.0mL/min.
Temp.: 30°C
Det.: UV @ 230nm

Sample:
Inj.: 10µL
Conc.: 333µg/mL
Solvent: acetonitrile:water (80:20)



LC_0118

pharmaceutical

When analyzing steroids by LC/mass spectrometry (MS), the extra retention offered by the Ultra C18 column may prove advantageous. LC/MS sensitivity can be enhanced by increasing the organic content of the mobile phase. The added retention of the Ultra C18 phase allows the use of mobile phases with higher organic content to speed elution of the analytes and increase LC/MS sensitivity. Keep in mind that increasing the organic solvent content of the mobile phase may alter the elution order of the components.

Polar Interaction

Hydrophobic interaction is only one mechanism for resolving related compounds. Phases that can introduce a polar mechanism of retention can sometimes provide selectivity not offered by C18 phases. The Ultra Cyano and Allure™ Basix phases have shown excellent utility in the analysis of steroids and provide an alternative selectivity to the C18 phase.

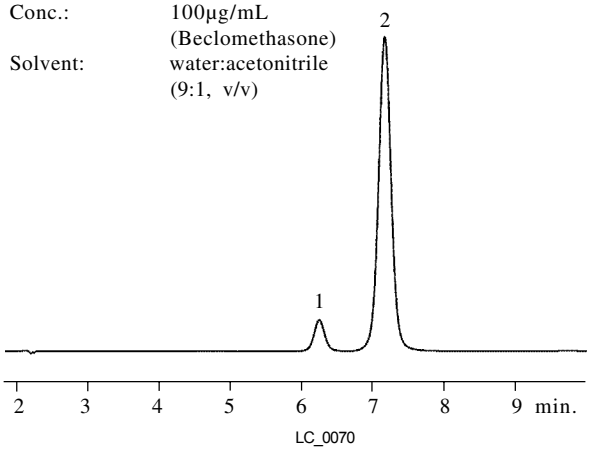
When measuring impurities by analytical chromatography or separating impurities by preparative chromatography, it often is better to have the impurity elute before the major analyte peak. If the major analyte peak tails badly or must be injected at high concentration, an impurity residing in the tail of the primary analyte peak may reduce fractional purity and analytical reproducibility. The Ultra Cyano and Ultra C18 phases demonstrate the outstanding peak shape and selectivity toward beclomethasone and des-chlorobeclomethasone (Figure 2). The elution order of

Figure 2

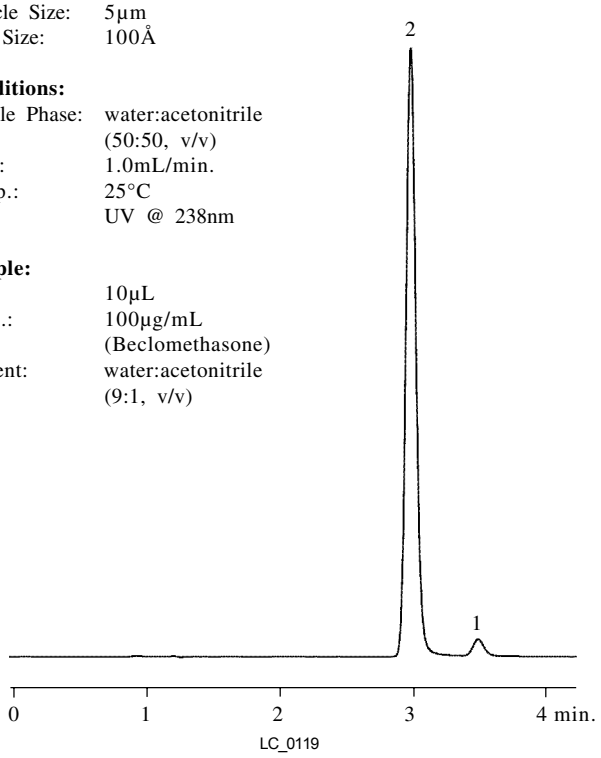
The elution order change exhibited by the Ultra Cyano column can aid in the separation of beclomethasone from its des-chlorinated impurity.

Peak List:
1. des-chlorobeclomethasone
2. beclomethasone

Column:	**Ultra Cyano**
Catalog #:	9106565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å
Conditions:	
Mobile Phase:	water:acetonitrile (70:30, v/v)
Flow:	1.0mL/min.
Temp.:	ambient
Det.:	UV @ 238nm
Sample:	
Inj.:	10µL
Conc.:	100µg/mL (Beclomethasone)
Solvent:	water:acetonitrile (9:1, v/v)



Column:	**Ultra C18**
Catalog #:	9174565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å
Conditions:	
Mobile Phase:	water:acetonitrile (50:50, v/v)
Flow:	1.0mL/min.
Temp.:	25°C
Det.:	UV @ 238nm
Sample:	
Inj.:	10µL
Conc.:	100µg/mL (Beclomethasone)
Solvent:	water:acetonitrile (9:1, v/v)



beclomethasone and des-chlorobeclomethasone is reversed on the Ultra Cyano column compared to the Ultra C18 column, thereby allowing the impurity to elute before the major component.

The analysis of the contraceptive mixture of ethynyl estradiol and norgestrel using Allure™ C18 and Allure™ Basix columns demonstrates excellent peak shape and selectivity towards these compounds, although the elution order is reversed (Figure 3). Because either column is capable of separating these compounds, sensitivity and sample matrix concerns will dictate the selection of the most appropriate column.

Conclusion

Factors such as sensitivity, purity, selectivity, and reproducibility influence column selection when creating a chromatographic method. No one column can provide all the necessary factors for every analysis. The Ultra and Allure™ column series provide a wide range of retention and selectivity characteristics to create rugged chromatographic methods. The choice of the best stationary phase must be determined during the course of method development to insure the appropriate analytical factors have been optimized.

Figure 3

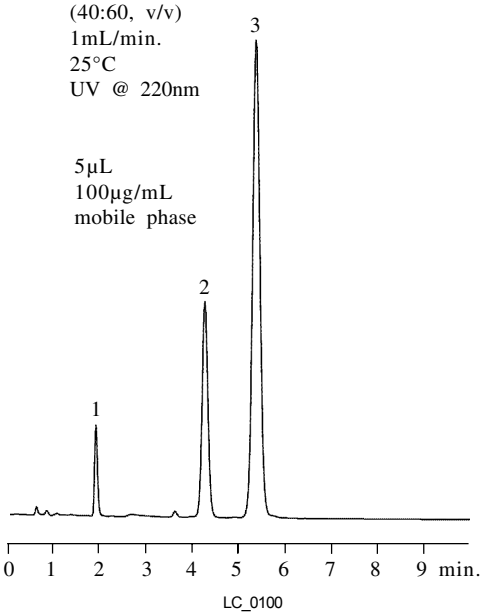
The Allure Basix column offers an alternative elution order along with excellent peak shape and selectivity for a common contraceptive mixture when compared to the Allure C18 column.

Peak List:
1. uracil (marker)
2. norgestrel
3. ethynyl estradiol

Column: **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile Phase: water:methanol (40:60, v/v)
Flow: 1mL/min.
Temp.: 25°C
Det.: UV @ 220nm

Sample:
Inj.: 5µL
Conc.: 100µg/mL
Solvent: mobile phase

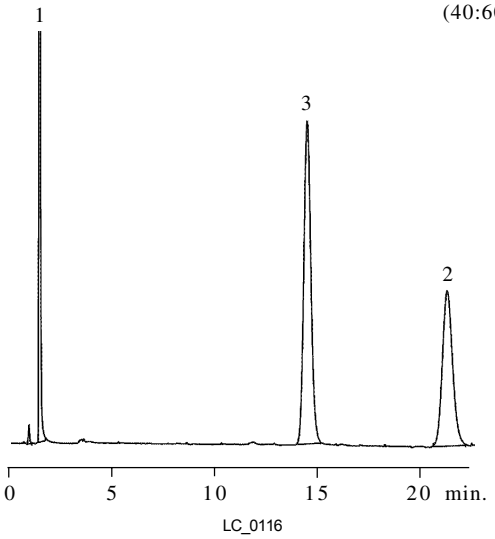


LC_0100

Column: **Allure™ C18**
Catalog #: 9164565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile Phase: water:methanol (25:75, v/v)
Flow: 1.0mL/min.
Temp.: 25°C
Det.: UV @ 220nm

Sample:
Inj.: 5µL
Conc.: 100µg/mL
Solvent: water:methanol (40:60)



LC_0116

Allure™ Basix

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9161531	9161532	9161533	9161535
50mm length	9161551	9161552	9161553	9161555
100mm length	9161511	9161512	9161513	9161515
150mm length	9161561	9161562	9161563	9161565
200mm length	9161521	9161522	9161523	9161525
250mm length	9161571	9161572	9161573	9161575

Allure™ C18

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9164531	9164532	9164533	9164535
50mm length	9164551	9164552	9164553	9164555
100mm length	9164511	9164512	9164513	9164515
150mm length	9164561	9164562	9164563	9164565
200mm length	9164521	9164522	9164523	9164525
250mm length	9164571	9164572	9164573	9164575

Ultra C4

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9102331	9102332	9102333	9102335
50mm length	9102351	9102352	9102353	9102355
100mm length	9102311	9102312	9102313	9102315
150mm length	9102361	9102362	9102363	9102365
200mm length	9102321	9102322	9102323	9102325

Ultra C18

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9174531	9174532	9174533	9174535
50mm length	9174551	9174552	9174553	9174555
100mm length	9174511	9174512	9174513	9174515
150mm length	9174561	9174562	9174563	9174565
200mm length	9174521	9174522	9174523	9174525
250mm length	9174571	9174572	9174573	9174575

Ultra Cyano

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9106531	9106532	9106533	9106535
50mm length	9106551	9106552	9106553	9106555
100mm length	9106511	9106512	9106513	9106515
150mm length	9106561	9106562	9106563	9106565
200mm length	9106521	9106522	9106523	9106525
250mm length	9106571	9106572	9106573	9106575

RESTEK pharmaceutical

#59511A

Applications note

Improved HPLC Analysis of Analgesics

Analgesics are the largest selling class of pharmaceuticals. Many people use analgesics in prescription form or in simple over-the-counter remedies. Headaches, inflammation, colds, flu, congestion, fever, sneezing, coughing, and muscle aches are among the list of symptoms that can be treated with analgesics.

Symptoms, however, often arrive in multiples. For multiple symptoms, analgesics combined with pain relief, fever reduction, and other beneficial compounds have been created. Many of these compounds can be analyzed simultaneously using high performance liquid chromatography (HPLC), thereby improving laboratory efficiency and productivity. With the selection of the proper LC column phase, separation becomes a simpler and more manageable task that does not rely upon extensive preparation procedures or use of ion pairing agents, which often are described in pharmaceutical compendiums.

Popular compounds for multi-active analgesics include aspirin, salicylic acid, salicylamide, acetaminophen, ibuprofen, naproxen, guaifenesin, codeine, oxycodone, hydrocodone, and caffeine. The Restek Ultra Phenyl, Allure™Acidix, and Allure™ Basix HPLC column phases separate mixtures of these pharmaceuticals in a productive and cost effective manner. The selective chemistry of these phases provide powerful separation mechanisms.

The interaction of codeine with active residual silanol sites on improperly deactivated column phases can cause extreme tailing for this compound. A properly end-capped and base-deactivated phenyl phase, such as the Ultra Phenyl phase, can significantly reduce tailing of the codeine peak. In Figure 1, the Ultra Phenyl column provides excellent peak shape and superior resolution of guaifenesin and codeine with a run time of less than six minutes. Furthermore, the method is MS-compatible because no mobile phase additives or ion pairing reagents are needed to reduce tailing.

Acidic silanols also can cause undesirable interaction with analytes containing basic functional groups such as amines and amides. The fully end-capped Allure™ Basix column was developed with specialized functional groups that provide the desired interaction with nitrogen-based functional groups such as amines and amides.

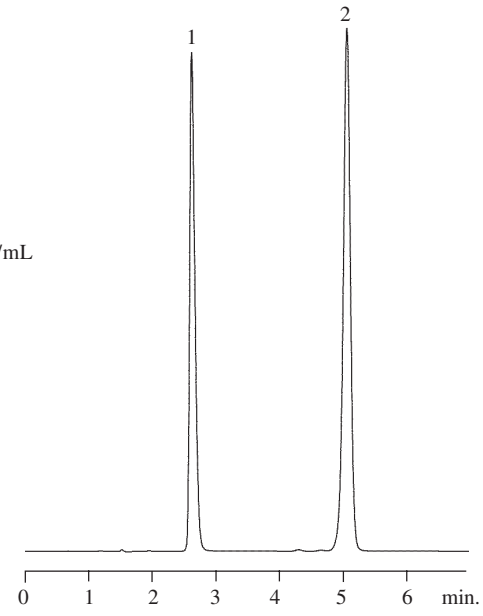
Figure 1

Excellent peak shape and superior resolution of guaifenesin and codeine using Ultra Phenyl column.

Peak List:
1. codeine
2. guaifenesin

Sample:

Inj.:	5µL
Conc.:	codeine 825µg/mL guaifenesin 375µg/mL
Solvent:	methanol:water (50:50)



Column:	**Ultra Phenyl**
Catalog #:	9105565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å
Conditions:	
Mobile Phase:	10mM ammonium formate, pH 2.5: acetonitrile (80:20,v/v)
Flow:	1.2mL/min.
Temp.:	25°C
Det.:	UV @ 280nm

Figure 2 displays a four-component mixture of acetaminophen (a secondary amide); and codeine, oxycodone, and hydrocodone (tertiary amines). Admixtures of acetaminophen and one of the above tertiary amines are used as popular prescription painkillers. The Allure™ Basix column separates all these analytes within 8.5 minutes, showing excellent resolution and peak shape without the need for the docusate sodium modifier recommended in certain method compendiums.

Acetaminophen also is included in many over-the-counter remedies for congestion. In Figure 3, the popular mix of nitrogen-based compounds, such as acetaminophen, pseudoephedrine, and caffeine are separated. The Allure™ Basix column performs well, separating all compounds in under 9.5 minutes with excellent shape and resolution, and without the need of ethanesulfonic acid as an ion-pairing agent.

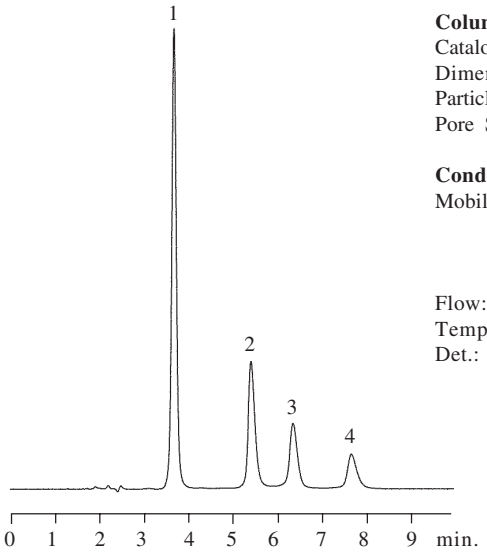
Some of the oldest analgesics are not bases, but acids. Acetaminophen falls into the category of a weak acid. Figure 4 displays the analysis of acetaminophen on the Allure™ Acidix column. The selective interaction of this column with acidic solutes, such as acetaminophen, results in excellent peak shape and an analysis time of under four minutes.

Figure 2

The Allure™ Basix column separates acetaminophen and tertiary amines within 8.5 minutes, resulting in excellent resolution and peak shape.

Peak List:
1. acetaminophen
2. codeine HCl
3. oxycodone HCl
4. hydrocodone bitartrate

Sample:
Inj.: 5µL
Conc.: 100µg/mL
Solvent: mobile phase



Column: **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

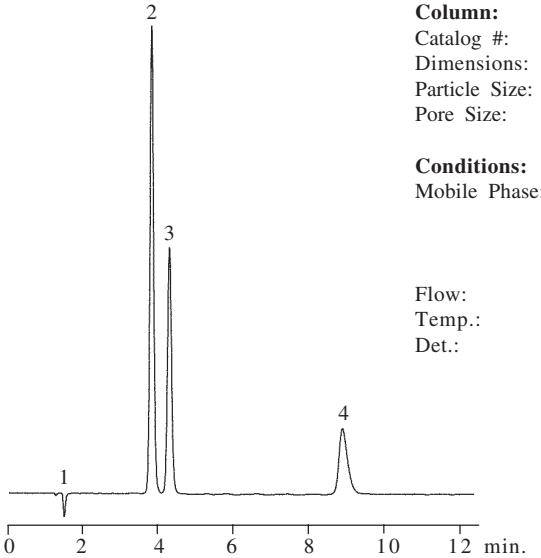
Conditions:
Mobile Phase: 20mM ammonium acetate, pH 4.5: acetonitrile (85:15, v/v)
Flow: 1.0mL/min.
Temp.: 30°C
Det.: UV @ 280nm

Figure 3

The Allure™ Basix column separates acetaminophen mixed with nitrogen-based compounds in under 9.5 minutes, with excellent resolution and peak shape.

Peak List:
1. sample solvent (marker)
2. caffeine
3. acetaminophen
4. pseudoephedrine

Sample:
Inj.: 5µL
Conc.: 100µg/mL
Solvent: mobile phase



Column: **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile Phase: 10mM ammonium acetate, pH 6.9: methanol (75:25, v/v)
Flow: 1.0mL/min.
Temp.: 25°C
Det.: UV @ 210nm

Figure 5 displays the analysis of aspirin and salicylic acid, which also are easily separated on the Allure™ Acidix phase without the need of 1-heptane sulfonic acid or special solid phase supports referenced by the USP. This separation uses LC/MS compatible buffers and will out-perform the reproducibility of any method employing ion-pair reagents.

Ibuprofen and naproxen are medications frequently recommended for pain caused by inflammation. Figure 6 displays the separation of these closely related carboxylic acids in under seven minutes using an Allure™ Acidix column.

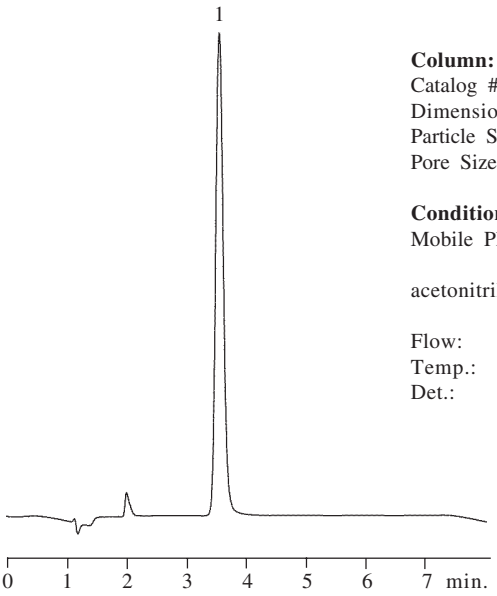
The Restek Ultra Phenyl, Allure™ Acidix, and Allure™ Basix columns provide excellent separation of analgesics without the need of additional mobile phase modifiers. The Food and Drug Administration (FDA) is now requiring "grandfathered" methods that suffer from low accuracy and precision to be upgraded to state-of-the-art methods. These phases provide the means to update many of the older methods used in pharmaceutical compendia, which will reduce laboratory costs, improve laboratory productivity, and minimize additive obstacles to LC/MS compatibility.

Figure 4

Acetaminophen elutes with excellent peak shape in under 4 minutes using the Allure™ Acidix column.

Peak List:
1. acetaminophen

Sample:
Inj.: 10µL
Conc.: 1mg/mL
Solvent: water:acetonitrile (1:1, v/v)



Column: **Allure™ Acidix**
Catalog #: 9162565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

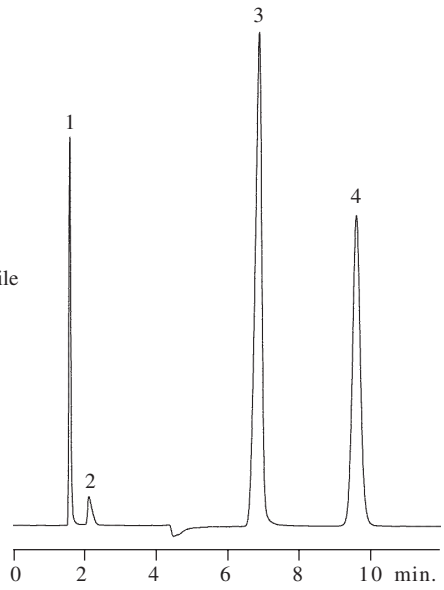
Conditions:
Mobile Phase: 20mM ammonium acetate pH 4.5: acetonitrile (95:5, v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 220nm

Figure 5

Aspirin and salicylic acid are easily separated on the Allure™ Acidix column.

Peak List:
1. toluene
2. unknown
3. salicylic acid
4. aspirin

Sample:
Inj.: 10µL
Conc.: 200µg/mL
Solvent: buffer : acetonitrile (1:1, v/v)



Column: **Allure™ Acidix**
Catalog #: 9162565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

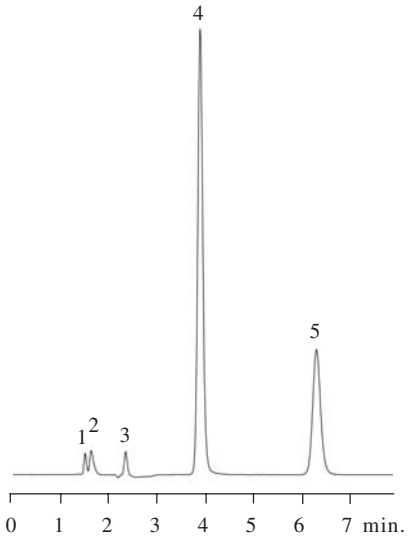
Conditions:
Mobile Phase: 20mM ammonium acetate pH 4.5: acetonitrile (20:80, v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 220nm

Figure 6

Closely related carboxylic acids are quickly separated in under 7 minutes using the Allure™ Acidix column.

Peak List:
1. unknown
2. unknown
3. uracil
4. ibuprofen
5. naproxen

Sample:
Inj.: 10µL
Conc.: 100µg/mL (ibuprofen and naproxen)
Solvent: ammonium acetate buffer : acetonitrile (1:1, v/v)



Column: **Allure™ Acidix**
Catalog #: 9162565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

Conditions:
Mobile Phase: 20mM ammonium acetate pH 4.5: acetonitrile (30:70, v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 220nm

Allure™ Acidix Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9162531	9162532	9162533	9162535
50mm length	9162551	9162552	9162553	9162555
100mm length	9162511	9162512	9162513	9162515
150mm length	9162561	9162562	9162563	9162565
200mm length	9162521	9162522	9162523	9162525
250mm length	9162571	9162572	9162573	9162575

Allure™ Basix Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9161531	9161532	9161533	9161535
50mm length	9161551	9161552	9161553	9161555
100mm length	9161511	9161512	9161513	9161515
150mm length	9161561	9161562	9161563	9161565
200mm length	9161521	9161522	9161523	9161525
250mm length	9161571	9161572	9161573	9161575

Ultra Phenyl Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9105531	9105533	9105533	9105535
50mm length	9105551	9105552	9105553	9105555
100mm length	9105511	9105512	9105513	9105515
150mm length	9105561	9105562	9105563	9105565
200mm length	9105521	9105522	9105523	9105525
250mm length	9105571	9105572	9105573	9105575

RESTEK ISO 9001 REGISTERED

RESTEK pharmaceutical

Applications note

The Ultra IBD Column Allows HPLC Separation of Polar and Non-Polar Analytes from the Same Sample

The ability to retain and separate polar and non-polar analytes in the same sample can be difficult in high performance liquid chromatography (HPLC) analyses. Restek's Intrinsically Base Deactivated (IBD) Ultra IBD column can provide the retention needed to simultaneously separate polar and non-polar analytes to more expediently perform analyses within a single method. The Ultra IBD phase is composed of an alkyl chain and a non-ionic functional group. The alkyl chain ensures retention of more hydrophobic analytes, and the intrinsic base deactivation of the functional group reduces tailing often seen with basic analytes. The nature of the functional group also allows retention of charged analytes. The Ultra IBD column is the best choice for separating mixtures of acids, bases, zwitterions, and neutral analytes.

Ultra IBD Column Retains Analytes Across a Wide Range of pH and Mobile Phase Compositions

The base deactivation and polar interaction capability of the Ultra IBD phase remains steady across the pH stability range of the silica. Therefore, the polar retentive capability of the phase is not decreased as the pH is decreased; the retention is stable for a given buffer concentration and aqueous/organic ratio.

Using 100% water can cause chain collapse and loss of retention in standard C18 phases, thereby requiring at least 5% organic in the mobile phase. Because the Ultra IBD phase is immune to stationary phase collapse reverse phase gradients starting with 100% water can be used for maximum retention of analytes. Unlike standard alkyl phases, the Restek Ultra IBD phase results in a rugged HPLC column that can withstand these separation extremes.

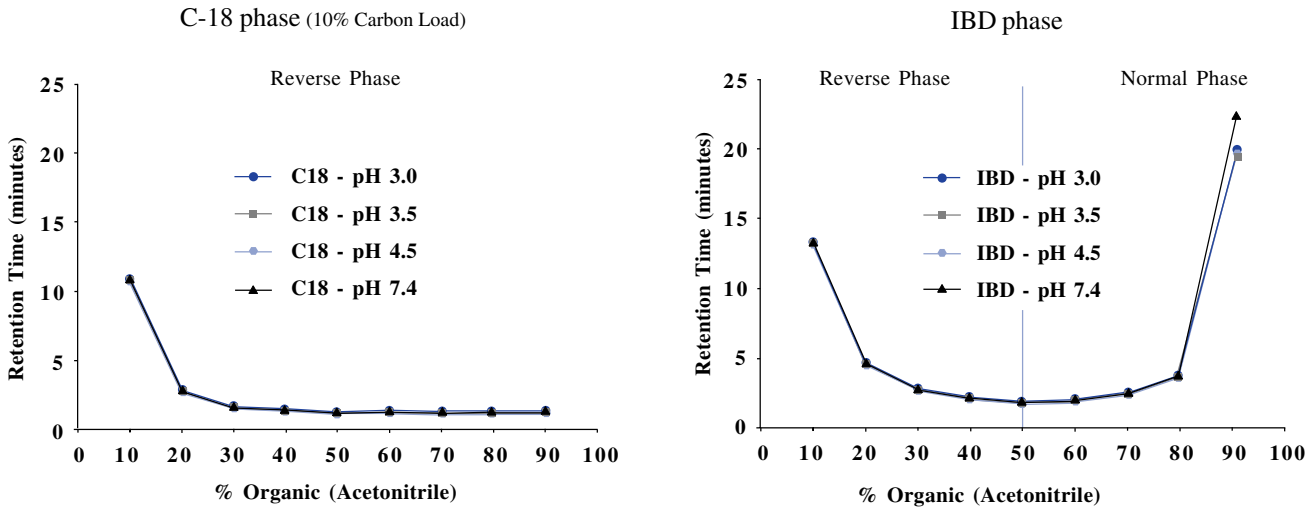
We compared the separation ability of the Ultra IBD column to that of a C18 column using varying pH and mobile phase ratios (Figure 1). Cephaloridine was analyzed because it contains both polar and non-polar (hydrophobic) functional groups. The data demonstrates the ability of the phase to retain the analyte under a wide range of mobile phase conditions.

The comparison revealed that the C18 column cannot successfully retain cephaloridine when acetonitrile exceeds 40% in the mobile phase. Upon reaching 40% acetonitrile, cephaloridine elutes with the solvent front. The C18 column is only capable of hydrophobic interaction by a reverse phase mechanism, and so cannot exhibit the characteristic U-shaped retention profile shown by the Ultra IBD column.

Figure 1

The Ultra IBD column retains analytes by hydrophobic and polar interactions, whereas the C18 column is only capable of retention by hydrophobic interaction

Cephalordine Retention vs % Organic - Aqueous Portion is Ammonium Formate (20 mM)



pharmaceutical

By contrast, the Ultra IBD column reaches a minimum retention time at 50% acetonitrile, closely duplicating the hydrophobic interactions of the C18 column. As the organic ratio increases above 50%, polar interactions in the normal phase mode allow retention of the cephaloridine analyte. The retention of cephaloridine reaches a usable maximum at 90% acetonitrile. Furthermore, the U-shaped retention profile is not adversely altered by the pH of the buffer. The separation is a near mirror image across the reverse phase and normal phase modes. Only at the extreme of 90% acetonitrile is a small variation seen in analyte retention due to pH changes. Even then, the variation is less than 2.8 minutes at pH 7.4. The normal phase retention is greater than the reverse phase retention. Both modes, however, provide similar peak shape and retention time (Figure 2).

Note: As with any HPLC column phase capable of performing a reverse or normal phase separation, the user should take care to ensure the retention mechanism is either reverse or normal phase.

Ultra IBD Column Solvent Focusing Ability Improves Detection

Loading multiple samples onto the head of the column concentrates the analyte to improve detection. The injections are then eluted through the system using a decreased solvent level ratio. This ability to load and maintain a sample at the column head is called solvent focusing.*

When cephaloridine was injected with a mobile phase of either 100% acetonitrile or 100% buffer, no peak eluted from the system—even after 90 minutes. To elute the analyte from the system, the mobile phase ratio was altered to 80%. The peak then eluted near its isocratic run time. Cephaloridine remained intact near the start of the column and did not begin appreciably eluting through the column until another mobile phase ratio was selected.

Due to its solvent focusing capabilities, an Ultra IBD column can be loaded at either the 100% aqueous (reverse phase) or 100% organic (normal phase) solvent level to concentrate the sample directly onto the head of the column.

Furthermore, this solvent focusing capability allows use of a gradient system to better remove impurities from a sample mixture that have adsorbed onto the column. More hydrophilic impurities theoretically could be eluted from the analytes of interest with a simple aqueous flush. The process could be reversed using 100% organic for truly neutral analytes.

* The ability to perform solvent focusing will be affected by the chemical nature of the analyte.

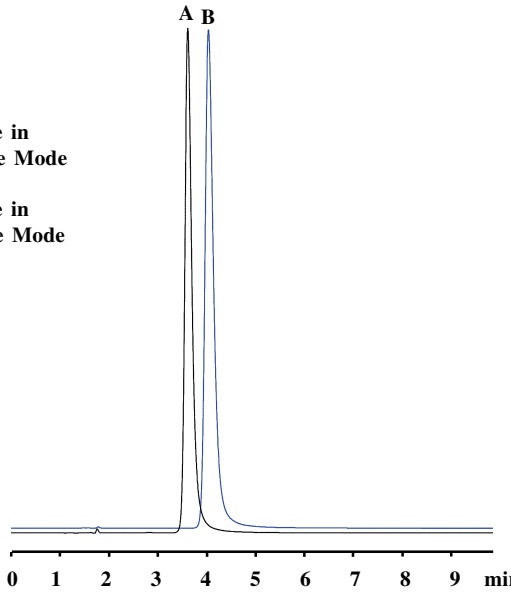
Conclusion

The unique functionality of the Ultra IBD column creates an ideal tool for the separation and detection of acids, bases, and zwitterions within complex mixtures. Because the phase interactions are bimodal, a wide range of compounds can be retained and separated by either reverse or normal phase mechanics. At the extreme range of the reverse or normal modes, the solvent focusing ability of the Ultra IBD column can be used to concentrate an analyte or to purify analytes within complex matrices.

See back for product listing.

Figure 2

Both normal and reverse phase modes provide good peak shape and retention time on Ultra IBD column.



Peak List:

A. Cephaloridine in Reverse Phase Mode

B. Cephaloridine in Normal Phase Mode

Column:	Ultra IBD, 5µm, 4.6mm x 150mm, 100 Å (cat.# 9175565)
Mobile Phase:	
Reverse Phase	20mM Ammonium Acetate, pH 4.0:Acetonitrile (80:20)
Normal Phase:	20mM Ammonium Acetate, pH 4.0:Acetonitrile (20:80)
Inj.:	5uL, 1.0mg/mL Cephaloridine
Det.:	254nm
Temp.:	27°C

Application notes: LC-0101 & LC-0102

Trident™ Integral HPLC Guard Column System Offers Maximum Protection Against Contaminants and Particulate Matter

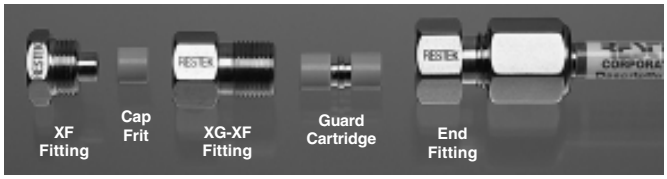
High backpressure is one of the most common problems encountered when performing HPLC analysis. Normal column backpressure is observed after a new column has been installed and equilibrated with the mobile phase. Unfortunately, this pressure will often increase with use because of particulates collecting on the column inlet frit.

The source of these particles can be from sample impurities, mobile phase contaminants, and the injector or autosampler rotor seal. The presence of particles can result in increased backpressure, split peaks, tailing, and eventually over-pressure shut-down. In some circumstances, these problems can be corrected by back-flushing the column. However, in many cases it results in an unusable column.

To reduce backpressure problems, all samples and mobile phase solvents must be filtered before use; and rotor seals should be changed on a routine basis. Along with these preventative measures, it is advisable to use column prefilters such as the Trident™ column protection system. When using a prefilter, particles build up on its inexpensive, replaceable frit, instead of the permanent frit at the head of the column.

The system's foundation consists of the analytical column configured with our exclusive Trident™ end fitting and XF fitting. This configuration contains the standard internal frit as well as a replaceable cap frit, which can be easily changed without disturbing the packed bed. Changing the external frit can reverse the effects of accumulated particles, such as high backpressure or peak distortion. To obtain this basic configuration, simply order any Restek HPLC column that has a (-700) suffix catalog number.

For maximum protection against contaminants and particulate matter, the system can be configured with both an integral guard cartridge and a replaceable external frit. To obtain this configuration, simply order any Restek HPLC column that has a (-700) suffix catalog number, the XG-XF male fitting (cat.# 25026), and the appropriate pack of guard cartridges (see right).



Trident™ HPLC Guard Column Fittings and Frits

Description	cat.#
XG-XF Fitting for 1cm Guard Cartridge	25026
Replacement XF Filter Fitting	25024
Replacement Cap Frits, 2µm	25022
Replacement Cap Frits, 0.5µm	25023

Trident™ HPLC Guard Column Cartridges

	(10 x 2.1mm)	(10 x 4.0mm)
Guard Cartridge	**cat.#**	**cat.#**
Allure™ Acidix	916250212	916250210
Allure™ Basix	916150212	916150210
Allure™ C18	916450212	916450210
Allure™ Silica	916050212	916050210
Ultra Amino	910750212	910750210
Ultra C1	910150212	910150210
Ultra C4	910250212	910250210
Ultra C8	910350212	910350210
Ultra C18	917450212	917450210
Ultra Cyano	910650212	910650210
Ultra IBD	917550212	917550210
Ultra Phenyl	910550212	910550210
Ultra Silica	910050212	910050210

Product Listing

Ultra IBD, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365
200mm length	9175321	9175322	9175323	9175325

Ultra IBD, 3µm Columns with Trident™ Inlet

Particle Size: 3µm		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	—	9175332-700	9175333-700	9175335-700
50mm length	—	9175352-700	9175353-700	9175355-700
100mm length	—	9175312-700	9175313-700	9175315-700
150mm length	—	9175362-700	9175363-700	9175365-700
200mm length	—	9175322-700	9175323-700	9175325-700

Ultra IBD, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

Ultra IBD, 5µm Columns with Trident™ Inlet

Particle Size: 5µm		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	—	9175532-700	9175533-700	9175535-700
50mm length	—	9175552-700	9175553-700	9175555-700
100mm length	—	9175512-700	9175513-700	9175515-700
150mm length	—	9175562-700	9175563-700	9175565-700
200mm length	—	9175522-700	9175523-700	9175525-700
250mm length	—	9175572-700	9175573-700	9175575-700

Ultra IBD Guard Cartridges

Dimensions	cat.#	Qty.
10 x 2.1mm	917550212	3
10 x 4.0mm	917550210	3
20 x 4.0mm	917550220	2

RESTEK HPLC

Applications note

Single-Column Method for HPLC Analysis of Organic Acids in Fruit Juices, Using an Allure™ Organic Acids Column

Organic acids play multiple roles in food and beverage systems: they are important flavor compounds, and they are indicators of product quality. Organic acid profiles are monitored to determine the purity of certain fruit juices. In some food and beverage systems they are added as acidulants, to control the pH of a product. Certain organic acids also can be used as antimicrobial agents; for example, propionic acid can be used to slow mold growth. Malic acid, citric acid, and others are found in fruits. Oxalic acid is present in spinach and rhubarb; grapes contain tartaric acid.

Analysis of polar organic acids can be difficult on conventional reversed phase columns. A highly aqueous mobile phase is needed to increase interaction between the acids and the stationary phase, but stationary phases in conventional C18 columns collapse in 100% aqueous mobile phases. The Allure Organic Acids column was designed to enhance retention and selectivity in challenging applications such as this. Polar embedded groups allow the alkyl groups in Allure Organic Acids columns to remain extended in 100% aqueous mobile phases; retention is stable and reproducible.

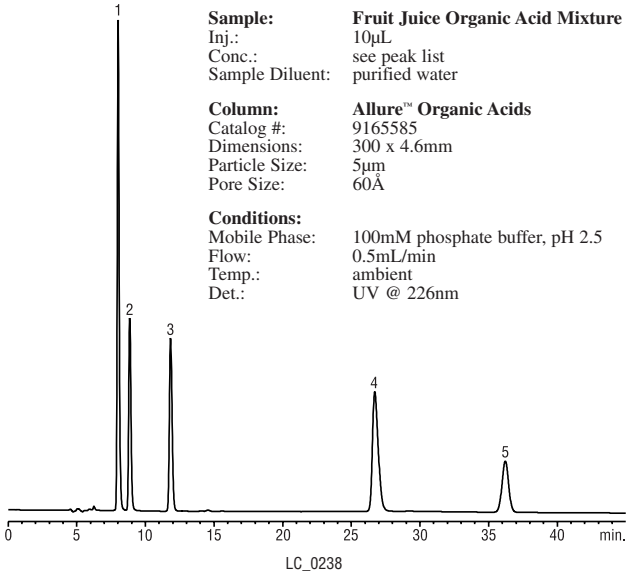
The fruit juice industry in the US alone is worth over $12 billion per year[1] and is many times that worldwide. As with other industries in which there is a large potential for profit, unscrupulous juice producers and traders have found ways to replace or extend more valuable juices. This can be done by substituting sugars for juice solids, or by diluting higher cost juices with less expensive ones. For example, white grape juice and pear juice have been used to extend other, more costly juices. To detect these adulterations, a number of laboratories employ fruit juice authenticity testing.

Because juices are chemically quite complex, several complementary tests should be performed to verify authenticity. These can include determining sugar profile and sorbitol content; minerals; anthocyanin pigments; phenolics; oligosaccharides; carbon stable isotope ratio for various components; and organic acid profile. High performance liquid chromatography (HPLC) is a powerful tool in analyses of many of these components. With these complex matrices, the resolving power of HPLC is invaluable for accurately quantifying components.

Organic acids give fruit products their characteristic tartness. Since the organic acids content varies in composition and in concentrations among different fruit juices, organic acid content can be used to identify a product and verify its purity. For example, malic acid is a major component of the organic acid profile of apple juice. If apple juice has been diluted, e.g., with sugar water, the malic acid content will be low. In grape juice, tartaric acid is present at relatively high levels. Cranberry juice, on the other

Figure 1

Excellent separation of tartaric and quinic acids by an Allure™ Organic Acids column.



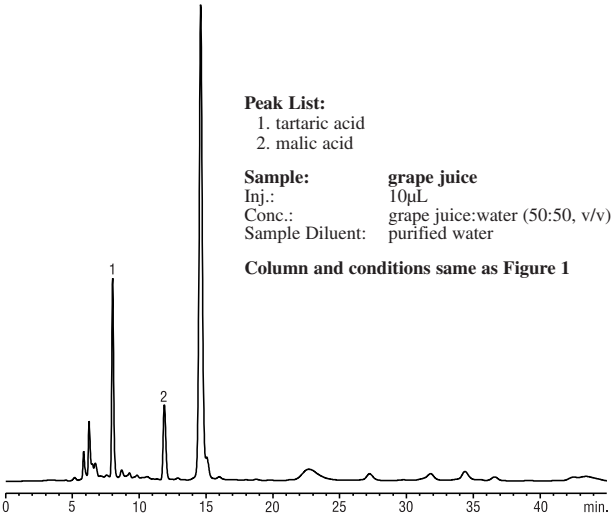
LC_0238

Peak List:	Conc.
1. tartaric acid	1mg/mL
2. quinic acid	1mg/mL
3. malic acid	1mg/mL
4. citric acid	1mg/mL
5. fumaric acid	0.005mg/mL

Sample:	**Fruit Juice Organic Acid Mixture**
Inj.:	10µL
Conc.:	see peak list
Sample Diluent:	purified water
Column:	**Allure™ Organic Acids**
Catalog #:	9165585
Dimensions:	300 x 4.6mm
Particle Size:	5µm
Pore Size:	60Å
Conditions:	
Mobile Phase:	100mM phosphate buffer, pH 2.5
Flow:	0.5mL/min
Temp.:	ambient
Det.:	UV @ 226nm

Figure 2

Tartaric and malic acids, the primary organic acids in grape juice, resolved by an Allure™ Organic Acids column.



LC_0237

Peak List:
1. tartaric acid
2. malic acid

Sample:	**grape juice**
Inj.:	10µL
Conc.:	grape juice:water (50:50, v/v)
Sample Diluent:	purified water

Column and conditions same as Figure 1

hand, contains quinic acid. A cranberry juice that contains measurable amounts of tartaric acid would be suspect. Thus, it is critical to both identify and quantify the organic acids in a fruit juice to determine if the juice is described truthfully.

The organic acid content of fruit juices, such as cranberry juice and apple juice, can be determined using AOAC method 986.13.[2] In this procedure, reversed phase HPLC with a UV detector is used to quantify organic acids. Because several of the organic acids are extremely difficult to resolve, this procedure calls for two reversed phase C18 columns in series. A 100% aqueous mobile phase (phosphate buffer at pH 2.4) is used to maximize the interaction between the acids and the stationary phase.

Now there is a simpler and more reliable approach. A single 30cm Allure Organic Acids column effectively resolves key organic acids, such as tartaric and quinic acids, using the chromatographic conditions specified in AOAC method 986.13!

Figure 1 shows a separation of typical fruit juice organic acids, including tartaric, quinic, malic, citric, and fumaric acids. Note the baseline resolution between tartaric and quinic acids. This superior performance makes interpretation of analytical data more reliable. Similarly, note the distinct organic acid profiles for grape juice and cranberry juice cocktail in Figures 2 and 3.

Analysis of polar organic acids can be difficult on conventional reversed phase columns, even when using highly aqueous mobile phases and two reversed phase columns in series for the separation. In contrast, an Allure Organic Acids column provides enhanced retention and selectivity for these compounds, allowing the separation to be performed on one 30cm column. Retention is stable and reproducible, even with a 100% aqueous mobile phase, as specified in AOAC method 986.13. If you are monitoring fruit juice quality, and want a trouble-free analysis with accurate results, we highly recommend an Allure Organic Acids column.

References

1. *Authenticity of Apple Juice* Technical Bulletin #2 (1996), Analytical Chemical Services of Columbia, Inc.
2. Official Methods of Analysis (2000), AOAC International, 17th edition, method #986.13.

HPLC Catalog

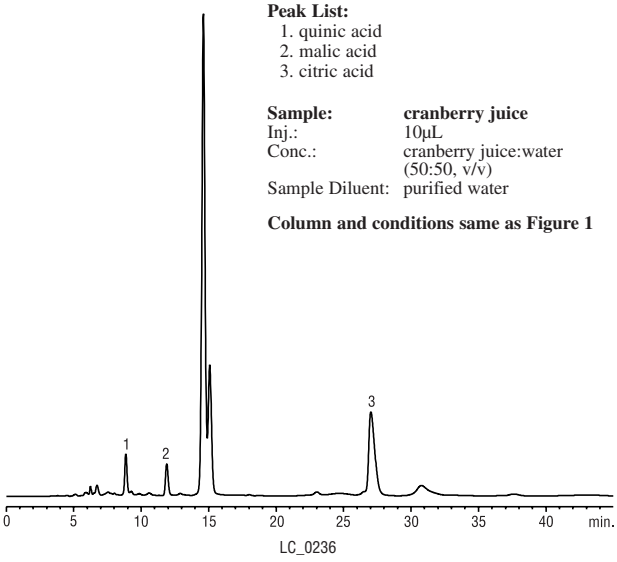
Ability to manufacture silica, synthesize many stationary phases, and perform high-density bonding has made Restek a significant supplier of HPLC columns. This 116-page catalog lists columns in four silica lines, including LC/MS columns and other special-purpose columns, microbore columns, and preparative columns.Many application chromatograms. (lit. cat.# 59241A)

Foods, Flavors, & Fragrances In-Review

Abstracts of 30+ Restek publications on foods - flavors - fragrances applications. Check the publications you want on the pre-paid postcard - or access them on our website. (lit. cat.# 59489)

Figure 3

Quinic, malic, and citric acids in cranberry juice cocktail.



Allure™ Organic Acids Column

Physical Characteristics:

particle size:	5µm, spherical, non-endcapped phase
pore size:	60Å
pH range:	2.5 to 7.5
temperature limit:	80°C

Dimension	qty.	cat.#
300mm x 4.6mm	ea.	9165585

Fruit Juice Organic Acid Mixture

citric acid	2000µg/mL
fumaric acid	10
malic acid	2000
quinic acid	2000
tartaric acid	2000

In water, 1mL/ampul

Each	5-pk.
35080	35080-510

In water, 5mL/ampul

Each	5-pk.
35081	35081-510

Foods, Flavors, & Fragrances

A 48-page overview of the foods, flavors, fragrances applications for which Restek HPLC and GC columns have been used. Applications include amino acids, carbohydrates, chiral separations, essential oils, fats and oils, organic acids, preservatives, vitamins, and more. (lit. cat.# 59260)

#59539

RESTEK environmental

Applications note

GC Analysis of US EPA Method 504.1 Organochlorine Pesticides Using the Rtx®-CLPesticides and Rtx®-CLPesticides2 Columns

United States Environmental Protection Agency (US EPA) Method 504.1 is a common test performed by environmental laboratories for the analysis of 1,2-dibromoethane (EDB), 1,2-dibromo-3-chloropropane (DBCP), and 1,2,3-trichloropropane (TCP) in drinking water. This method uses a salting-out microextraction and dual-column gas chromatography with electron capture detection (GC/ECD). Because the workload of Method 504.1 usually does not warrant the dedication of an instrument, many laboratories use the same instruments for the analysis of chlorinated pesticides and herbicides. Therefore, when selecting a set of capillary columns for this analysis, it is important that they work well for a number of different methods.

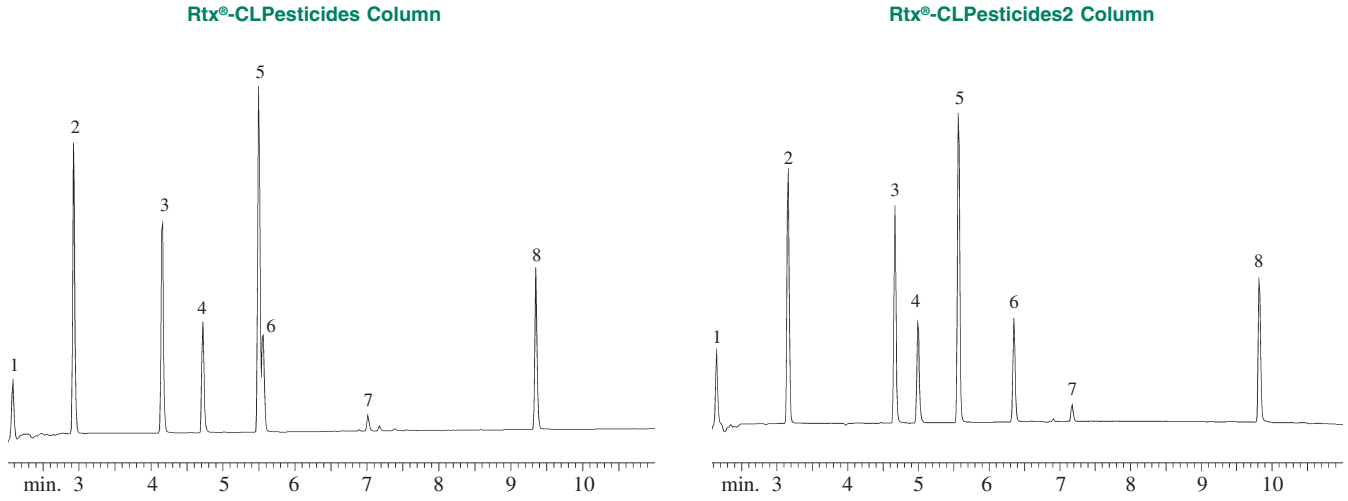
Restek has designed the Rtx®-CLPesticides and the Rtx®-CLPesticides2 columns specifically for the analysis of organochlorine pesticides (EPA Methods 608 and 8081), and they also provide excellent separation for the compounds listed in Method 504.1. In addition to the analytes listed in Method 504.1, these stationary phases also work well for the analysis of herbicides and polychlorobiphenyls (PCBs) because they are highly selective for compounds that contain electronegative substituents.

Figure 1 shows the analysis of the Method 504.1 target compounds on the Rtx®-CLPesticides and the Rtx®-CLPesticides2 columns, connected in parallel using a glass 'Y' Press-Tight® connector. This configuration is important to ensure that both the primary and confirmation column analyses are performed simultaneously under the same conditions, using the same injection port.

Method 504.1 requires that EDB, DBCP, and TCP be fully resolved from the common interference compounds (i.e., chloroform, bromodichloromethane, chlorodibromomethane,

Figure 1

Rtx®-CLPesticides and Rtx®-CLPesticides2 columns fully resolve Method 504.1 organochlorine pesticides in 10 minutes.



1. chloroform
2. bromodichloromethane
3. chlorodibromomethane
4. 1,2-dibromoethane (EDB)
5. 1,1,1,2-tetrachloroethane
6. bromoform
7. 1,2,3-trichloropropane
8. 1,2-dibromo-3-chloropropane (DBCP)

30m, 0.32mm ID, 0.50µm Rtx®-CLPesticides (cat.# 11139) and 30m, 0.32mm ID, 0.25µm Rtx®-CLPesticides2 (cat.# 11324), 0.32mm ID guard column (cat.# 10044), universal angled 'Y' Press-Tight® connector (cat.# 20403). Direct injection using a Uniliner® sleeve (cat.#20335). **On-column concentration:** 10pg each compound. **Oven temp.:** 35°C (hold 2 min.) to 300°C @ 12°C/min.; **Inj./det. temp.:** 200°C/300°C; **Carrier gas:** helium, 12psi constant pressure.

environmental

1,1,1,2-tetrachloroethane, and bromoform). The Rtx®-CLPesticides2 column fully resolved these compounds. The coelution of 1,1,1,2-tetrachloroethane and bromoform on the Rtx®-CLPesticides column is between two interference compounds, which does not effect the identification or quantification of EDB, DBCP, or TCP.

In summary, these columns are the complete solution for the analysis of chlorinated pesticides, herbicides, and Method 504.1 organochlorine pesticides. If you are involved in the analysis of any of these methods, and would like more information, please contact Restek's Technical Service Team at 800-356-1688 or 814-353-1300, ext. 4.

Product Listing

Rtx®-CLPesticides Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.25	340°C	11120	11123
0.32mm	0.50	340°C	11136	11139
0.53mm	0.50	340°C	11137	11140

ID	df (µm)	Stable to	10m	20m
0.18mm	0.14	340°C	42101	42102

Rtx®-CLPesticides2 Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.20	340°C	11320	11323
0.32mm	0.25	340°C	11321	11324
0.53mm	0.42	340°C	11337	11340

ID	df (µm)	Stable to	10m	20m
0.18mm	0.14	340°C	42301	42302

Rtx®-CLPesticides Column Kits

These kits include both a CLPesticides and CLPesticides2 column, a Universal Angled 'Y' Press-Tight® Connector, and a 5m guard column. (Note: Columns are not preconnected in these kits.)

Description	cat.#
0.53mm ID Rtx®-CLPesticides Kit	11197
0.32mm ID Rtx®-CLPesticides Kit	11198
0.25mm ID Rtx®-CLPesticides Kit	11199

504.1 Calibration Mix

1,2-dibromo-3-chloropropane
1,2-dibromoethane
1,2,3-trichloropropane

200µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30239	30239-510	
w/data pack	30239-500	30239-520	30339

5m Phenylmethyl-Deactivated Guard Columns

ID (mm)	cat.#
0.25	10043
0.32	10044
0.53	10045

Universal 'Y' Press-Tight® Connectors

20405, each	20406, 3-pk.

Universal Angled 'Y' Press-Tight® Connectors

20403, each	20404, 3-pk.

Uniliner® Direct Injection Sleeves for HP GCs

4mm ID x 6.3mm OD x 78.5mm Length	
20335, each	20336, 5-pk.

Restek Trademarks: Press-Tight, Rtx, Uniliner.

RESTEK ISO 9001 REGISTERED

RESTEK environmental

Applications note

Extraction of Oil and Grease from Wastewater Using US EPA Method 1664 and Resprep™ Oil and Grease SPE Disks and Flow Filters

Increasing environmental concerns about the effects of released chlorofluorocarbons (CFCs) in the atmosphere, and in an effort to reduce discharges during routine laboratory procedures, the US Environmental Protection Agency (EPA) has mandated the elimination of Freon® (CFC-113) extraction solvent for industrial waste samples. This has recently resulted in the release of an alternative procedure for US EPA Method 413.1—the US EPA Method 1664, Oil and Grease Analysis in Wastewater. The updated method describes an *n*-hexane liquid-liquid extraction and allows use of alternative extraction and concentration techniques such as SPE if the performance specifications of the method are not met.

The *n*-hexane extraction process has several difficulties. As with all liquid-liquid extractions, emulsions are problematic. With industrial discharge samples, this problem may become extreme and result in poor sample partitioning or samples too viscous to process at all. Because *n*-hexane is lighter than water, it makes routine separatory funnel processes difficult to perform. Fortunately, some of these difficulties may be overcome using the SPE procedure.

By decanting the supernatant from the sample first and using an appropriate prefilter, most complex samples can be extracted in less than an hour. The SPE system also can be automated using vacuum manifolds. This allows simultaneous processing of up to six samples, thereby improving sample throughput and lab efficiency.

The following instructions are general guidelines for SPE extraction of oil and grease from wastewater. Sample volume, solvent type, pH adjustment, and conditioning may be changed to adapt to specific methods as needed.

Please note: This method is based on the US EPA Method 1664, but is not intended to replace or substitute the method.

Please refer directly to the published EPA method for additional information for the preparation of samples to be analyzed according to EPA Method 1664.

EPA methods are available from NTIS (National Technical Information Service), U.S. Department of Commerce, Springfield, VA 22161, phone: (703) 487-4650.

List of Materials:

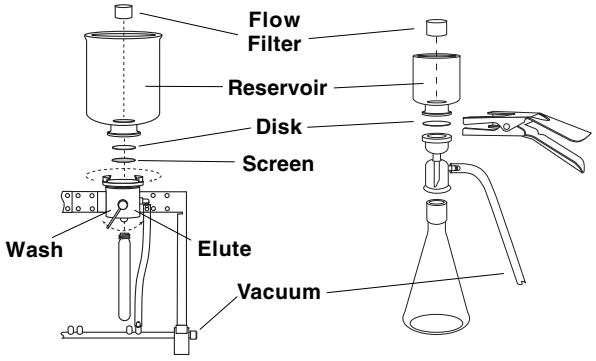
Disk (cat.# 26022); Filter (cat.# 26024); Manifolds (cat.#s 26025, 26026, 26027); Dry Prep (cat.#s 26075, 26076); 25mm Filter (cat.# 26073). If using glass filter beads, see *end note* for procedural modifications.

Sample Preparation:

1. Adjust sample to pH 2.0 with 6N HCl
2. If sample is high in suspended solids, allow solids to settle. When adding the sample, tilt the sample container to allow particulates to settle on one side. This is suggested to aid in the decanting of the liquid portion of the sample.

Extraction Disk Conditioning:

Proper disk conditioning is critical for a successful extraction. Conditioning provides a good interface between the sorbent and the sample matrix. Failure to condition the disk properly may result in erratic and low recoveries.



1. Place disk on manifold, ripple side up. Connect reservoir securely to disk base.
2. Place the Resprep™ Flow Filter in the bottom of the reservoir, flush with the edge of the glass.
3. Wash the disk with 25mL of n-hexane.
   Note: *Always run solvents down the sides of the glassware when washing or eluting. Apply a light vacuum (~5in. Hg) and pull approximately 1mL through the disk. Vent the vacuum and allow the disk to soak for 2 minutes.*
4. Apply vacuum to pull the remaining solvent through the disk. Allow the disk to dry.

environmental

5. Repeat steps 3 and 4 one more time.

6. Add 25mL of methanol to the reservoir. Apply a light vacuum (~5in. Hg) and pull approximately 1mL through the disk. Vent the vacuum and allow the disk to soak for 1 minute.

7. Apply vacuum and pull methanol through the disk until the methanol surface is 1–2mm above the surface of the disk or prefilter.

8. Add 10mL of reagent-grade water to the reservoir. Apply a light vacuum and pull the water through the disk until the surface is covered with about 1–2mm of water.

9. Repeat steps 7 and 8 one more time.
Note: *It is important that the disk is not allowed to dry out before introducing the sample. Drying of the disk at this point could lead to decreased yields.*

Sample Extraction:

1. Pour or decant the sample into the reservoir and apply vacuum. Decant and extract as much liquid as possible before adding sediment to the reservoir. Do not let the disk dry before adding sediment.
Note: *Use an appropriate vacuum to keep the extraction time to more than 10 minutes, as faster extraction times will reduce recoveries.*

2. After sample extraction is complete, remove as much residual water as possible from the disk by applying vacuum to dry the disk for 10 minutes.

Sample Drying:

1. Use acetone (5–6mL) to remove any remaining water from the disk.

2. Leaving an open vacuum, add 5mL acetone around the lower portion of the reservoir and to the top of the disk. Apply maximum vacuum to dry the disk for 5 minutes.
Note: *Do not allow acetone to sit in the reservoir.*

3. If 5mL does not remove all remaining water, try an additional 1mL, but do not exceed a total of 6mL.
Note: *Acetone may reduce yields if used in large quantities or for long durations.*

Sample Elution:

1. Put receiving vessel in place. Elute twice with 25mL of *n*-hexane. Smaller volumes of solvent may be used if the elution techniques have been validated. When adding elution solvent, rinse down the sides, washing the walls of the reservoir in the process.

2. Carefully apply vacuum to pull a few drops of *n*-hexane through the disk, and then release the vacuum. Allow the *n*-hexane to soak the disk for two minutes before applying full vacuum to remove residual *n*-hexane and dry the disk.

3. Repeat the process in steps 1 and 2 with a second aliquot of eluting solvent.

* Optional Step: It may be desirable to further dry and filter the combined hexane eluates before transferring them to a pre–weighed vaporation dish. Pass the hexane through a small or large sodium sulfate-containing Dry Prep tube (cat.# 26075 or 26076), and a 0.45µm PTFE syringe tip filter (cat.# 26073), connected to the outlet of the Dry Prep tube.

4. Transfer eluted hexane sample to the pre-weighed evaporating dish. Rinse collection vessel with two additional 5mL aliquots of n-hexane, add rinse to evaporating dish.

5. Evaporate the *n*-hexane, at a temperature of 30–40°C, from the pre-weighed dish until a constant weight is reached.

End Note: *When using glass filter beads instead of the prefilter, modifications to the procedure are required. Due to the difference in physical properties, it is necessary to reduce all quantities of the solvents used to 10mL instead of 25mL during the disk conditioning and sample elution. In the sample drying process, start with 4–5mL of acetone. If any water remains in the sample, try using up to 6mL.*

Product Listing:

Description	cat. #
Oil & Grease SPE Disk, 47mm (20pk.)	26022
Flow Filter, 47mm (20pk.)	26024
Resprep™ Maxi-Manifold, Complete Station	26026
Resprep™ Maxi-Manifold, 1-Station	26025
Individual Maxi-Manifold Station	26027
Replacement Reservoir, 1L	26028
Dry Prep I Sodium sulfate drying tube - small (50pk.)	26075
Dry Prep II Sodium sulfate drying tube - large (50pk.)	26076
25mm Syringe Tip Filter, PTFE, 0.45µm	26073

US EPA Method 1664 Oil & Grease Reference Mix

	Each	5-pk.	10-pk.
	31457	31457-510	
w/data pack	31457-500	31457-520	31557

RESTEK pharmaceutical

Applications note

HPLC Analysis of Basic Pharmaceutical Compounds on an Ultra Cyano Phase

Pharmaceuticals designed for use as antihypertensive agents are challenging samples for high performance liquid chromatography (HPLC) methods development. The challenge arises when the basic functional group in the sample interacts with a residual silanol group in the column packing, causing peak tailing and difficult quantitation.

When compared to C18 phases, a bonded base-deactivated cyanopropyl phase exhibits the best peak shape for basic pharmaceutical compounds. However, cyano phases on traditional Type A silica can be unstable under certain pH conditions (Scheme 1). In order to combat these problems, we've developed new deactivation and bonding chemistries for the Ultra Cyano HPLC column, which increase phase stability and eliminate potential hydrolysis when incorporated with a highly pure, Type B silica support. The high bonding density (8% carbon) increases the retention/capacity factor for analytes, resulting in better resolution of complex mixtures.

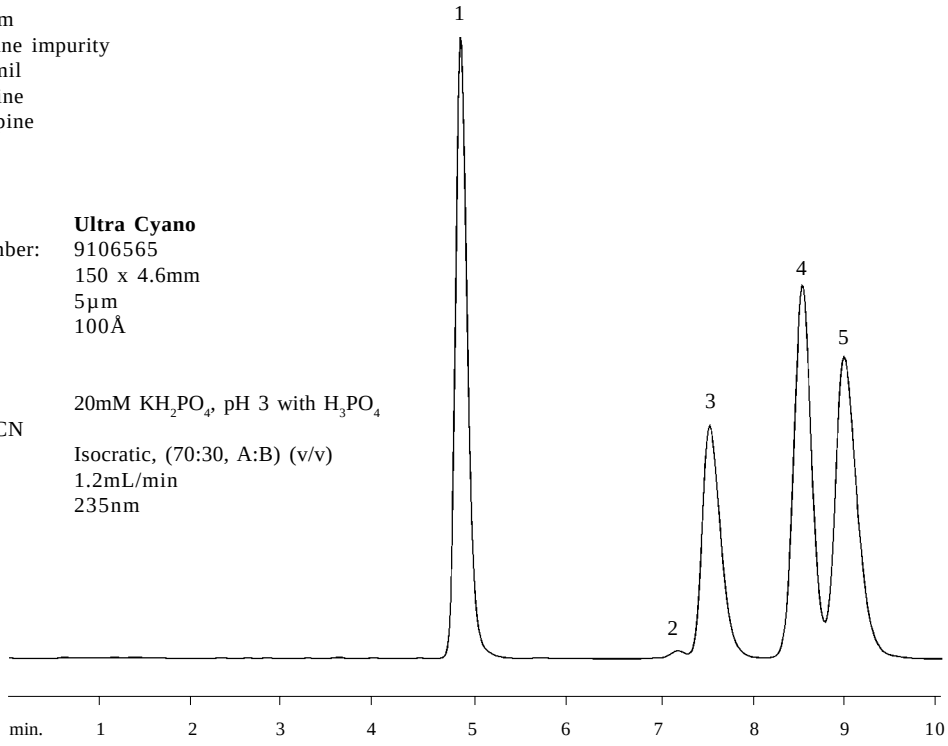
Figure 1 illustrates the separation of calcium channel blockers, a common analysis containing compounds with these basic functional groups, using an Ultra Cyano HPLC column. The 150 x 4.6mm column produces good resolution in less than ten minutes and excellent peak symmetry without tailing. Through changes to the deactivation, bonding chemistry, and bonding density, Ultra Cyano HPLC columns provide a superior chromatographic solution to difficult pharmaceutical analyses.

Figure 1

HPLC Separation of Popular Calcium Channel Blockers Shows Good Peak Shape and Resolution in 10 minutes

Peak List:
1. diltiazem
2. nifedipine impurity
3. verapamil
4. nifedipine
5. nicardipine

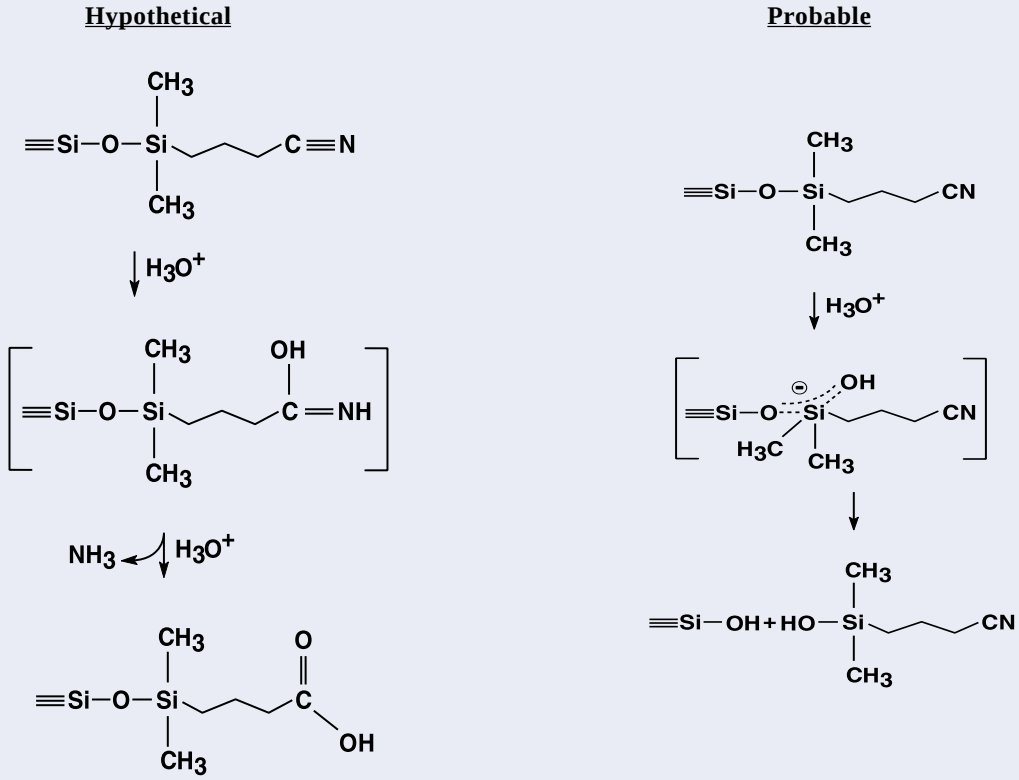
Column:	**Ultra Cyano**
Catalog Number:	9106565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å
Conditions:	
Solvent A:	20mM KH_2PO_4, pH 3 with H_3PO_4
Solvent B:ACN	
Mode:	Isocratic, (70:30, A:B) (v/v)
Flow Rate:	1.2mL/min
Wavelength:	235nm



pharmaceutical

Scheme 1

Hydrolysis of Cyano Bonded HPLC Phase Causes Instability



Ultra Cyano, 5µm Particle size				
Length (mm)	**1.0mm ID cat.#**	**2.1mm ID cat.#**	**3.2mm ID cat.#**	**4.6mm ID cat.#**
30	9106531	9106532	9106533	9106535
50	9106551	9106552	9106553	9106555
100	9106511	9106512	9106513	9106515
150	9106561	9106562	9106563	9106565
200	9106521	9106522	9106523	9106525
250	9106571	9106572	9106573	9106575

Ultra Cyano, 5µm, w/ Trident Guard Inlet Fitting			
Length (mm)	**2.1mm ID cat.#**	**3.2mm ID cat.#**	**4.6mm ID cat.#**
30	9106532-700	9106533-700	9106535-700
50	9106552-700	9106553-700	9106555-700
100	9106512-700	9106513-700	9106515-700
150	9106562-700	9106563-700	9106565-700
200	9106522-700	9106523-700	9106525-700
250	9106572-700	9106573-700	9106575-700

Ultra Cyano Guard Cartridges		
Length (mm)	**cat.#**	**Qty.**
10 x 2.1	910650212	3
10 x 4.0	910650210	3
20 x 4.0	910650220	2

Also available in 3µm particles.

RESTEK

Food & Flavor

Applications note

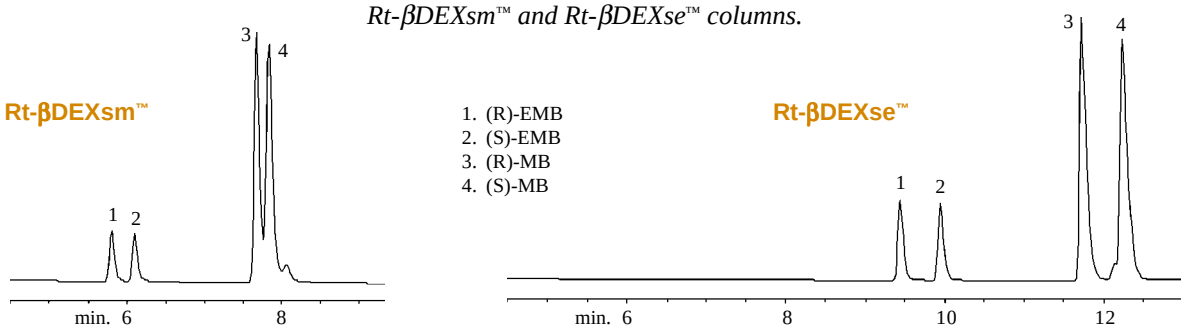
GC Analysis of Chiral Flavor Compounds in Apple Juices Using the Rt-βDEXsm™ and Rt-βDEXse™ Columns

The fruit market has been estimated at over $12 billion per year in the U.S., and the incidence of adulterated/mislabeled juices is at least 10%, costing over $1 billion per year.[1] This small percentage means that sugar, color, organic acids, minerals, and flavors are being sold either partially or wholly in place of the juice.[2] Many natural flavoring materials are much more expensive to produce than the corresponding artificial materials and they command much higher prices.[3]

Many flavor constituents are chiral compounds that usually exist as one predominant enantiomer in nature; whereas those from a synthetic origin often occur as a racemic mixture. Chiral gas chromatography (GC), which determines enantiomer ratio, is a good method to detect synthetic adulterants. Ethyl-2-methylbutyrate and 2-methylbutyrate are important constituents contributing to apple flavor, and both are naturally predominant as the (S) isomer in apple juices.[3] Analysis of apple essence on modified cyclodextrin chiral columns can detect additions of synthetic ethyl-2-methylbutyrate.[4] The Rt-βDEXsm™ column (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin) and the Rt-βDEXse™column (2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin) can separate the enantiomers of 2-methylbutyrate and ethyl-2-methylbutyrate (Figure 1). In addition, the Rt-βDEXsm™ column also can resolve the optical isomers of 2-methylbutyric acid, a chiral acid that is used in significant quantities as a flavor substance.[5] The enantiomers are not well resolved on the Rt-βDEXse™ column (Figure 2). Natural 2-methylbutyric acid has been investigated in many fruits such as apples, apricots, pineapples, and strawberries, and in all cases was found to be present in the almost enantiopure (S) form.[5]

Figure 1

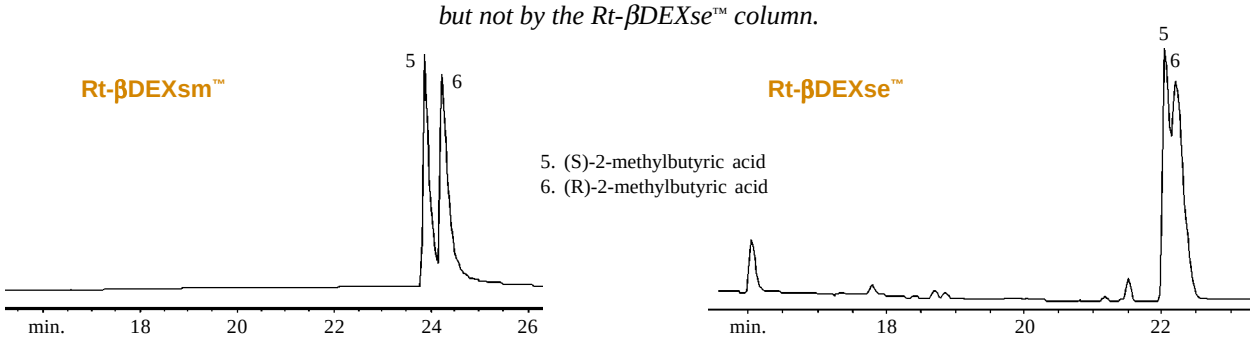
Enantiomeric resolution of racemic ethyl-2-methylbutyrate and 2-methylbutyrate is achieved on Rt-βDEXsm™ and Rt-βDEXse™ columns.



30m, 0.32mm ID, 0.25µm a) Rt-βDEXsm™ and b) Rt-βDEXse™ columns (cat.#'s 13104 and 13106). 10µL direct injection; **Oven temp.:** 40°C (hold 1 min.) to 220°C @ 2°C/min.; **Inj. & det. temp.:** 220°C; **Carrier gas:** hydrogen @ 80cm/sec.

Figure 2

Enantiomeric resolution of racemic 2-methylbutyric acid is provided by the Rt-βDEXsm™ column, but not by the Rt-βDEXse™ column.



30m, 0.32mm ID, 0.25µm a) Rt-βDEXsm™ and b) Rt-βDEXse™ columns (cat.#'s 13104 and 13106). 10µL direct injection; **Oven temp.:** 40°C (hold 1 min.) to 220°C @ 2°C/min.; **Inj. & det. temp.:** 220°C; **Carrier gas:** hydrogen @ 80cm/sec.

Analysis of Apple Juices

Sample Preparation

The flavor compounds were obtained by liquid-liquid extraction. Twenty milliliters of methylene chloride were added to 16–20 oz. of juice in a 500mL separatory funnel and were then shaken. After the organic layer (on the bottom) was removed, the previous step was repeated two more times. The final organic extract was then funneled through a bed of sodium sulfate and concentrated to 4mL in a Kuderna-Danish concentrator with a hot water bath at 70°C.

Analysis

The enantiomers of ethyl-2-methylbutyrate, 2-methylbutyrate, and 2-methyl butyric acid for several brands of apple juices were analyzed on both Rt-βDEXsm™ and Rt-βDEXse™ columns. Ten microliters of sample were introduced via direct injection. A 1.5m guard column was connected to the 4mm open-top Uniliner® sleeve and to the beta-cyclodextrin column to accommodate the large volume injection and to protect the analytical column. Some spectral confirmation was conducted by GC/mass spectrometry (MS).

Results

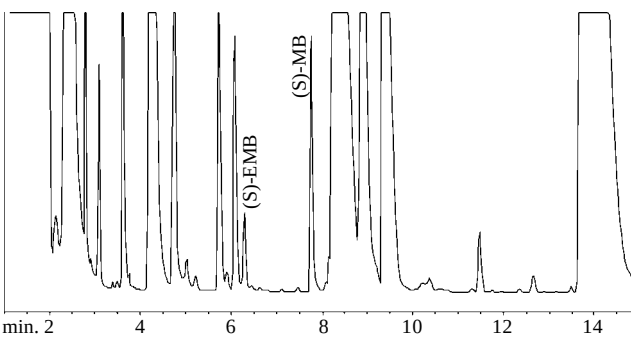
The data revealed that most of the tested samples of apple juice contained almost enantiomerically pure (S)-ethyl-2-methylbutyrate and (S)-2-methylbutyrate, as shown in Figure 3, concluding that there was no flavor adulteration of the juice. Figure 4 shows the same brand spiked with racemic ethyl-2-methylbutyrate and 2-methylbutyrate.

Figure 5B illustrates the example of matrix interference in some juices. The 2-methylbutyrate may at first appear to be racemic, because in this case (R)-2-methylbutyrate coelutes with 3-methylbutyrate on the Rt-βDEXse™ column. In Figure 4, the 3-methylbutyrate is not present in Brand "X" apple juice, but is in Brand "Y".

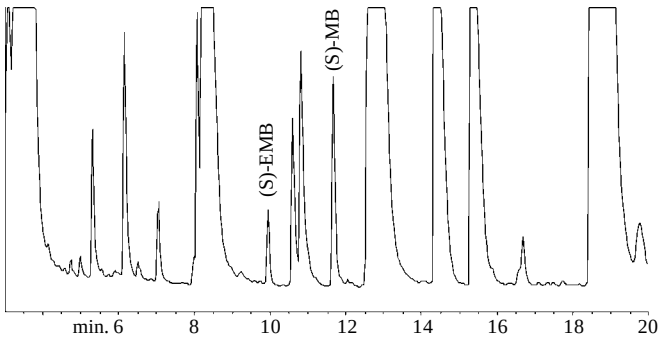
Figure 3

Detection of (S)-ethyl-2-methylbutyrate and (S)-2-methylbutyrate in Brand "X" apple juice, on the Rt-βDEXsm™ and Rt-βDEXse™ columns respectively, indicates enantiomerically pure flavor.

A) Rt-βDEXsm™



B) Rt-βDEXse™

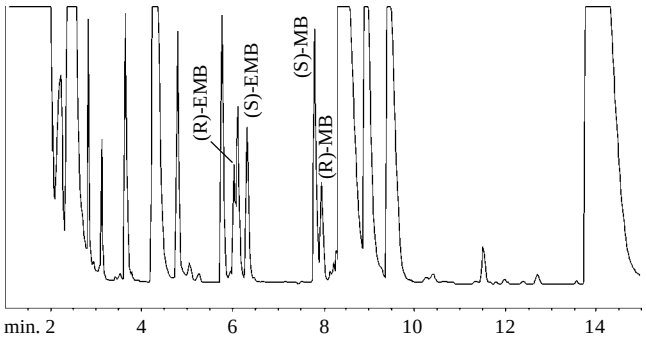


30m, 0.32mm ID, 0.25µm a) Rt-βDEXsm™ and b) Rt-βDEXse™ columns (cat.#'s 13104 and 13106). 10µL direct injection; **Oven temp.:** 40°C (hold 1 min.) to 220°C @ 2°C/min.; **Inj. & det. temp.:** 220°C; **Carrier gas:** hydrogen @ 80cm/sec.

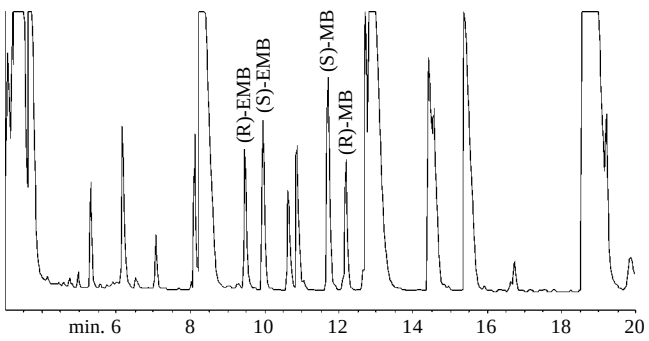
Figure 4

The analysis of Brand "X" apple juice spiked with both racemates of ethyl-2-methylbutyrate and 2-methylbutyrate shows how the presence of racemic mixtures would look.

A) Rt-βDEXsm™



B) Rt-βDEXse™



30m, 0.32mm ID, 0.25µm a) Rt-βDEXsm™ and b) Rt-βDEXse™ columns (cat.#'s 13104 and 13106). 10µL direct injection; **Oven temp.:** 40°C (hold 1 min.) to 220°C @ 2°C/min.; **Inj. & det. temp.:** 220°C; **Carrier gas:** hydrogen @ 80cm/sec.

A second column, the Rt-βDEXsm™ column, confirms the presence of 3-methylbutyrate and not racemic 2-methylbutyrate. Figure 4A shows what the presence of both racemates would look like from Brand "X" apple juice on the Rt-βDEXsm™ column. Figure 5A reveals the enantiomerically pure (S)-2-methylbutyrate on the Rt-βDEXsm™ column. This second column does not have the interference of 3-methylbutyrate and, therefore, is a good confirmational column, especially when identification confirmation by GC/MS is not available.

Inspection of 2-methylbutyric acid in all samples of juice revealed almost complete enantiomeric purity of (S)-2-methylbutyric acid. Figure 6 illustrates 2-methylbutyric acid in Brand "Z" apple juice and in cranberry/apple juice.

The Rt-βDEXsm™ and Rt-βDEXse™ columns reveal the enantiomeric ratio of key chiral flavor components in apple juice, especially in a dual-column system. Inspection of ethyl-2-methylbutyrate, 2-methylbutyrate, and 2-methylbutyric acid do not reveal any flavor adulteration of the five brands of juices analyzed, but the ability to do so has been demonstrated with chiral capillary GC.

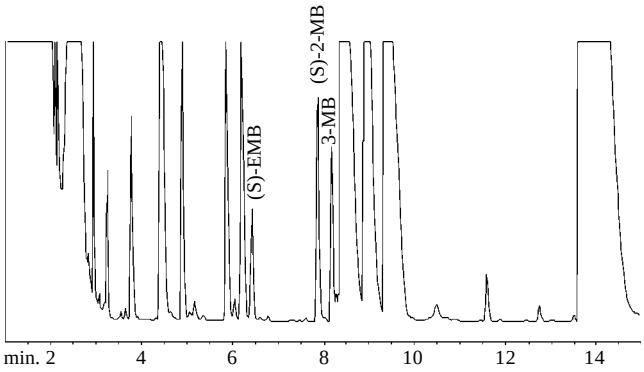
References

1. Nagy, S., and Wade, R., "Methods to Detect Adulteration of Fruit Juice Beverages," *Agroscience,* 1995, p. 213.
2. ibid.
3. Rouseff, R.L., and Leahy, M.M., Eds., "Fruit Flavors, Biogenesis, Characterization, and Authentication," *ACS Publication,* 1995, pp. 70-77.
4. Mosandl, Schmarr, *J Agr. Food Chemistry,* 1991.
5. Werkoff, P., Brennecko, S., Bretscheider, W., Guntert, M., Hopp, R., Surburg, H., "Chirospecific Analysis in Essential Oil, Fragrance, and Flavor Research," *Lebensmittel-Untersüchung und-Forshung,* Springer-Verlag, 1993.

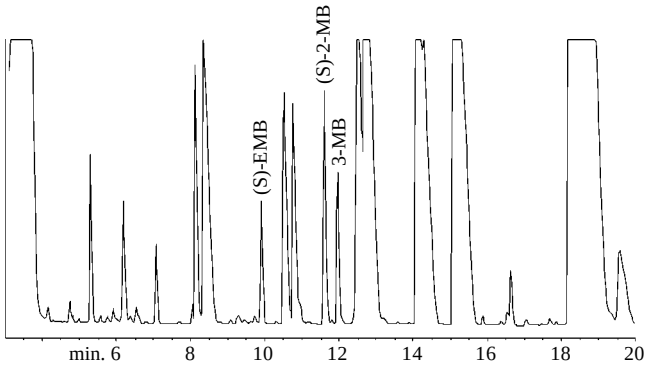
Figure 5

Dual-column analysis confirms the enantiomeric purity of (S)-2-methylbutryate.

A) Rt-βDEXsm™



B) Rt-βDEXse™

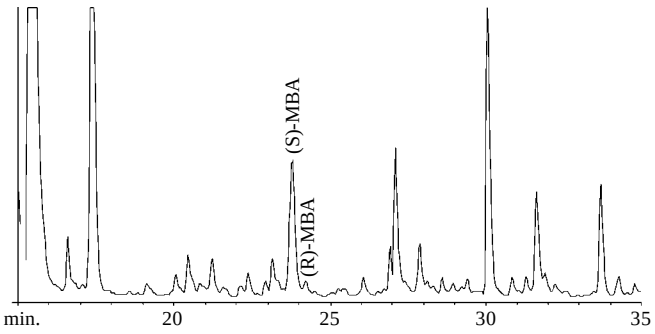


30m, 0.32mm ID, 0.25µm a) Rt-βDEXsm™ and b) Rt-βDEXse™ columns (cat.#'s 13104 and 13106). 10µL direct injection;
Oven temp.: 40°C (hold 1 min.) to 220°C @ 2°C/min.; **Inj. & det. temp.:** 220°C; **Carrier gas:** hydrogen @ 80cm/sec.

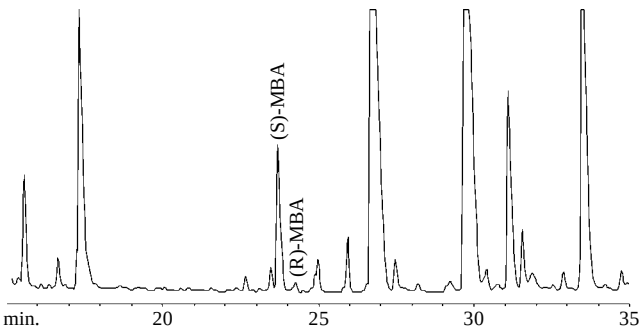
Figure 6

The Rt-βDEXsm™ column confirms enantiomeric purity of (S)-2-methylbutyric acid in apple juice and cranberry/apple juice.

A) Brand "Z" Apple Juice



B) Cranberry/Apple Juice



30m, 0.32mm ID, 0.25µm Rt-βDEXsm™ column (cat.# 13104). 10µL direct injection;
Oven temp.: 40°C (hold 1 min.) to 220°C @ 2°C/min.; **Inj. & det. temp.:** 220°C; **Carrier gas:** hydrogen @ 80cm/sec.

Product Listing

Rt-βDEXsm™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13105
0.32mm	0.25	40 to 230°C	13104

Rt-βDEXse™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13107
0.32mm	0.25	40 to 230°C	13106

Restek offers a wide range of cyclodextrin columns for the analysis of many chiral compounds.

Rt-βDEXsa™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13109
0.32mm	0.25	40 to 230°C	13108

Rt-βDEXsp™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13111
0.32mm	0.25	40 to 230°C	13110

Rt-βDEXm™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13100
0.32mm	0.25	40 to 230°C	13101

Rt-βDEXcst™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13103
0.32mm	0.25	40 to 230°C	13102

Rt-γDEXsa™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13113
0.32mm	0.25	40 to 230°C	13112

For more information on chiral analysis, visit our web site at **www.restekcorp.com** to view *A Guide to the Analysis of Chiral Compounds by GC.*

To Optimize Chiral Separations Use:

1) Faster linear velocities (80cm/sec.) with hydrogen carrier gas.
2) Slower temperature ramp rates (1–2°C/min.).
3) Appropriate minimum operating temperature (40 or 60°C).
4) On-column concentrations of 50ng or less.

Direct Injection Sleeves for HP/Finnigan GCs

Description	Each	5-pk.
4mm ID Uniliner®	20335	20336
4mm ID Cyclo-Uniliner®	20337	20338
4mm ID Open-top Uniliner® w/wool	20843	20844

RESTEK environmental

Applications note

GC Analysis of US EPA Method 8081A Chlorinated Pesticides Using Rtx®-CLPesticides & Rtx®-CLPesticides2 Columns

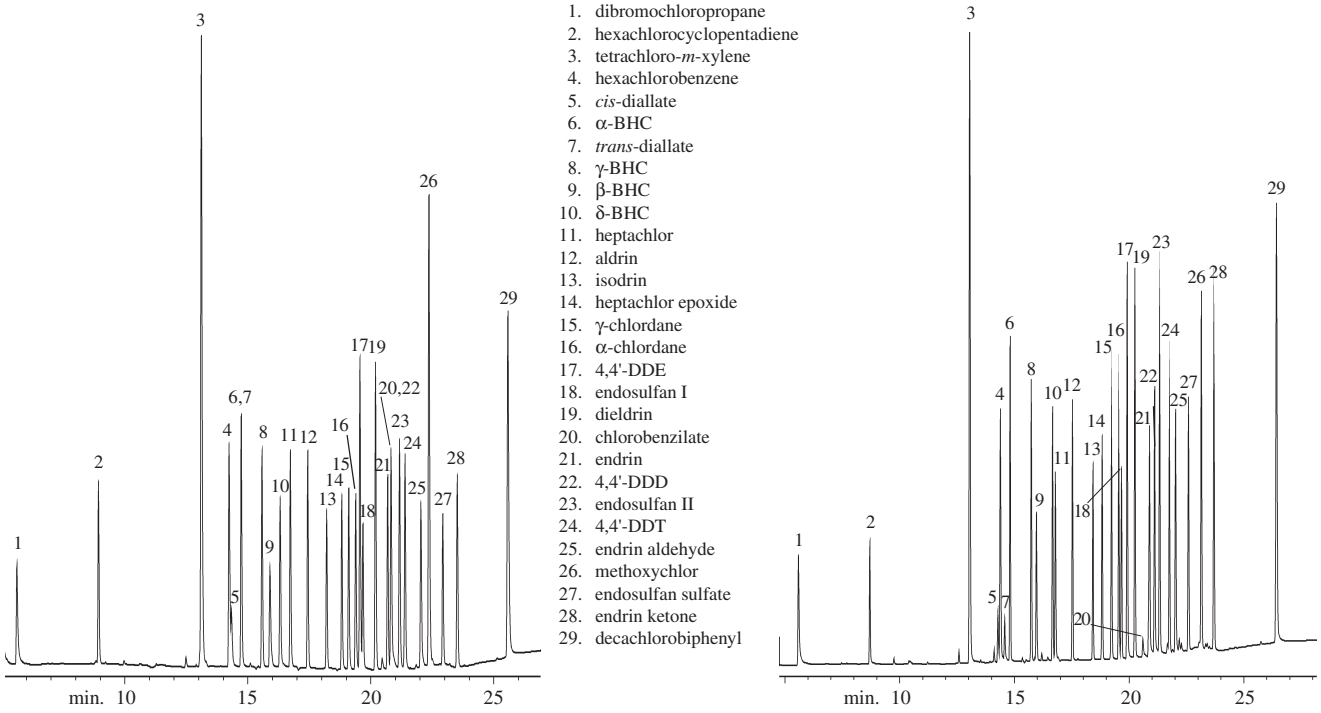
In the environmental industry, the chlorinated pesticide analytical methods often are the most challenging to perform. Analysts struggle with linearity, breakdown, and lengthy calibrations; as well as column bleed, column reactivity, and poor separation. Restek has addressed many of these issues with the development of the Rtx®-CLPesticides and the Rtx®-CLPesticides2 capillary columns. These columns were designed specifically for the separation of chlorinated pesticides, to be used in parallel for simultaneous quantitation and confirmation by gas chromatography/electron capture detection (GC/ECD). We have reported the performance and separation for these columns in several articles, but many laboratories are now dealing with the latest version of the chlorinated pesticides method—SW-846, 8081A. This method adds several new target analytes to the 20 common single-component pesticides contained in earlier versions.

The Rtx®-CLPesticides and Rtx®-CLPesticides2 achieve baseline separation of the 20 chlorinated pesticides listed in US Environmental Protection Agency (EPA) Method 8081 (Figure 1). They also have a high maximum operating temperature, excellent inertness, low bleed, and operate under the same flow and temperature conditions. This facilitates installing them as a column pair into a single injection port, thereby minimizing maintenance concerns with the injection ports.

Because the Rtx®-CLPesticides and Rtx®-CLPesticides2 columns were designed with selectivity for neutral, halogenated compounds, they are easily adapted for the analysis of the extended list of pesticides in Method 8081A. Figure 1 also shows the separation of the nine additional single-component compounds listed in Method 8081A. Using a guard column and splitting the

Figure 1

Excellent Separation of US EPA Method 8081A Chlorinated Pesticides Using Restek's Rtx®-CLPesticides and Rtx®-CLPesticides2 Columns.



30m, 0.32mm ID, 0.50µm Rtx®-CLPesticides and 30m, 0.32mm ID, 0.25µm Rtx®-CLPesticides2 columns (cat.#'s 11139 & 11324).
On-column concentration: 16-160pg; **Oven temp.:** 80°C (hold 1 min) to 300°C @ 10°C/min (hold 15 min);
Inj. port: Direct, Uniliner® sleeve (cat.# 20335); **Detector:** ECD, 300°C with Anode Purge;
Dead time: 1.9 min; **Head pressure:** 8.7psi (constant); **Flow rate:** 1.3mL/min @ 120°C, helium.

environmental

flow into the two columns with a glass 'Y' Press-Tight® connector, these chromatograms were acquired simultaneously.

The combination of the Rtx®-CLPesticides and Rtx®-CLPesticides2 columns provides unsurpassed performance for the analysis of chlorinated pesticides. They can be baked-out at the end of each analysis to remove high-boiling contaminants, without degrading the stationary phase. They do not have the problems associated with cyanopropyl phases such as on-column methoxychlor and DDT breakdown, and low maximum temperature. The Rtx®-CLPesticides and Rtx®-CLPesticides2 columns are a good choice for improving resolution and capacity for the analysis of dirty extracts, and for increasing throughput for chlorinated pesticide samples.

Product Listing

Rtx®-CLPesticides Columns

ID	df (µm)	Stable to	10m	20m
0.18mm	0.14	340°C	42101	42102
ID	**df (µm)**	**Stable to**	**15m**	**30m**
0.25mm	0.25	340°C	11120	11123
0.32mm	0.50	340°C	11136	11139
0.53mm	0.50	340°C	11137	11140

Rtx®-CLPesticides2 Columns

ID	df (µm)	Stable to	10m	20m
0.18mm	0.14	340°C	42301	42302
ID	**df (µm)**	**Stable to**	**15m**	**30m**
0.25mm	0.20	340°C	11320	11323
0.32mm	0.25	340°C	11321	11324
0.53mm	0.42	340°C	11337	11340

Rtx®-CLPesticides Column Kits

These kits include both a CLPesticides and CLPesticides2 column, a Universal Angled 'Y' Press-Tight® Connector, and a 5m guard column. (Note: Columns are not preconnected in these kits.)

Description	cat.#
0.53mm ID Rtx®-CLPesticides Kit	11197
0.32mm ID Rtx®-CLPesticides Kit	11198
0.25mm ID Rtx®-CLPesticides Kit	11199

5m Phenylmethyl-Deactivated Guard Columns

ID (mm)	cat.#
0.25	10043
0.32	10044
0.53	10045

Universal 'Y' Press-Tight® Connectors

Each	3-pk.
20405	20406

Universal Angled 'Y' Press-Tight® Connectors

Each	3-pk.
20403	20404

Organochlorine Pesticide Mix AB #2

aldrin	8µg/mL	dieldrin	16µg/mL
α-BHC	8	endosulfan I	8
β-BHC	8	endosulfan II	16
δ-BHC	8	endosulfan sulfate	16
γ-BHC (lindane)	8	endrin	16
α-chlordane	8	endrin aldehyde	16
γ-chlordane	8	endrin ketone	16
4,4'-DDD	16	heptachlor	8
4,4'-DDE	16	heptachlor epoxide (B)	8
4,4'-DDT	16	methoxychlor	80

In hexane/toluene (1:1), 1mL/ampul.

	each	5-pack	10-pack
	32292	32292-510	
w/data pack	32292-500	32292-520	32392

For more information on Chlorinated Pesticide Analysis, Request Lit. cat.# 59892.

Restek Trademarks: Rtx, Uniliner.

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clinical/forensic

Applicationsnote

GC Analysis of Commonly Abused Inhalants in Blood Using Rtx®-BAC1 and Rtx®-BAC2 Columns

Inhalant abuse is the intentional concentration and inhalation of volatile compounds found in commercial products. In recent years, inhalant abuse has become the method of choice for first-time drug users. In 1993, the average age for first-time inhalant abusers was 10.8 years, whereas the average age for first-time abusers of other drug substances was 12.5 years. In fact, almost 20% of eighth grade students have abused inhalants. Chronic inhalant abuse can lead to respiratory, cardiovascular, liver, and kidney disease. Acute respiratory and cardiovascular responses to inhalant abuse also can produce inhalant-induced sudden death syndrome.[1]

Inhalant abuse can be detected during screening of whole blood, serum or urine samples using headspace gas chromatography (GC) combined with flame ionization detection (FID). For this application, a GC equipped with an automated headspace sampler that simultaneously introduces a sample into two analytical columns was used. A dual-column configuration provides screening and confirmational data from the same injection. We used the Rtx®-BAC1 (30m, 0.53mm ID, 3.00µm df) and the Rtx®-BAC2 (30m, 0.53mm ID, 2.00µm df) columns—typically used in combination as a screening and confirmational column set for blood alcohol analysis. A useful extension of blood alcohol analysis using this column set is the detection of other volatile organic compounds (VOCs), such as those found in inhalants.

Optimal performance of these columns during headspace analysis depends on proper GC/headspace system set-up. Band broadening can occur if there is excess dead volume in the sample flow path prior to the sample reaching the head of the column. Low-volume inlet sleeves or injection port interfaces significantly reduce the amount of excess volume at the exit end of the transfer line and will help to maintain narrow symmetrical peak shapes. Higher carrier gas flow rates through the transfer line also are important in maintaining good peak shape. Our experiments showed that carrier gas flow rates between 15 and 25mL/minute were the most efficient for transferring the sample from the headspace sampler to the head of the column in a tight sample bandwidth.

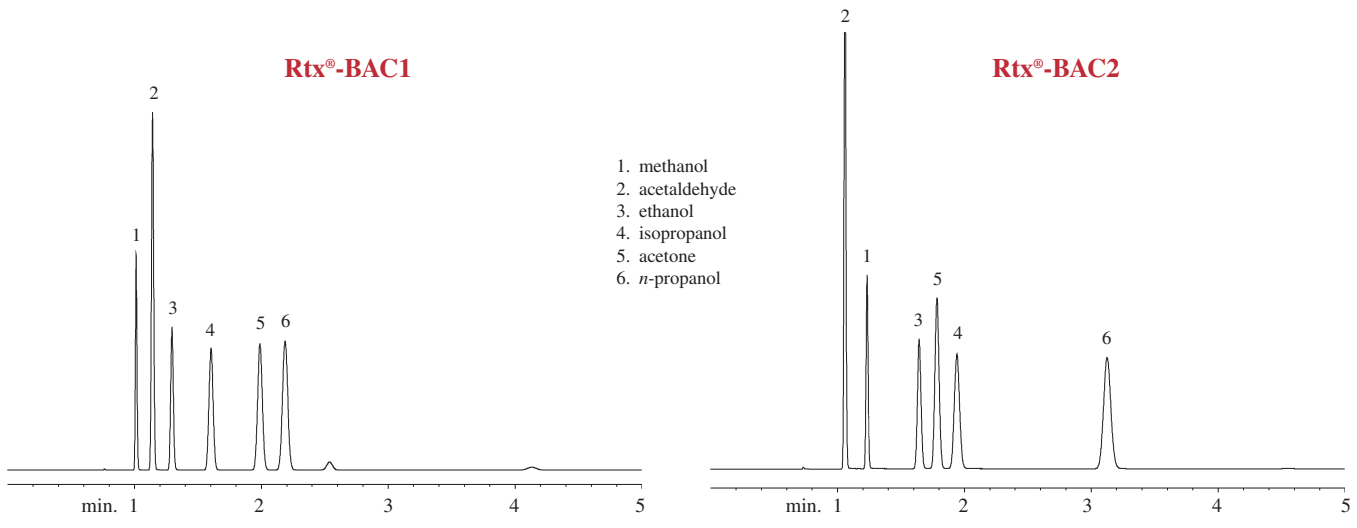
The following classes of commonly abused inhalant compounds were analyzed to determine retention times for each compound.

Blood Alcohol Analysis

Although ethanol is not commonly abused as an inhalant, it is the primary volatile substance detected in screening for volatile organic compounds as a result of alcoholic beverage ingestion. Other compounds monitored during blood alcohol analysis include low molecular weight alcohols and their metabolites (Figure 1).

Figure 1

Baseline resolution on the Rtx®-BAC1 and Rtx®-BAC2 columns for all compounds tested for during blood alcohol screening.



30m, 0.53mm ID, 3.0µm Rtx®-BAC1 (cat.# 18001) and 30m, 0.53mm ID, 2.0µm Rtx®-BAC2 (cat.# 18000). 1.0mL headspace sample; **Oven temp.:** 40°C (hold 5 min.) to 240°C @ 5°C/min.; **Inj. & det. temp.:** 240°C; **Carrier gas:** He; **Linear velocity:** 65cm/sec.

clinical/forensic

Anesthetics

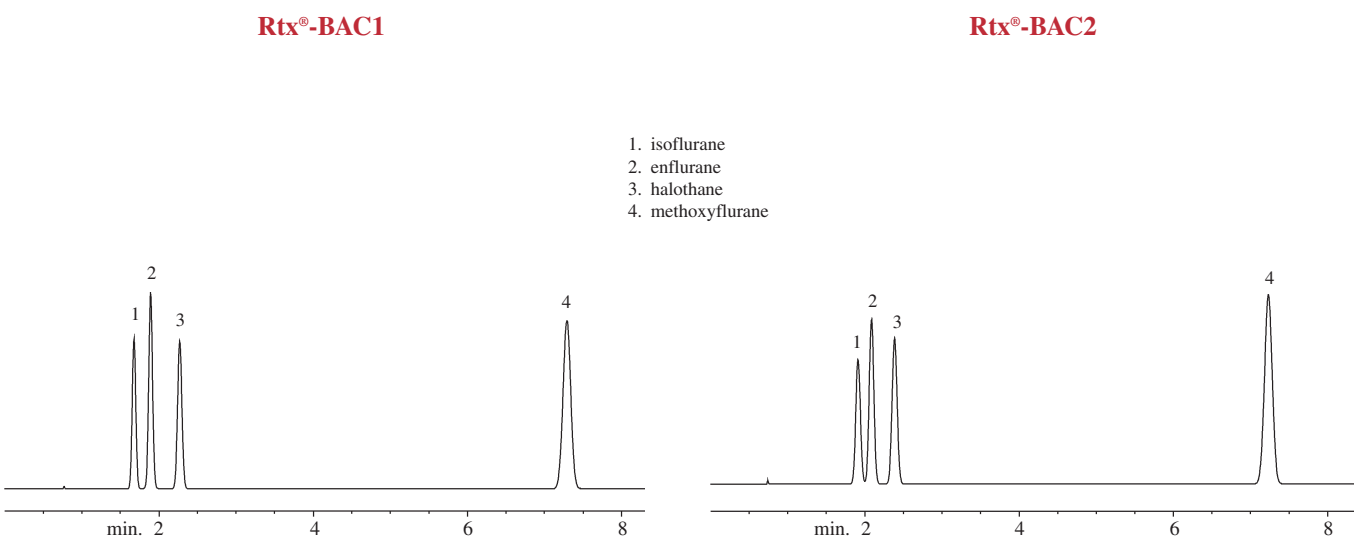
Volatile anesthetics belong to a group of low molecular weight halogenated compounds. The abuse of volatile anesthetics is uncommon but has been reported among hospital personnel and others with access to anesthetic agents (Figure 2).

Alkyl Nitrites

Alkyl nitrites are abused for their vasodilation properties. Abuse has centered on the ability of alkyl nitrites to produce short-lived highs and possible aphrodisiac sensations. Analysis of alkyl nitrites in biological samples is complicated by the fact that alkyl nitrites are rapidly hydrolyzed to their corresponding alcohol. Analytical methods should take this into account by monitoring for both the parent compound and the corresponding alcohol (Figure 3).

Figure 2

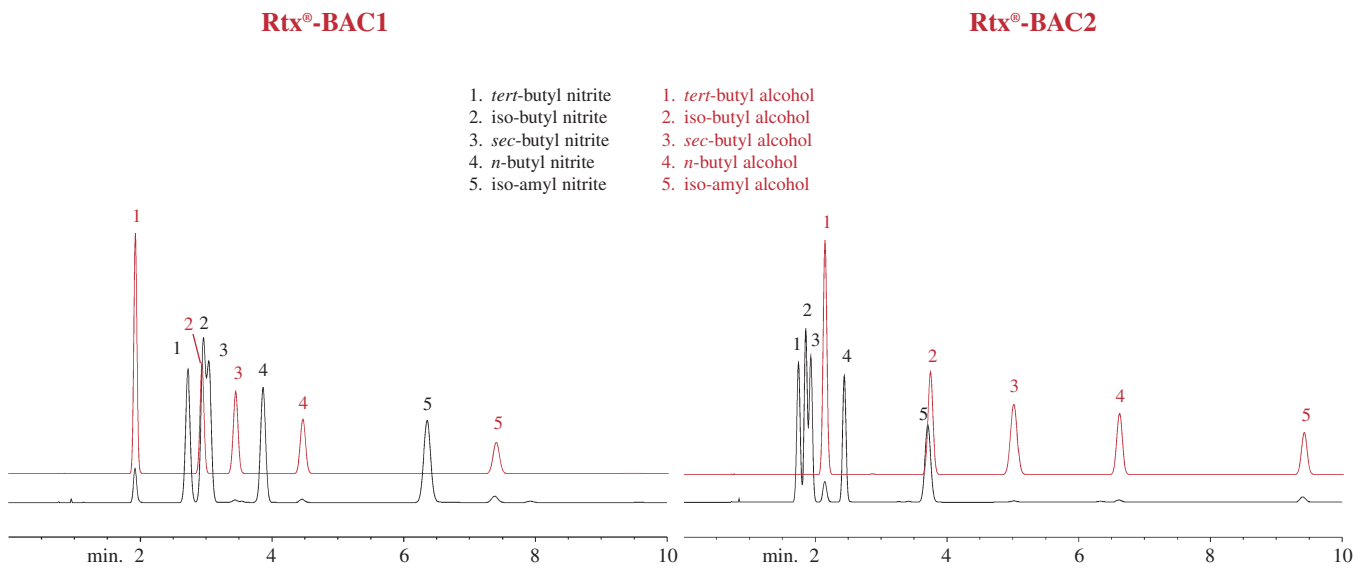
Fast analysis times for volatile anesthetics on the Rtx®-BAC1 and Rtx®-BAC2 columns.



30m, 0.53mm ID, 3.0µm Rtx®-BAC1 (cat.# 18001) and 30m, 0.53mm ID, 2.0µm Rtx®-BAC2 (cat.# 18000). 1.0mL headspace sample; **Oven temp.:** 40°C (hold 5 min.) to 240°C @ 5°C/min.; **Inj. & det. temp.:** 240°C; **Carrier gas:** He; **Linear velocity:** 65cm/sec.

Figure 3

Complete screening and confirmation for alkyl nitrites and metabolites on the Rtx®-BAC1 and Rtx®-BAC2 columns.



30m, 0.53mm ID, 3.0µm Rtx®-BAC1 (cat.# 18001) and 30m, 0.53mm ID, 2.0µm Rtx®-BAC2 (cat.# 18000). 1.0mL headspace sample; **Oven temp.:** 40°C (hold 5 min.) to 240°C @ 5°C/min.; **Inj. & det. temp.:** 240°C; **Carrier gas:** He; **Linear velocity:** 65cm/sec.

Industrial Solvents

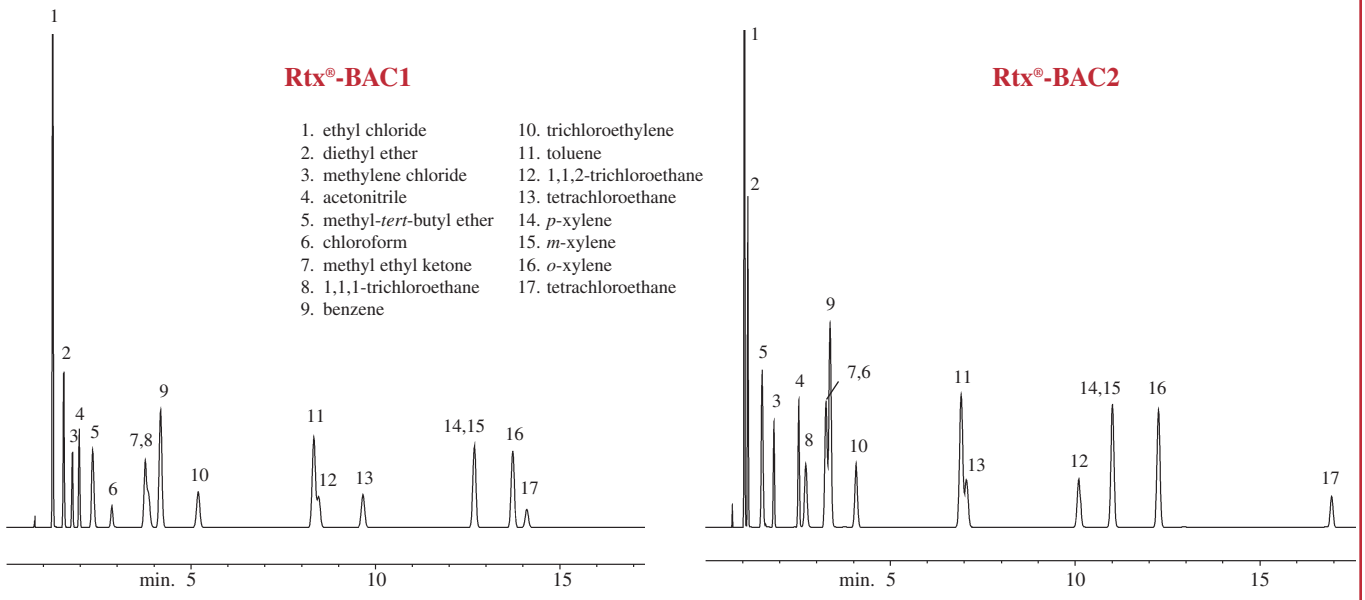
Industrial solvents are the most likely VOCs to be abused. Industrial solvents are available in purified form or as solvents in many household products. Toluene is the most commonly abused industrial solvent and is used in many products such as paints or glues (Figure 4).

Petroleum Hydrocarbons

Distilled petroleum fractions are used as carrier solvents for many commercial products. Distilled fractions, like mineral spirits, are used in products like pesticides, herbicides, paints, lacquers, thinners, and household cleaning products. Inhalation, ingestion or adsorption through the skin can produce detectable concentrations of a variety of individual hydrocarbons (Figure 5).

Figure 4

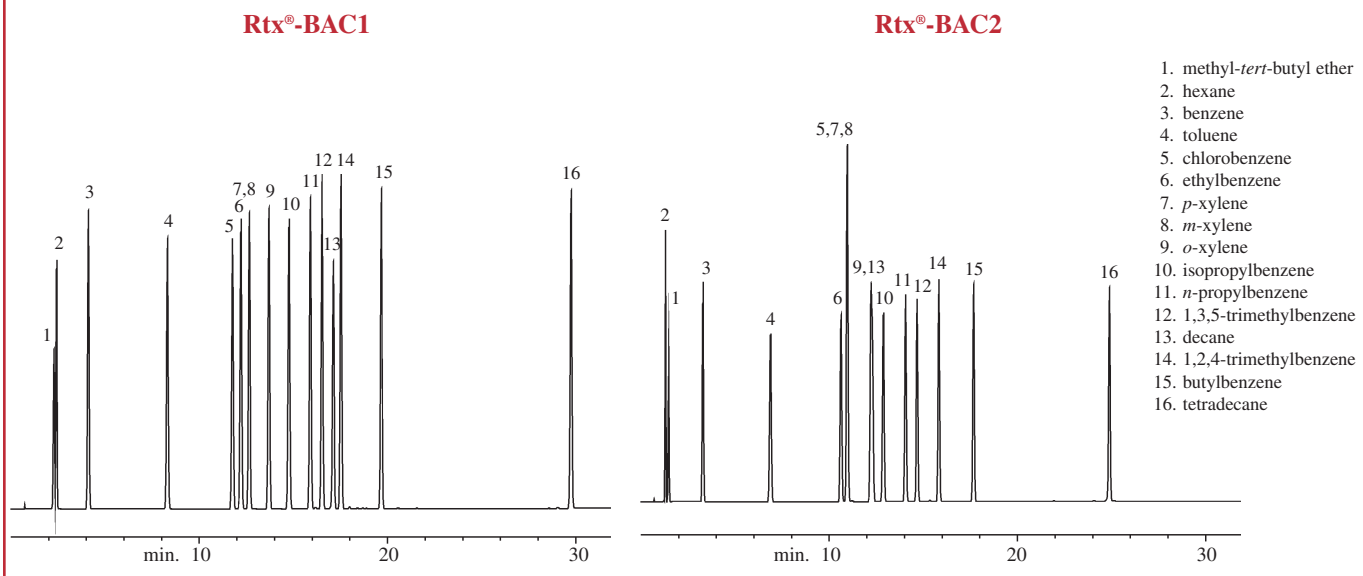
Elution order changes for common industrial solvents on the Rtx®-BAC1 and Rtx®-BAC2 columns.



30m, 0.53mm ID, 3.0µm Rtx®-BAC1 (cat.# 18001) and 30m, 0.53mm ID, 2.0µm Rtx®-BAC2 (cat.# 18000). 1.0mL headspace sample; **Oven temp.:** 40°C (hold 5 min.) to 240°C @ 5°C/min.; **Inj. & det. temp.:** 240°C; **Carrier gas:** He; **Linear velocity:** 65cm/sec.

Figure 5

The Rtx®-BAC1 and Rtx®-BAC2 columns have good thermal stability for analyzing higher boiling hydrocarbons.



30m, 0.53mm ID, 3.0µm Rtx®-BAC1 (cat.# 18001) and 30m, 0.53mm ID, 2.0µm Rtx®-BAC2 (cat.# 18000). 1.0mL headspace sample; **Oven temp.:** 40°C (hold 5 min.) to 240°C @ 5°C/min.; **Inj. & det. temp.:** 240°C; **Carrier gas:** He; **Linear velocity:** 65cm/sec.

Results

All of the compounds involved in the study exhibited good peak shape and response when analyzed by GC on either stationary phase. Both columns were programmed to 240°C without an appreciable increase in stationary phase bleed, and they analyzed some of the higher boiling point compounds found in the heavier petroleum distillates. Each stationary phase exhibited a unique selectivity for individual compounds. Coeluting compounds on one stationary phase were resolved on the complimentary stationary phase.

Conclusions

Inhalant abuse can be detected accurately by analyzing biological samples using headspace sampling combined with gas chromatography. When used in a dual-column setup, the Rtx®-BAC1 and Rtx®-BAC2 columns are very effective for identifying and confirming the presence of VOCs for detection of inhalant abuse.

References

1. National Institute on Drug Abuse, Research Report Series, Inhalant Abuse, June 6, 1996.

Table I: Inhalant Retention Times (Columns and Conditions Listed in Figure 1)

Compound	Rtx®-BAC1 Elution Order	Rtx®-BAC1 Ret. Time (min.)	Rtx®-BAC2 Elution Order	Rtx®-BAC2 Ret. Time (min.)
methanol	1	1.017	5	1.237
acetaldehyde	2	1.146	1	1.063
ethyl chloride	3	1.275	2	1.071
ethanol	4	1.299	8	1.648
diethyl ether	5	1.574	4	1.167
isopropanol	6	1.607	15	1.945
isoflurane	7	1.661	13	1.922
methylene chloride	8	1.805	11	1.849
Freon® 113	9	1.864	3	1.145
enflurane	10	1.891	16	2.081
tert-butyl alcohol	11	1.926	17	2.154
acetone	12	1.992	10	1.787
acetonitrile	13	1.997	20	2.553
n-propanol	14	2.191	25	3.130
halothane	15	2.267	18	2.383
methyl *tert*-butyl ether	16	2.366	7	1.554
hexane	17	2.495	6	1.386
tert-butyl nitrite	18	2.736	9	1.750
chloroform	19	2.870	27	3.290
sec-butyl alcohol	20	2.962	30	3.793
isobutyl nitrite	21	2.973	12	1.853
sec-butyl nitrite	22	3.059	14	1.939
isobutyl alcohol	23	3.460	32	5.100
tetrahydrofuran	24	3.736	24	2.845
methyl ethyl ketone	25	3.768	26	3.271
ethyl acetate	26	3.800	23	2.785
carbon tetrachloride	27	3.842	21	2.565
1,1,1-trichloroethane	28	3.869	22	2.729
n-butyl nitrite	29	3.879	19	2.469
benzene	30	4.186	28	3.392
n-butyl alcohol	31	4.565	33	6.747
trichloroethylene	32	5.205	31	4.084
isoamyl nitrite	33	6.377	29	3.728
methoxyflurane	34	7.279	36	7.219
isoamyl alcohol	35	7.428	38	9.447
toluene	36	8.358	34	6.944
1,1,2-trichloroethane	37	8.498	39	10.138
methyl isobutyl ketone	38	9.510	37	7.964
tetrachloroethylene	39	9.681	35	7.081
chlorobenzene	40	11.810	41	11.012
ethylbenzene	41	12.279	40	10.704
p-xylene	42	12.726	42	11.038
m-xylene	43	12.727	43	11.046
o-xylene	44	13.733	44	12.280
tetrachloroethane	45	14.106	50	16.968
isopropylbenzene	46	14.845	46	12.962
n-propylbenzene	47	15.966	47	14.124
1,3,5-trimethylbenzene	48	16.565	48	14.711
decane	49	17.166	45	12.369
1,2,4-trimethylbenzene	50	17.586	49	15.904
butylbenzene	51	19.739	51	17.732
tetradecane	52	29.806	52	24.950

Product Listing

Rtx®-BAC1 GC Columns

length	ID	df (µm)	cat.#
30m	0.53mm	3.0	18001
30m	0.32mm	1.8	18003

Rtx®-BAC2 GC Columns

length	ID	df (µm)	cat.#
30m	0.53mm	2.0	18000
30m	0.32mm	1.2	18002

Environmental

Applications note

Lit. cat.# 59550

Sample Preparation and Analysis of Organo Tin Compounds

Organo tin compounds recently have become of environmental interest because of their addition to several lists of endocrine disrupters,[1] and the attribution of shellfish growth abnormalities to the use of tributyl tin as a marine antifouling agent. Although this interest primarily has focused on the butyl tin series to date, concern has grown to include other organo tin compounds such as triphenyl tin, a crop pesticide. However, the US Environmental Protection Agency (EPA) has not adopted methods for the preparation and analysis of tributyl tin and its breakdown products of dibutyl tin and monobutyl tin. This presents the laboratory with several challenges. Difficulties arise in the preparation of sample matrices, ranging from water and seawater to soil and biota. The tin compounds also are known to exhibit ligand exchange. When the compounds are mixed in solution, the exchange will cause instability, which ultimately accounts for the short shelf-life of multi-component standards. Finally, these compounds are applied and found as chlorides. This leads to a wide range in polarities and creates difficulty in gas chromatography (GC) separation—the most common technique used in the environmental testing industry.

Because an "EPA accepted" method does not exist for this analysis, most laboratories have decided to not accept requests or to subcontract the analysis to one of the few laboratories that do perform this test. Laboratories often are under the impression that the analysis will require complex techniques, resulting in a considerable capital expenditure. However, this does not have to be the case. In fact, most laboratories could perform this analysis using the equipment they already have.

Generally, laboratories must achieve a detection limit of 50pg/L in water. The addition of organo tin compounds to the list of endocrine disrupters may lower that limit to 1 or 2pg/L. This detection requirement entails either a large-volume injection, additional concentration of the extract, or the use of a more sensitive detector. The steps presented in this application take all of these factors into account, while allowing adaptation for a commercial laboratory.

This method is the result of a combination of methods previously reported.[2,3,4] It was designed for use with equipment that exists in most commercial environmental laboratories. The method is applicable to water, soil, and biota samples, and is compatible with the monoalkyl through tetraalkyl tin compounds. The following is a step-by-step extraction procedure for the various matrices.

Sample Extraction Methods:

Water Samples:

1. Measure approx. 1000mL of sample into 2L-separatory funnel, record volume.
2. Adjust sample to less than pH 2 using HCl.
3. Add surrogate and/or matrix spike compounds.
4. Extract using 0.1% tropolone in hexane extraction solvent; use three serial additions of solvent, 60mL each.
   a. Extraction solvent is made by dissolving 4g tropolone into 4L of hexane.
   b. Tributyl (trialkyl) tin can be extracted with methylene chloride, but di and mono butyl tin require the addition of a chelating agent.
5. Collect extract into Kuderna-Danish (KD) concentrator, passing through granular sodium sulfate, 10-60 mesh.
6. Rinse with extraction solvent.
7. Concentrate extract to approximately 5mL and transfer to 20mL vial.

Soil Samples:

1. Measure approximately 30g of sample into a 400mL beaker. Record weight.
2. Add 10mL of 1:1 HCl:DI water.
3. Add surrogate and/or matrix spike compounds.
4. Extract using 0.1% tropolone in hexane extraction solvent using sonication for 2 minutes; use three serial additions of extraction solvent, 60mL each.
   a. Extraction solvent is made by dissolving 4g tropolone into 4L bottle of hexane.
   b. Tributyl (trialkyl) tin can be extracted with methylene chloride, but di and mono butyl tin require the addition of a chelating agent.
5. Collect extract into Kuderna-Danish (KD) concentrator, passing through granular sodium sulfate drying funnel.
6. Rinse funnel with extraction solvent.
7. Concentrate extract to approximately 5mL and transfer to 20mL vial.

Biota Samples

Follow procedure for soils, except use a homogenizer (tissuemizer) for the extraction and, if necessary, reserve a sample for percent lipids determination.

Deactivation Procedure

Prior to analysis by GC, it is critical to both derivatize the organo tin chlorides and process the sample extracts using some type of sorbent cleanup. Although several techniques are available, the following procedure works well for all compounds in any matrix. (Note: This method uses a commercially available Grignard reagent. Because this reagent is moisture-sensitive, keep exposure to the atmosphere to a minimum by handling the solution in a syringe.) The goal of this reaction is to displace all chlorides on the tin and replace them with an n-hexyl group. For this method, it is very important to remove as much of the potential contaminants as possible. If these compounds are not extracted, they will cause considerable interference, especially in soil or biota matrices. The primary interference is caused by sulfur-containing compounds. These occur in a relatively high concentration in comparison with the organo tin compounds. The large-capacity cleanup described below will remove these non-target compounds. For your convenience, SPE tubes designed for this sample extract cleanup are available from Restek (cat.# 24049).

Sample Extract Derivatization and Cleanup:

1. Add 0.8mL of 2M n-hexyl magnesium bromide.
2. Place vial on wrist shaker for 30 min., or agitate by hand.
3. Place vial in ice bath, and add HCl to dissolve the precipitate.
4. Pipet off the hexane layer and transfer to KD concentrator.
5. Concentrate extract to approximately 1mL.
6. Add extract to 16gm/5gm Florisil®/silica-gel SPE tube. (Restek cat.# 24049). May use prep-scale chromatography column.
7. Elute with 100mL hexane, collecting eluent in KD concentrator.
8. Spike internal standard as appropriate.
9. Concentrate extract to 1.0mL.

Prior to final volume adjustment, internal standards can be added. Tetra-n-propyl tin is the recommended internal standard. All standards used in this method are solutions of organo tin chlorides. Calibration standards must be either derivatized by the same procedure, or they can be purchased as neat-derivatized material from Restek. (Contact our analytical reference materials group at 814-353-1300 or 800-356-1688.) Tropolone and Grignard reagents are available from Aldrich. Since alkyl tin compounds are known to exhibit ligand exchange, the samples and standards should be kept in a cool (4°C), dark place until used. If properly stored, the chemical standards should be stable for six months; but if left at room temperature, peaks from additional organo tin compounds may be observed in the chromatogram. These peaks are caused by compounds exchanging alkyl groups. If this is observed, replace the standards. The use of at least one surrogate compound, such as tripentyl tin chloride, is suggested to allow for extraction recovery calculations.

Sample Analysis

Once the organo tin chlorides have been extracted and derivatized to tetraalkyl tin compounds and the extracts are cleaned using the listed procedure, the GC analysis is relatively simple. The flame photometric detector (FPD) must be operated under fuel-rich conditions for efficient conversion of the alkyl tin compounds to tin hydrides. The only modification needed is the installation of a 610nm wavelength filter in the FPD to collect the molecular emission for tin hydride. This filter can be obtained from Oriel Corporation, Stratford, CT, (203) 377-8282 (cat.# 53925) or other optics supply companies.

Figures 1 and 2 show a simultaneous analysis using two capillary columns of differing selectivity. This provides both a primary and a confirmation analysis from a single injection. The compounds are injected at 500pg on-column.

Using the procedure detailed in this Applications Note, a commercial laboratory[5] was able to obtain the data shown in Table I. The results easily meet the method detection limit requirements of 50ng/L. Should your laboratory need to meet a 1ng/L detection limit, some modification of the method will be necessary. The simplest and least expensive way to meet a lower detection limit would be to switch from using an FPD to a Pulsed FPD (PFPD) detector. This detector can give a sensitivity enhancement of 10 to 100 times over a standard FPD. Figure 3 shows the chromatogram obtained from an injection of an organo tin calibration standard with 5pg on-column for each compound. A comparison of this chromatogram to Figures 1 and 2 reveals that a similar signal-to-noise ratio can be obtained with 100 times less analyte. This demonstrates an increase in sensitivity (or lower reporting limits) of roughly 100 times using the PFPD and the preparation method outlined above.

This method is adaptable to most commercial laboratories and uses common glassware and equipment. If this is your first time using this method, you will not need to purchase a specialized instrument because the PFPD detector provides the sensitivity to meet even the lowest of proposed detection limits. The use of equipment such as tandem mass spectrometers (MS/MS/MS) or microwave induced plasma detectors (MIP or AED) are no longer necessary. Both are expensive and require much more operator skill. Finally, Restek is available to provide additional training on this and other methods to eliminate the need for literature and vendor research—please contact us for more information.

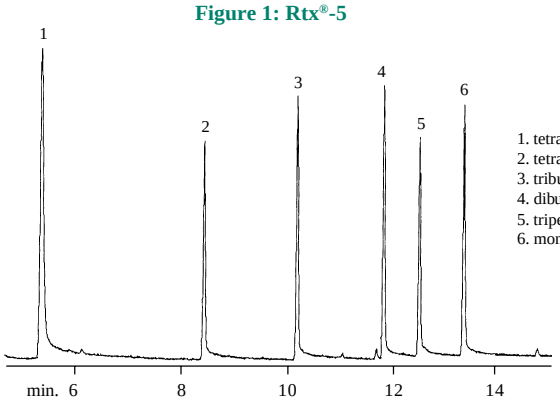
References

1. *Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis*, EPA/630/R-96/012, Feb. 1997.
2. Unger, M.A, MacIntyre, W.G., Greaves, J., and Huggett, R.J., *Chemosphere*, 15 (4), pp 461-70, 1986.
3. *Sampling and Analytical Methods of the National Status and Trends Program*, National Benthic Surveillance and Mussel Watch Projects, 1984-1992, Vol. IV, NOAA Technical Memorandum, NOS ORCA 71.
4. Jaima Conference, Tokyo, Japan. Nov. 1998.
5. ITS Environmental, 55 South Park Drive, Colchester, VT 05446.

Figures 1 & 2

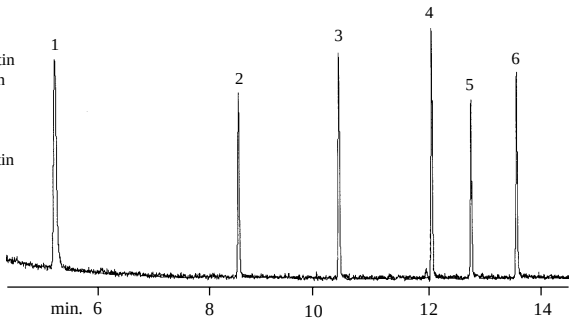
500pg of Derivatized Organo Tin Compounds Separated Well on Rtx®-5 and Rtx®-35 Columns Using GC/FPD.

Figure 1: Rtx®-5



1. tetrapropyl tin
2. tetrabutyl tin
3. tributyl tin
4. dibutyl tin
5. tripentyl tin
6. monobutyl tin

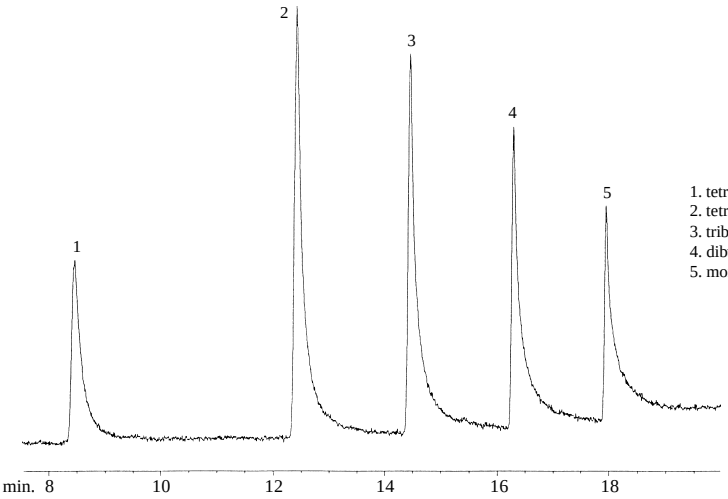
Figure 2: Rtx®-35



30m, 0.32mm ID, 1.0µm Rtx®-5 and Rtx®-35 columns (cat.# 10254 and 10454) 3µl direct injection using a Uniliner® (cat.# 20335). **Concentration:** ~500pg on-column. **Head pressure:** 15psi, constant. **Oven temp.:** 100°C (hold 1 min.) to 285°C @ 10°C/min. hold 10 min.; **Inj. & det. temp.:** 250°C; **Carrier gas:** He

Figure 3

PFPD Increases Sensitivity by 100 times When Analyzing Organo Tin Compounds Using this Method.*



1. tetrapropyl tin
2. tetrabutyl tin
3. tributyl tin
4. dibutyl tin
5. monobutyl tin

**Tailing is a function of this detector.*

30m, 0.32mm ID, 1.0µm Rtx®-35 (cat.# 10454). Direct injection using a Uniliner® (cat.# 20335). **Concentration:** 5pg on-column. . **Head pressure:** 15psi, constant. **Oven temp.:** 100°C (hold 1 min.) to 285°C @ 10°C/min. (hold 10 min.); **Detector:** PFPD Model 5380 courtesy of O.I. Analytical Corp., College Station, TX.

Table I

Commercial Laboratory Results Using the Proposed Method.[5]

Compound	Water Extraction Recovery (%)	Soil Extraction Recovery (%)	Restek SPE-Cleanup Recovery (%)	MDL Liquid (ng/L)	MDL Soil (µg/Kg)
tetrabutyl tin	83	86	92	29.9	0.45
tributyl tin	110	96	99	20.9	0.39
dibutyl tin	75	66	96	15.7	0.46
tripentyl tin (SSTD)	NA	NA	101	NA	NA
monobutyl tin	38	36	118	19.6	0.14

Product Listing

Rtx®-5 Column

ID	df (µm)	temp. limits	30-Meter
0.32mm	1.00	-60 to 330/350°C	10254

Rtx®-35 Column

ID	df (µm)	temp. limits	30-Meter
0.32mm	1.00	0 to 280/300°C	10454

New Analytical Reference Materials for GC Analysis of Organo Tins

- Most complete line of organo tin calibration mixes available.
- Organo tin analysis technical training offered.

Surrogate Mixtures

2000µg/mL in methylene chloride, 1mL/ampul

Tri-n-propyltin Chloride Mixture

	Each	5-pk.	10-pk.
	31476	**31476-510**	
w/data pack	**31476-500**	**31476-520**	**31576**

Tripentyltin Chloride Mixture

	Each	5-pk.	10-pk.
	31477	**31477-510**	
w/data pack	**31477-500**	**31477-520**	**31577**

Individual Calibration Compounds for Environmental Analysis and the Electronics Industry

2000µg/mL in methylene chloride, 1mL/ampul

Tributyltin Chloride Mixture

	Each	5-pk.	10-pk.
	31478	**31478-510**	
w/data pack	**31478-500**	**31478-520**	**31578**

Calibration Mixes for Environmental Analyses

2000µg/mL ea. in methylene chloride, 1mL/ampul

Butyltin Chlorides Calibration Mixture

tetrabutyltin, tributyltin chloride, dibutyltin dichloride, butyltin trichloride.

	Each	5-pk.	10-pk.
	31472	**31472-510**	
w/data pack	**31472-500**	**31472-520**	**31572**

Phenyltin Chlorides Calibration Mixture

tetraphenyltin, triphenyltin chloride, diphenyltin dichloride, phenyltin trichloride.

	Each	5-pk.	10-pk.
	31473	**31473-510**	
w/data pack	**31473-500**	**31473-520**	**31573**

Internal Standard Mixtures

2000µg/mL in methylene chloride, 1mL/ampul

Tetra-n-propyltin Mixture

	Each	5-pk.	10-pk.
	31474	**31474-510**	
w/data pack	**31474-500**	**31474-520**	**31574**

Tetrapentyltin Mixture

	Each	5-pk.	10-pk.
	31475	**31475-510**	
w/data pack	**31475-500**	**31475-520**	**31575**

Resprep™ Organo Tin SPE Cartridge

Suitable for high-capacity cleanup of butyl and phenyl tin compounds from soil, water, and biota.

Organo Tin
16 gm Florisil®/5gm Silica Gel (mixed bed) cat.# 24049 16-pk.

Resprep™ 12- & 24-Port Tube Manifold

Complete manifolds include glass basin with built-in vacuum regulator, polypropylene top plate with 12 or 24 individual control valves, 12- or 24-position collection rack, and 12 or 24 Teflon® sample guides.

Complete Resprep™-12 Port Manifold: cat.# 26077

Complete Resprep™-24 Port Manifold: cat.# 26080

RESTEK ISO 9001 REGISTERED

Petrochemical

Applications note

Lit. cat.# 59551

GC Analysis of Petroleum Products by Simulated Distillation, Using MXT® Sim Dist Columns

The American Society for Testing and Materials (ASTM) has released a new gas chromatographic (GC) method for petroleum distillation, "Boiling Range Distribution of Petroleum Distillates in the Boiling Range of 174°C to 700°C by Gas Chromatography (ASTM D6352)." This method extends the boiling point range specified in ASTM D2887 (55°C to 538°C). Determining the boiling range distribution of distillate fractions provides manufacturers with a better understanding of the composition of feed stocks and products related to petroleum refining processes.

Simulated distillation (Sim Dist) is a technique in which the GC is calibrated with alkane standards of published boiling points to perform a direct comparison to the boiling points of the sample components. Polyethylene standards such as Polywax® 655 or Polywax® 1000, which contain even-numbered hydrocarbons between C20 and C100, are used to calibrate the GC system. The retention times of the known alkanes are calibrated versus the published boiling points, and analysis of high molecular weight fractions are compared using special software. Analyzing high-boiling compounds (up to 700°C) requires an on-column or temperature-programmable injector to minimize injector discrimination, and a flame ionization detector (FID).

The capillary column used for the analysis is very important. The recommended column is coated with a non-polar methyl silicone phase that can withstand repeated cycles of high temperatures (430-450°C). However, the polyimide coating that maintains the flexibility of fused silica tubing rapidly breaks down at oven temperatures above 360°C and is unsuitable for high-temperature GC. Aluminum-clad tubing overcomes the problems associated with the polyimide, but has its own limitations. When repeatedly programmed to temperatures above 400°C, the aluminum sheath may become brittle. The most durable and inert capillary columns available are Restek MXT® columns, which are manufactured using Silcosteel® tubing—rugged metal tubing that is treated to provide the inertness of fused silica.

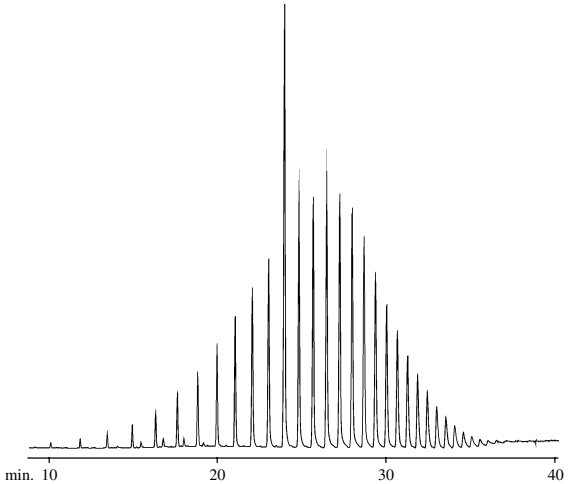
The liquid phase in the column also must withstand temperatures over 400°C, with minimum breakdown of the polymer. The phase must demonstrate low bleed and retention time stability that is repeatable with temperature programmed analysis. The methyl silicone polymer in the MXT®-1 Sim Dist column is synthesized to demanding quality control standards using a proprietary synthesis process. This process eliminates residual catalysts that could cause degradation and increase bleed from the polymer. The polymer then is carefully fractionated to remove low molecular weight fragments, providing a tight mono-modal distribution that results in extremely low bleed. The polymer is fully characterized for long-term reproducibility using techniques such as RI, FTIR, Kovats Indices, % crosslinking, coating efficiency, and a five-day thermal bake-out to ensure column longevity. Figure 1 shows the separation of Polywax® 655 on an MXT®-1 Sim Dist column.

Although the ASTM D6352 recommends a methyl silicone liquid phase, another phase also has gained acceptance by analysts performing simulated distillation—a carborane dimethyl polysiloxane, or MXT®-500 Sim Dist polymer. This has been designed by incorporating carborane into the backbone of the methyl silicone polymer chain to promote thermal stability (see Figure 2). The MXT®-500 Sim Dist stationary phase is the most stable phase available for high-temperature simulated distillation analyses and will outlast a 100% methyl silicone stationary phase. Figure 3 shows the separation of Polywax® 655 on the MXT®-500 column.

However, the trade-off for increased thermal stability produces a subtle polarity difference between the methyl silicone and the carborane stationary phase. The difference in polarity of the stationary phases causes a shift in the calculated boiling range

Figure 1

MXT®-1 Sim Dist Column Demonstrates Excellent Symmetry and Low Bleed for High-Temperature Sim Dist Calibration.



6m, 0.53mm ID, 0.15µm MXT®-1 (cat.# 70101). 1.0µL injection of Polywax® 655 in CS_2, cool on-column. **Oven temp.:** 40°C to 430°C @ 10°C/min. (hold 10 min.); **Det. temp.:** 430°C; **FID sensitivity:** 5 x 10^{-10} AFS; **Carrier gas:** hydrogen; **Linear velocity:** 45cm/sec. set @ 40°C.

distribution for petroleum samples containing aromatic hydrocarbons. The MXT®-500 Sim Dist stationary phase has increased relative retention of aromatic hydrocarbons compared to the methyl silicone MXT®-1 Sim Dist stationary phase. This causes the calculated boiling points of aromatics to be slightly higher using the MXT®-500 Sim Dist phase, although they are closer to the true value (see Table I).

While both columns can be operated to 430°C, the MXT®-500 Sim Dist column has lower bleed and longer lifetime. The MXT®-1 Sim Dist column offers methyl silicone polarity that matches published data.

Figure 2

MXT®-500 Sim Dist Polymer—designed by incorporating carborane into the backbone of the methyl silicone polymer chain to promote thermal stability.

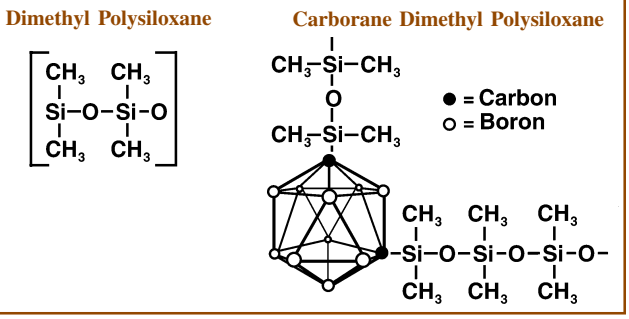


Table I

Differences in the Calculated Boiling Points of Aromatics Using the MXT®-500 Sim Dist Phase.

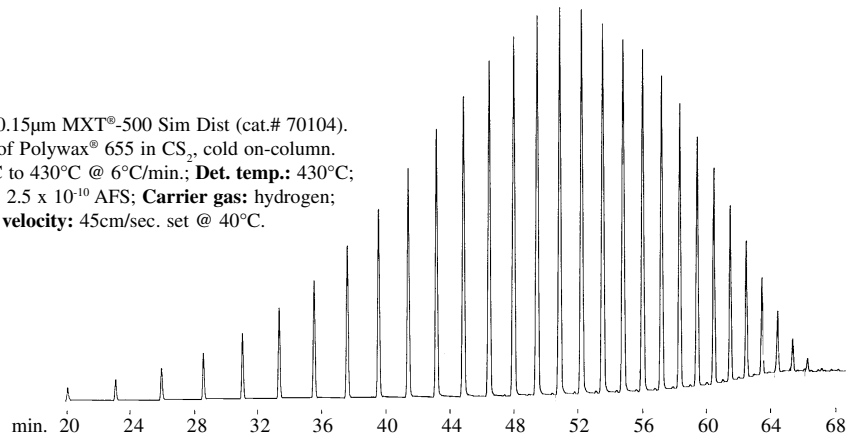
Compound	Boiling Point °C (°F)	MXT®-1 Δ °C (°F)	MXT®-500 Δ °C (°F)
toluene	128 (231)	-1 (-2)	8 (14)
p-xylene	157 (282)	-2 (-3)	4 (8)
naphthalene	236 (424)	-19 (-34)	-4 (-8)
acenaphthene	297 (534)	-28 (-51)	-11 (-19)
chrysene	465 (837)	-74 (-134)	-48 (-87)
coronene	543 (977)	-59 (-106)	-23 (-42)

High-temperature GC analyses challenge the limits of existing tubing and stationary phase technology. Restek's MXT® tubing is ideally suited to the task when compared to fused silica or aluminum clad tubing. The high temperatures necessary for simulated distillation analyses also push GC polymers to the limit of thermal stability. The MXT®-500 and MXT®-1 Sim Dist columns are proven performers under these extreme conditions. When properly conditioned to 430°C, these columns give stable baselines, low bleed, and repeatable retention times for simulated distillation and other high-temperature GC analyses.

Figure 3

MXT®-500 Sim Dist Column is an Excellent Choice for Lower Bleed and Extended Lifetime.

6m, 0.53mm ID, 0.15µm MXT®-500 Sim Dist (cat.# 70104).
1.0µL injection of Polywax® 655 in CS_2, cold on-column.
Oven temp.: 40°C to 430°C @ 6°C/min.; **Det. temp.:** 430°C;
FID sensitivity: 2.5 x 10^{-10} AFS; **Carrier gas:** hydrogen;
Linear velocity: 45cm/sec. set @ 40°C.



Product Listing

Description	Cat.#
MXT®-1 Sim Dist (6m, 0.53mm ID, 0.15µm)	70101
MXT®-500 Sim Dist (6m, 0.53mm ID, 0.15µm)	70104

Description	Cat.#
Polywax® Standard (655 Calibration Material, 1g)	36225

Industrial Chemicals
Applications note
Lit. cat.# 59552

GC/MS Analysis of Azo Dye Compounds

Some consumer goods that come into contact with the human body, such as clothing, jewelry, and toys, may contain azo dyes that metabolize into carcinogenic aryl amines. Consumer safety regulations in Germany prohibit the distribution of all consumer goods containing azo dyes,[1] and other European countries may follow suit in the near future. In 1996, the German government institute BgVV published four methods for azo dye analysis, including a gas chromatography (GC) method.[2]

Analysts must overcome several obstacles when performing a GC analysis of aryl amines. First, response factors rise for the first several injections before reaching an equilibrium state, especially when using standard sleeve, wool, and column deactivation. Restek's proprietary amine deactivation for inlet liners and wool, when used in combination with the Rtx®-5 Amine column, will eliminate the adsorption that causes this "priming effect." As a result, the reproducibility of the analysis also will improve. Second, the inability to obtain pure, stable calibration standards is a problem. Raw material characterization and purification is essential for the preparation of suitable calibration standards. Restek's azo dye standards offer the most pure and stable calibration available. The following describes an optimized gas chromatography/mass spectrometry (GC/MS) method of azo dye analysis, based on the German method (Figure 1).

Sample Preparation

1g of material (e.g. leather or cotton) is solvated in 17mL of 60mM, pH 6 sodium citrate buffer at 70°C for 30 minutes. Add 3mL of 20% sodium dithionite to the sample and heat at 70°C for 30 minutes to chemically reduce the azo dyes into aryl amines.

Sample Extraction

Samples may be extracted and concentrated for analysis using solid phase extraction (SPE) or liquid/liquid extraction.

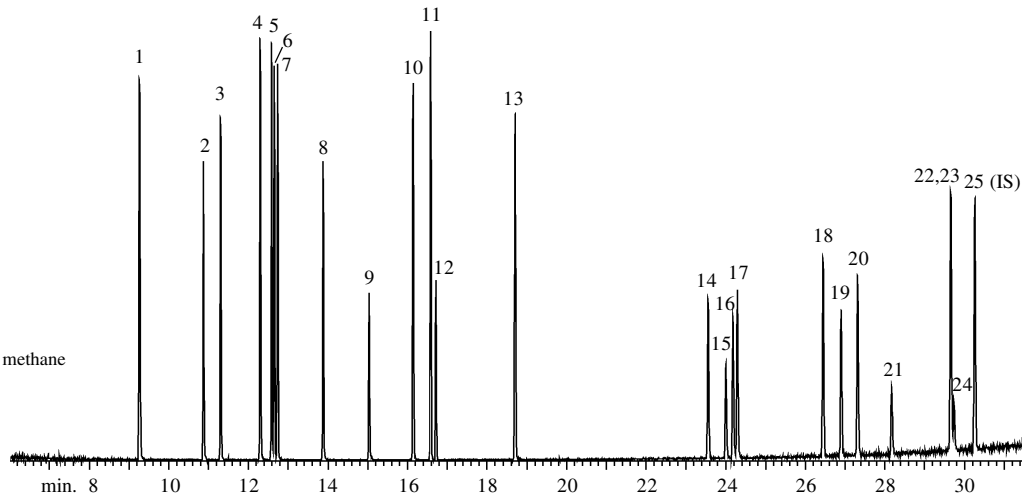
Procedure #1, Solid Phase Extraction

This procedure uses E. Merck Extrelut® 20 cartridges or equivalent. The aqueous, chemically-reduced samples are loaded onto the activated cartridges and allowed to stand for 5 minutes.

Figure 1

Restek's proprietary amine deactivated sleeves, fused silica wool, and Rtx®-5 Amine columns combine to give exceptional response for azo dyes, even when analyzed at the low 10µg/mL concentration.

1. *o*-toluidine
2. 2-methoxyaniline
3. 4-chloroaniline
4. 2-methoxy-5-methylaniline
5. 2,4,5-trimethylaniline
6. 3-chloro-2-methylaniline
7. 4-chloro-2-methylaniline
8. 2,4-diaminotoluene
9. 2,4-diaminoanisole
10. 2-naphthylamine
11. 2-aminobiphenyl
12. 2-methyl-5-nitroaniline
13. 4-aminobiphenyl
14. 4-phenylazoaniline
15. 4-aminophenylether
16. benzidine
17. bis-(4-aminophenyl)-methane
18. 4'-amino-2,3'-dimethylazobenzene
19. 4,4'-diamino-3,3'-dimethyldiphenyl methane
20. 3,3'-dimethylbenzidine
21. 4-aminophenylthioether
22. 3,3'-dichlorobenzidine
23. 4,4'-methylene-bis(2-chloroaniline)
24. 3,3'-dimethyoxybenzidine
25. 3,3',5,5'-tetramethyl benzidine (IS)



30m, 0.25mm ID, 0.25µm Rtx®-5 Amine (cat.# 12323). 1µL splitless injection using a 4mm gooseneck sleeve with base deactivated wool for HP GCs (cat.# 20798-211.1). **Concentration:** 10µg/mL in ethylacetate. **Oven temp.:** 50°C to 200°C @ 10°C/min. to 270°C @ 5°C/min.; **Inj./det. temp.:** 250°/300°; **Carrier gas:** helium @ 1mL/min.; **Detector type:** MSD; **Splitless hold time:** 1 min.

The target analytes are eluted from the cartridge with two additions of 40mL methyl-*tert*-butyl ether. The two fractions are combined and evaporated to about 1mL on a rotary evaporator under vacuum at 50°C. The extracts are then redissolved in 1mL of methanol for GC analysis.

Procedure #2, Liquid/Liquid Extraction

The chemically-reduced sample also may be concentrated by liquid/liquid extraction. The aqueous sample first is adjusted with sodium hydroxide to a pH greater than 10. Sodium chloride is added until saturation. The sample then is extracted twice with 25mL of methyl-*tert*-butyl-ether. The ether fractions are combined and evaporated to dryness. Finally, the extract is reconstituted in 2mL of methanol for GC/MS analysis.

Your Chromatographic Problems Solved

Problem: Priming Effects

Analysts commonly see priming effects in this analysis. While standard sleeve, wool, and column deactivations work well to decrease this in most situations, they do not perform well with amines in splitless injections at low concentrations.

Solution: Amine Deactivation

Restek has developed the ideal GC products to eliminate the resulting priming effects in the analysis of azo dye. The use of Restek's proprietary amine-deactivated sleeves, amine-deactivated wool, and an Rtx®-5 Amine column will ensure the highest level of system inertness and excellent reproducibility. The priming effect when using standard deactivated products was evaluated by comparing the response factors during repetitive runs. The results indicated that the response factors for the first injection were up to 10% lower than the tenth injection. However, the comparison analysis that used amine deactivation showed no priming effect. The amine deactivations also improved repeatability and lowered standard deviations to < 5%.

Problem: Calibration Standards

A lack of accurate, stable calibration standards presents another obstacle to the analysis of azo dyes. Many of the target compounds are not commercially available in a pure, stable form. Some are only available as the hydrochloric, carbonate, or sulfate salts of the free base. While these salts have better stability as a neat material, they do not make good raw materials for GC calibration standards. Standards prepared from the salt forms of azo dyes will yield poor response and peak shape when analyzed using GC. The salt forms also will cause difficulty in assigning the actual weight/weight purity. The best standards for GC analysis are prepared from the free base forms of the azo dye target analytes.

Solution: Calibration Standard Purity

To ensure accurate preparation of calibration standards, these target compounds must be tested and re-purified. One of the best methods for final purification of the free base is sublimation. When performed under an inert atmosphere, this produces a very pure material for final preparation of a calibration standard. Using multiple purity assays is required to detect impurities. While GC will detect only organic impurities, differential scanning calorimetry (DSC) will detect both organic and inorganic impurities. All of the raw materials for Restek's azo dye mixes are carefully tested and purified to provide the most accurate and stable calibration standards available.

Conclusion

When using the German azo dye method and Restek's amine-deactivated column, sleeve, and wool, analysts can achieve reproducible and accurate results.

References

1. *German Consumers Goods Act of July 15, 1994, Lebensmittel-und Bedarfsgegenständegesetz §35 LMBG*
2. *BgVV, Bundesgesundhbl. 2/96*

Product Listing

Rtx®-5 Amine Column

30m, 0.25mm ID, 0.25µm, cat.# 12323

Base-Deactivated Fused Silica Guard Columns

5m, 0.25mm ID	cat.# 10000, ea.	cat.# 10000-600, 6-pk.

4mm Gooseneck Base-Deactivated Inlet Sleeves Packed with Base-Deactivated Wool

GC	each	5-pk.	25-pk.
HP	20798-211.1	20799-211.5	20800-211.25
Varian	20904-211.1	20905-211.5	20906-211.25
CE*	20945-211.1	20946-211.5	20947-211.25

*5mm Gooseneck base-deactivated inlet sleeve.

Azo Dye Mix #1

Compounds 1-24 from Figure 1 chromatogram
10µg/mL ea. in ethyl acetate, 1mL/ampul

	Each	5-pk.	10-pk.
	31466	**31466-510**	
w/data pack	**31466-500**	**31466-520**	**31566**

Internal Standard for Azo Dye Method

3,3',5,5'-tetramethylbenzidine mix
1000µg/mL in ethyl acetate, 1mL/ampul

	Each	5-pk.	10-pk.
	31467	**31467-510**	
w/data pack	**31467-500**	**31467-520**	**31567**

RESTEK ISO 9001 REGISTERED

RESTEK

Food & Flavor

Applications note

Grape Flavor Analysis Using an Rt-γDEXsa™ GC Column

The enantiomeric ratios of certain chiral compounds found in flavorings can sometimes reveal adulteration. Two chiral indicators in grape flavor are methyl-3-hydroxybutyrate and ethyl-3-hydroxybutyrate. Methyl-3-hydroxybutyrate is racemic and (R)-ethyl-3-hydroxybutyrate is at least 77% predominant in natural grape flavor.[1] Extreme alterations of these ratios can indicate that the flavor is not completely authentic.

Although the exact mechanisms of compound-cyclodextrin interaction are not known, it is evident that the size of the cyclodextrin cavity is important. Perhaps some compounds may be too large to effectively interact with the cavity size of beta-cyclodextrin, which is composed of seven glucopyranose units. Gamma-cyclodextrins are composed of eight glucopyranose units and so possess a larger cavity, which may be more interactive with larger chiral molecules (see **Figure 1**).

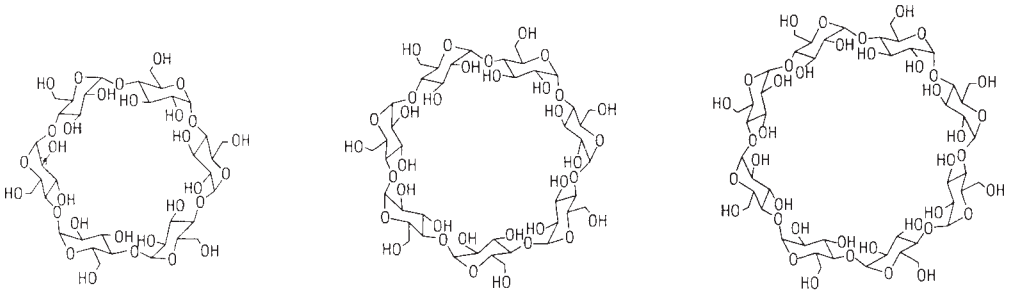
Beta-cyclodextrin stationary phases provide enantioselectivity for a variety of chiral compounds, but not for methyl-3-hydroxybutyrate and ethyl-3-hydroxybutyrate. **Figure 2** (on next page) illustrates the analysis of these compounds on three beta-cyclodextrin stationary phases: the Rt-βDEXm™ column (2,3,6-tri-O-methyl-β-cyclodextrin), the Rt-βDEXsm™ column (2,3-di-O-methyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin), and the Rt-βDEXsa™ column (2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin). The peaks tail and exhibit no chiral separation on the Rt-βDEXm™ column. Different derivatives on the cyclodextrin molecule can help increase selectivity of enantiomers. Although the Rt-βDEXsm™ and Rt-βDEXsa™ columns show improved enantioselectivity for methyl-3-hydroxybutyrate and ethyl-3-hydroxybutyrate, the peaks still tail and separation of the optical isomers is not baseline-resolved.

Analysis of methyl-3-hydroxybutyrate and ethyl-3-hydroxybutyrate on the new Rt-γDEXsa™ column reveals excellent chiral selectivity for both, as shown in two types of grape juice in **Figure 3** (on next page). The Rt-γDEXsa™ contains 2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-γ-cyclodextrins that are dissolved into cyanopropyl phenyl stationary phase (Rtx®-1701). This composition promotes thermal stability to a maximum temperature of 230°C and longevity that is comparable to other capillary columns. This column is available in 0.32mm ID and 0.25mm ID for direct interfacing into a mass spectrometer.

Previous articles have demonstrated the utility of beta-cyclodextrin columns for flavor analysis using gas chromatography (GC), but sometimes the larger gamma-cyclodextrin cavities provide better enantioselectivity for chiral indicating compounds. Restek's Rt-γDEXsa™ column provides better separation of specific chiral compounds in grape flavor than most beta-cyclodextrin phases.

Figure 1

*Structures of beta- and gamma-2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-cyclodextrins.*



α-Cyclodextrin *β-Cyclodextrin* *γ-Cyclodextrin*

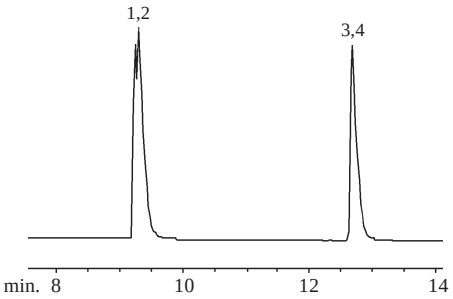
Food & Flavor

Figure 2

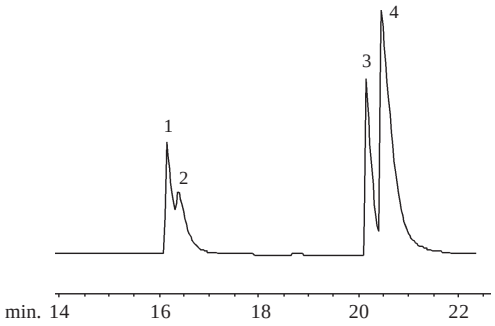
Beta-cyclodextrin columns do not provide optimum separation of methyl- and ethyl-3-hydroxy butyrate.

A) The Rt-βDEXm™ column exhibits no enantiomeric selectivity.

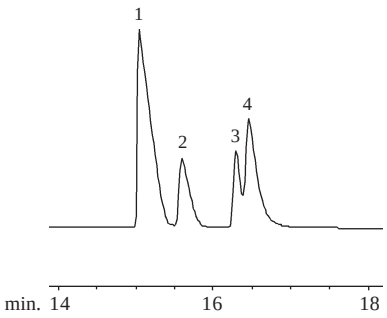
1. (S)-(+)-methyl-3-hydroxybutyrate
2. (R)-(–)-methyl-3-hydroxybutyrate
3. (S)-(+)-ethyl-3-hydroxybutyrate
4. (R)-(–)-ethyl-3-hydroxybutyrate



B) The Rt-βDEXsm™ column exhibits poor enantiomeric separation.



C) The Rt-βDEXsa™ column exhibits improved enantiomeric separation.



30m, 0.32mm ID, 0.25µm a) Rt-βDEXm™; b) Rt-βDEXsm™; and c) Rt-βDEXsa™ columns (cat.#s 13101, 13104, and 13108 respectively). 1.0µL split injection of methyl- and ethyl-3-hydroxybutyrate; **On-column concentration:** ~150ng/enantiomer; **Oven temp.:** 40°C (hold 1 min.) to 200°C @ 2°C/min.; **Inj. / det. temp.:** 200°C/230°C; **Carrier gas:** H_2; **Linear velocity:** 80cm/sec. set @ 40°C; **Split ratio:** 25:1.

Figure 3

The Rt-γDEXsa™ column provides excellent chiral selectivity for both methyl- and ethyl-3-hydroxybutyrate in the flavor extracts of grape juice and white grape juice.

Fig. 3a - grape juice

1. (S)-(+)-methyl-3-hydroxybutyrate
2. (R)-(–)-methyl-3-hydroxybutyrate
3. (S)-(+)-ethyl-3-hydroxybutyrate
4. (R)-(–)-ethyl-3-hydroxybutyrate

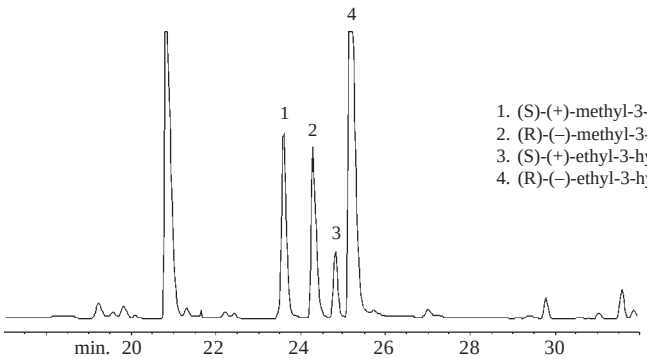
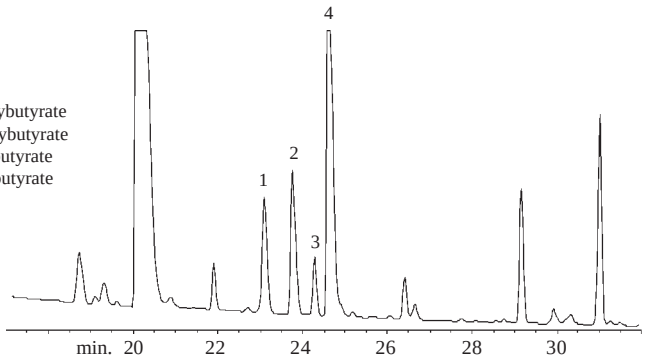


Fig. 3b - white grape juice



30m, 0.32mm ID, 0.25µm Rt-γDEXsa™ (cat.# 13112) 10µL direct injection of grape juice (Fig. 3a) and white grape juice (Fig. 3b) using a 4mm open-top Uniliner® sleeve (cat.# 20843). **Oven temp.:** 40°C (hold 1 min.) to 230°C @ 2°C/min.; **Inj. / det. temp.:** 200°C/230°C; **Carrier gas:** H_2; **Linear velocity:** 80cm/sec. set @ 40°C.

Comparing Grape Juices and Grape-Flavored Beverages

Extraction Procedure

Grape juice, white grape juice, grape drink, grape-flavored soda, and a grape-flavored sport drink were evaluated. Each 16–20oz. beverage was added to a 500mL separatory funnel. Thirty milliliters of methylene chloride were added to the sample in the separatory funnel, which was shaken for 3–5 minutes. The extract was then collected into a beaker. This procedure was repeated three times. The organic extract was then funneled through anhydrous sodium sulfate to eliminate water and transferred to a Kuderna-Danish collector with a Snyder column. This was immersed into a hot water bath of 65°C until the extract was concentrated to 4mL.

Analysis

Ten microliters of sample was introduced via direct injection. A 1.5m x 0.53mm guard column was connected to the 4mm open-top Uniliner® sleeve and to the 30m, 0.32mm ID, 0.25µm Rt-γDEXsa™ column, to accommodate the large volume injection and to protect the analytical column. Some spectral confirmation was conducted by GC/MS on a 30m, 0.25mm ID, 0.25µm Rt-γDEXsa™ column, using splitless analysis.

Results

The methyl-3-hydroxybutyrate was essentially racemic in both the grape and white grape juices, as shown in **Figure 3a** and **3b**. It was also racemic in one grape-flavored drink, but at much lower concentrations. Analysis on a 0.25mm ID Rt-γDEXsa™ column with a slower linear velocity provides resolution of benzaldehyde and (R)-methyl-3-hydroxybutyrate (**Figure 4**). The (R)-ethyl 3-hydroxybutyrate was predominant in both juices and grape-flavored drink. Neither chiral compound was detected in the grape-flavored soda or sport drink.

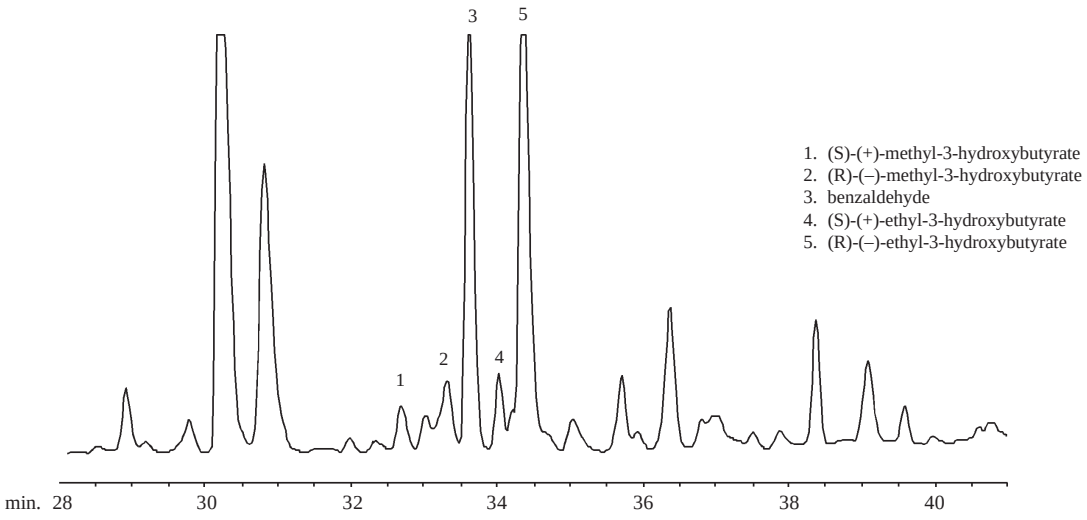
Beta-cyclodextrin phases can separate a variety of chiral indicating compounds in flavors, but are not effective in all applications. Using different cyclodextrin derivatives can help chiral selectivity, but going to a larger cyclodextrin sometimes is necessary. Switching from a 2,3-di-O-methyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin to a 2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-β-cylcodextrin column partially improved enantiomer separation for chiral indicating compounds in grape flavor. However, the 2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-γ-cyclodextrin column provided the best enantiomeric profile of these compounds. The Rt-γDEXsa™ column allows detection of racemic methyl-3-hydroxybutyrate and (R)-ethyl-3-hydroxybutyrate in juices and drinks that contain authentic grape flavor. This column can offer certain separations that cannot be achieved by beta-cyclodextrin columns and may be a more suitable alternative for your chiral analysis.

References

1. Dr. Joulain, Robertet Corp., “private communication.”

Figure 4

The 0.25mm ID Rt-γDEXsa™ column provides detection of benzaldehyde, and methyl-and ethyl-3-hydroxybutyrate.



30m, 0.25mm ID, 0.25µm Rt-γDEXsa™ (cat.# 13113) 10µL splitless injection of a grape-flavored drink. **Oven temp.:** 40°C (hold 1 min.) to 200°C @ 2°C/min.; **Inj. / det. temp.:** 200°C/230°C; **Carrier gas:** He; **Linear velocity:** 35cm/sec. set @ 40°C; **Splitless hold time:** 1 min.

Product Listing

Rt-γDEXsa™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13113
0.32mm	0.25	40 to 230°C	13112

Rt-βDEXsa™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13109
0.32mm	0.25	40 to 230°C	13108

Rt-βDEXm™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13100
0.32mm	0.25	40 to 230°C	13101

Rt-βDEXsm™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13105
0.32mm	0.25	40 to 230°C	13104

To Optimize Chiral Separations Use:

1) Faster linear velocities (80cm/sec.) with hydrogen carrier gas.
2) Slower temperature ramp rates (1–2°C/min.).
3) Appropriate minimum operating temperature (40 or 60°C).
4) On-column concentrations of 50ng or less.

Direct Injection Sleeves for HP/Finnigan GCs

Description	Each	5-pk.
4mm ID Uniliner®	20335	20336
4mm ID Cyclo-Uniliner®	20337	20338
4mm ID Open-top Uniliner® w/wool	20843	20844

Restek offers a wide range of cyclodextrin columns for the analysis of many chiral compounds.

Rt-βDEXse™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13107
0.32mm	0.25	40 to 230°C	13106

Rt-βDEXsp™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13111
0.32mm	0.25	40 to 230°C	13110

For more information on chiral analysis, visit our web site at **www.restekcorp.com** to view *A Guide to the Analysis of Chiral Compounds by GC.*

Rt-βDEXcst™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13103
0.32mm	0.25	40 to 230°C	13102

Environmental

Applications note

cat.# 59557

SPE Extraction for EPA Method 525.1

EPA Method 525.1 Overview

EPA Method 525.1 is used for the determination of organic compounds in drinking water by liquid-solid extraction and capillary column gas chromatograph/mass spectrometry. It is applicable to a wide range of organic compounds that are efficiently partitioned from the water sample onto a C18 organic phase chemically bonded to a solid silica matrix in a cartridge or disk.

SIMDisk™-*GF* extraction disks can be used in EPA Method 525.1

This procedure was followed to demonstrate method equivalency:

1 Sample Pretreatment:
Allow 1 liter of deionized water to equilibrate to room temperature in a narrow-mouth amber glass bottle. Adjust sample pH to less than 2 with 6M hydrochloric acid, being careful not to over acidify the sample. Addition of too much HC_1 can cause the formation of acidic extracts.

2 Apparatus Assembly:
Assemble the 47mm apparatus. Place the SIMDisk™-GF disk in the Diskcover™-47 filter support, wrinkled side up.

3 Disk Precleaning:
Add 5ml of methylene chloride to the top surface of the disk and immediately draw through under vacuum at 15 in. Hg (50 kPa). Continue to draw vacuum at 15 in. Hg (50 kPa) for 5 minutes to remove all solvent.

4 Disk Conditioning:
Add 5ml methanol to the top surface of the disk and immediately apply low vacuum (1- 2 in. Hg, 3-7 kPa). Draw through until the top surface of the methanol is just above the disk. Do not allow any air to pass through the disk or reach the top surface of the disk. Immediately add 5ml of deionized water to the disk and draw through at low vacuum until the water almost reaches the top surface of the disk. It is preferable to leave extra liquid above the disk rather than allow any air to contact the surface of the disk.

5 Sample Addition:
Add the sample onto the disk, directly to the film of water left from the conditioning step. Adjust the vacuum to 10 in. Hg (35 kPa) for a flow rate of approximately 100ml per minute until the entire sample has been passed through the disk.

6 Disk Drying:
After the sample has been processed, draw air through the disk under vacuum at approximately 15 in. Hg (50 kPa) for approximately 5 minutes.

7 Analyte Elution:
Release system vacuum. Insert the sample collection rack and collection vessels. Reassemble the apparatus. Add 5ml methylene chloride directly to the sample bottle and gently swirl to rinse all inner surfaces of the bottle. Allow the sample bottle to stand for 1 to 2 minutes and transfer the methylene chloride to the disk using a glass pipet. Rinse the reservoir sides in the process. Draw the solvent through the disk at 5 in. Hg (17 kPa). Repeat the bottle rinse and disk elution twice with fresh aliquots of methylene chloride, combining all eluates in the collection tube.

8 Final Analysis:
Remove water from sample eluate by passing it through approximately 3 grams of anhydrous sodium sulfate. Concentrate to 1ml and analyze 1µl by GC/MS.

SIMDisk™-*GF*:
cat.# 24004, 20-pack

EPA methods are available from the National Technical Information Service:
703-487-4650

Accuracy/Precision data from four determinations of Method 525.1 analytes at 2µg/L with Liquid-Solid SIMDisk™-GF 47mm extraction disk and the Finnigan MAT ITS40 Ion Trap MS

compound	target conc. (µg/L)	mean (µg/L)	std. dev. (µg/L)	% RSD	accuracy (% of target)	% REC. in method
acenaphthalene-d10	5	------	------	------	------	------
henanthrene-d10	5	------	------	------	------	------
chrysene-d12	5	------	------	------	------	------
hexachlorocyclopentadiene	2	1.6	0.30	2.1	80	55
dimethylphthalate	2	1.8	0.17	9.4	90	95
acenaphthylene	2	2.0	0.60	3.1	100	95
2-chlorobiphenyl	2	2.0	0.50	2.4	100	95
diethylphthalate	2	2.1	0.70	3.3	105	100
fluorene	2	2.1	0.60	3.1	105	110
2,3-dichlorobiphenyl	2	2.0	0.70	3.2	100	115
hexachlorobenzene	2	2.0	0.60	2.8	100	85
simazine	2	1.9	0.19	10.2	95	105
atrazine	2	2.1	0.16	7.5	105	110
pentachlorophenol	8	9.7	0.79	8.2	121	97
gamma-BHC	2	2.1	0.40	2.2	105	105
phenanthrene	2	2.2	0.40	1.9	110	120
anthracene	2	2.0	0.90	4.6	100	85
2,4,5-trichlorobiphenyl	2	1.9	0.40	1.9	95	85
alachlor	2	2.1	0.40	1.7	105	------
heptachlor	2	1.9	0.40	2.2	95	110
di-n-butylphthalate	2	2.5	0.24	9.5	125	110
2,2',4,4'-tetrachlorobiphenyl	2	1.9	0.20	1.2	95	75
aldrin	2	1.6	0.20	12.7	80	80
heptachlor epoxide (isomer B)	2	2.1	0.50	2.5	105	115
2,2',3',4,6-pentachlorobiphenyl	2	1.9	0.50	2.4	95	95
gamma-chlordane	2	1.9	0.80	4.1	95	110
pyrene	2	2.0	0.40	2.0	100	95
alpha-chlordane	2	1.9	0.50	2.8	95	100
trans-nonachlor	2	1.9	0.70	3.7	95	135
2,2',4,4',5,6'-hexachlorobiphenyl	2	1.7	0.14	8.0	85	80
endrin	2	2.2	0.50	2.2	110	90
butylbenzylphthalate	2	2.2	0.12	5.4	110	100
bis(2-ethylhexyl)adipate	2	1.8	0.21	11.9	90	80
2,2',3,3',4,4',6-heptachlorobiphenyl	2	1.8	0.40	1.9	90	70
methoxychlor	2	2.1	0.50	2.5	105	90
2,2',3,3',4,5',6,6'-octachlorobiphenyl	2	1.7	0.20	1.2	85	90
benzo(a)anthracene	2	1.9	0.20	0.9	95	90
chrysene	2	1.9	0.20	0.9	95	110
bis(2-ethylhexyl)phthalate	2	2.2	0.40	2.0	110	95
benzo(b)fluoranthene	2	2.0	0.90	4.2	100	------
benzo(k)fluoranthene	2	2.0	0.80	4.2	100	105
benzo(a)pyrene	2	2.0	0.10	5.1	100	40
perylene-d12	5	4.7	0.34	7.3	94	100
indeno(1,2,3-cd)pyrene	2	1.9	0.22	11.6	95	20
dibenzo(a,h)anthracene	2	1.8	0.20	11.3	90	15
benzo(g,h,i)perylene	2	1.8	0.18	9.9	90	35

RESTEK environmental

Applications note

Optimizing Chlorophenoxy Herbicides Analysis Using Rtx®-CLPesticides and Rtx®-CLPesticides2 Columns

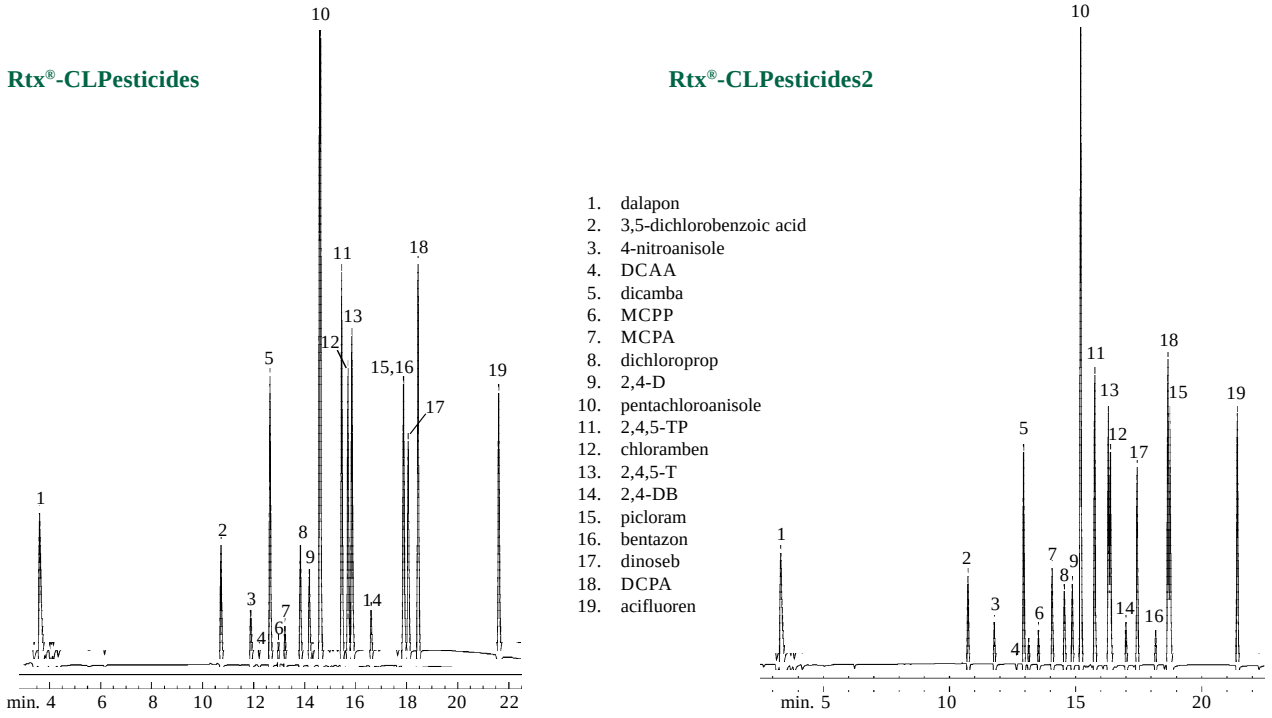
Chlorine-substituted phenoxyacetic acids, such as 2,4-D, MCPA, and 2,4,5-T [2,4-dichlorophenoxy acetic acid, (4-chloro-2-methylphenoxy) acetic acid, and 2,4,5-trichlorophenoxy acetic acid respectively], were introduced as selective weed killers in the 1940s. Due to their growth-regulating and herbicidal activities against broadleaf weeds, they have been commonly used for weed control on cereal crops, grasslands, and lawns. 2,4-D and 2,4,5-T also were used as the primary defoliant agents in Agent Orange during the Vietnam War. Today, chlorophenoxy herbicides still are used as commercially available lawn weed killers.[1]

Chlorophenoxy herbicides are applied as either esters or salts, which are easily metabolized by plants. The esters are oil soluble, but also can be applied as emulsions in water. The salts typically are highly soluble in water and are used as aqueous concentrates. Because the chlorophenoxy herbicides are spread on top of the soil or grass and then leach into the ground, there is great potential for groundwater contamination. Chlorophenoxy herbicides readily degrade in the environment and for many years were not considered an environmental or public concern. However, potential hazards to public health and environmental quality led to the development of US Environmental Protection Agency (EPA) methods for the analysis of these herbicides. US EPA Methods 615 (municipal/industrial wastewater) and 8151 (solid waste) were developed to monitor chlorophenoxy herbicides in environmental samples.[2,3]

Analysis of chlorophenoxy herbicides using gas chromatography (GC) is difficult. In their free acid form, these herbicides have limited volatility and are prone to irreversible adsorption. To overcome this problem, they are most frequently analyzed as methyl esters. Because these herbicides can be applied as several different types of esters or as a salt, they must first be converted to the free acid form, then derivatized into methyl esters for GC

Figure 1

The Rtx®-CLPesticides and Rtx®-CLPesticides2 columns give good resolution for all 18 chlorophenoxy herbicides in a single injection.



30m, 0.32mm ID, 0.50µm, Rtx®-CLPesticides column (cat.# 11139) and 30m, 0.32mm ID, 0.25µm Rtx®-CLPesticides2 column (cat.# 11324). 1.0µL direct injection of chlorophenoxy herbicides using a Uniliner® sleeve (cat.# 20335), on-column concentration 200-20,000pg/mL. **Oven temp.:** 80°C (hold 1 min.) to 300°C @ 10°C/min. (hold 10 min.); **Inj. / det. temp.:** 200°C/300°C; **Carrier gas:** helium; **Inlet pressure:** 12.5psi set @ 80°C; **ECD sensitivity:** 60kHz fused silica.

analysis. Methylation increases herbicide volatility and overcomes matrix interferences of herbicides extracted from soil. Despite this derivatization step, analysts still can experience the problems of poor resolution, matrix interference, and peak misidentification.

This analysis has been performed on a number of different GC column stationary phases, many of which suffer from slow analysis time, low thermal stability, or coelutions. In addition, many laboratories perform this analysis with the same instrument used for chlorinated pesticide analysis (US EPA 8081). The Rtx®-CLPesticides and Rtx®-CLPesticides2 columns provide unsurpassed separation of pesticides and herbicides, while allowing high-temperature operation to facilitate the removal of contaminants from the system by programming to 300°C or higher.

Figure 1 shows the separation of the method 8151 target compounds on the Rtx®-CLPesticides and the Rtx®-CLPesticides2 columns for simultaneous confirmation. These columns were connected using a glass "Y" connector (cat. # 20403) and a 5m guard column (cat. # 10044) installed into a single injection port. The injection port was configured with a direct injection liner (cat. # 20335), which improves the inertness and sample transfer of a splitless injection.

In summary, by configuring your instrument as described and using the Rtx®-CLPesticides and Rtx®-CLPesticides2 columns, the separation of the chlorophenoxy herbicides can be obtained on both columns simultaneously. The surrogates are not shown in these chromatograms because many laboratories use a variety of compounds as surrogates and internal standards for this analysis. The common compounds (1,4-dichlorobenzene, DCAA, and 4,4'-dibromooctafluorobiphenyl) are baseline resolved on both of these columns, so you may use either of them without compromising the chromatography. This same instrument configuration is also optimal for the analysis of chlorinated pesticides and PCBs.

References

1. Kaufamn, D.D., Kearney, P.C., Herbicides, Vol. 1, *Chemistry, Degradation, and Mode of Action*, 2nd Edition, Marcel Dekker, Inc., New York and Basel.
2. US EPA, *Organic Chemical Analysis of Municipal and Industrial Wastewater*; Method 615, "Determination of Chlorinated Herbicides in Industrial and Municipal Wastewater."
3. US EPA, SW-846 *Test Methods for Evaluating Solid Waste*, 3rd Edition, Final Update 1; Method 8150A, "Chloronated Herbicides by Gas Chromatography."

Product Listing

Rtx®-CLPesticides Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.25	-60 to 310/330°C	11120	11123
0.32mm	0.50	-60 to 310/330°C	11136	11139
0.53mm	0.50	-60 to 310/330°C	11137	11140

Rtx®-CLPesticides2 Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.20	-60 to 310/330°C	11320	11323
0.32mm	0.25	-60 to 310/330°C	11321	11324
0.53mm	0.42	-60 to 310/330°C	11337	11340

Universal Angled "Y" Press-Tight® Connectors

cat.# 20403 (ea.)	cat.# 20404 (3-pk.)

Universal "Y" Press-Tight® Connectors

cat.# 20405 (ea.)	cat.# 20406 (3-pk.)

Phenylmethyl Deactivated Guard Columns

ID	length	cat.#
0.25mm	5m	10043
0.32mm	5m	10044
0.53mm	5m	10045

For a complete listing of Analytical Reference Materials and Sample Preparation Products for EPA Methods 515, 8150, and 8151, see Restek's Product Guide, visit the web site (www.restekcorp.com), or call 800-356-1688, 814-353-1300, or your local Restek representative for information.

RESTEK environmental

Applications note

Florisil® SPE cleanup for organochlorine pesticides and polychlorinated biphenyl compounds (PCBs)

This procedure is adapted from the US Environmental Protection Agency (EPA) Contract Laboratory Program, Statement of Work for Organic Analyses, Multimedia, Multi-Concentration, OLM04.0. It is not intended to be a replacement or substitute for the official procedure. Please refer directly to the published procedure for additional information.

Preparing for Sample Cleanup

A. Determine the size of Florisil® solid phase extraction (SPE) tube needed for sample cleanup. If the gas chromatography (GC) autosampler can operate reliably with 1mL of sample extract, then a 500mg tube (cat.# 24031 & 24032) is used and the required final volume is 1mL. If the GC autosampler requires a larger sample volume, prepare 2mL of sample extract using a 1g tube (cat.# 24034). Manual injection requires only 1mL final extract and a 500mg tube. See *Tips for Better Results* below.

B. Every lot of Florisil® adsorbent must be quality control-tested for activity level before using for sample cleanup. Add 0.5mL of 2,4,5-trichlorophenol solution (0.1µg/mL in acetone) and 0.5mL of Standard Mixture A (midpoint concentration) to 4mL of hexane. Reduce the final volume to 0.5mL using nitrogen. Place mixture onto the top of a washed Florisil® cartridge, and elute it with 9mL of hexane:acetone (90:10) v/v. Use two additional 1mL hexane rinses to ensure quantitative transfer of standard from the Florisil® tube. Reduce the final volume to 1mL using nitrogen, and analyze the solution by GC/electron capture detector (ECD) using at least one of the GC columns specified for sample analysis. Determine the recovery and percent recovery for each analyte. The check sample must be analyzed on a GC/ECD and meet the initial calibration and calibration verification technical acceptance criteria. The Florisil® lot is acceptable if all pesticides are recovered at 80% to 120%, if the recovery of 2,4,5-trichlorophenol is less than 5%, and if no peaks interfering with the target analytes are detected.

Tips for Better Results

- *1g tubes will give the most consistent results regardless of final sample volume.*
- *Flow rate during elution should be either dropwise or gravity feed (no vacuum). This will reduce trichlorophenol breakthrough.*

Procedure for Sample Cleanup Using Florisil® SPE Tubes

1. Attach the vacuum manifold (cat.# 26077 or 26080) to a vacuum pump, install a trap between the manifold and the vacuum source. Adjust the vacuum pressure in the manifold to <20" Hg. Place one Florisil® tube onto the vacuum manifold for each sample extract.

2. Prior to cleanup of samples, the tubes must be washed with hexane:acetone (90:10). This is accomplished by placing the tube on the vacuum manifold, pulling a vacuum, and passing at least 5mL of the hexane:acetone solution through the tube. While the tubes are being washed, adjust the vacuum applied to each tube so that the flow rate through each cartridge is approximately equal. Do not allow the cartridges to dry after they have been washed.

3. After the tubes on the manifold are washed, release the vacuum and place a rack containing labeled 10mL collection vessels inside the manifold.

4. After the collection vessels are in place, restore the vacuum to the manifold. Transfer a volume of extract equal to the required final volume (1 or 2mL)* from each sample, blank or matrix spike to the top frit of the appropriate Florisil® tube. Use a syringe or volumetric pipette to transfer the extract to the cleanup tube.

5. Elute the pesticides and PCBs in the extract concentrates through the column with 8mL of hexane:acetone (90:10) and collect into the 10mL collection vessels that are held in the vacuum manifold rack.

6. Transfer the elute in each collection vessel to a clean, appropriate vessel for nitrogen blowdown. Perform two additional 1mL hexane rinses to ensure quantitative transfer of the tube elute.

7. Adjust the extract to the final volume (1 or 2mL) by using either nitrogen blowdown or a micro Snyder column. Measure the final volume with a syringe or by transferring the extract to a volumetric flask. The extract is ready for GC/ECD analysis.

**This volume must equal the final volume after Florisil® cleanup.*

environmental

Product Listing

Rtx®-CLPesticides Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.25	-60 to 310/330°C	11120	11123
0.32mm	0.50	-60 to 310/330°C	11136	11139
0.53mm	0.50	-60 to 310/330°C	11137	11140

Rtx®-CLPesticides2 Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.20	-60 to 310/330°C	11320	11323
0.32mm	0.25	-60 to 310/330°C	11321	11324
0.53mm	0.42	-60 to 310/330°C	11337	11340

Resprep™ SPE Cartridges

(All cartridges are polypropylene and have polyethylene frits unless otherwise noted):

Florisil®			
3mL	500mg	cat.# 24031	50-pk.
3mL†	500mg	cat.# 24032*	50-pk.
6mL	1000mg	cat.# 24034	30-pk.
6mL†	500mg	cat.# 26086**	30-pk.
6mL†	1000mg	cat.# 26085**	30-pk.

†These cartridges are specified in the CLP Method.
**Stainless steel frits*
***Glass cartridges with Teflon® frits.*

Resprep™ 12- & 24-Port Tube Manifold

Complete manifolds include glass basin with built-in vacuum regulator, polypropylene top plate with 12 or 24 individual control valves, 12- or 24-position collection rack, and 12 or 24 Teflon® sample guides.

Complete Resprep™-12 Port Manifold: cat.# 26077

Complete Resprep™-24 Port Manifold: cat.# 26080

Florisil® Cartridge Check Mix

1000µg/mL in acetone, 1mL/ampul

	Each	5-pk.	10-pk.
	32017	32017-510	
w/data pack	32017-500	32017-520	32117

RESTEK ISO 9001 REGISTERED

Environmental

Applications note

cat.# 59563

Extraction of Paraquat & Diquat from Water Using Resprep™-C8–47 Extraction Disks

Please note: **This method is based on the sample extraction procedure from Section 11.3 of EPA Method 549.1, but is not intended to be a replacement or substitute for the official EPA Method 549.1 procedure. Please refer directly to the published EPA method for additional information for the preparation of samples to be analyzed according to EPA Method 549.1.**

EPA methods are available from NTIS (National Technical Information Service), U.S. Department of Commerce, Springfield, VA 22161, phone: (703) 487-4650.

EPA methods are available from the National Technical Information Service: 703-487-4650

Solution Preparation

The following reagent solutions are used in the extraction procedure —

Conditioning Solution A: Dissolve 0.500gm of cetyl trimethyl ammonium bromide and 5mL of concentrated ammonium hydroxide in 500mL of deionized water and dilute to 1000mL in a volumetric flask.

Conditioning Solution B: Dissolve 10.0gm of 1-hexanesulfonic acid (sodium salt) and 10mL of concentrated ammonium hydroxide in 250mL of deionized water and dilute to 500mL in a volumetric flask.

Sodium Hydroxide Solution (10% w/v): Dissolve 50gm of sodium hydroxide in 400mL of deionized water and dilute to 500mL in a volumetric flask.

Hydrochloric Acid Solution (10% v/v): Add 50mL of concentrated hydrochloric acid to 400mL of deionized water and dilute to 500mL in a volumetric flask.

Disk Eluting Solution: Add 13.5mL of orthophosphoric acid and 10.3mL of diethylamine to 500mL of deionized water and dilute to 1000mL in a volumetric flask.

Ion-pair Concentrate: Dissolve 3.75gm of 1-hexanesulfonic acid in 15mL of disk eluting solution and dilute to 25mL with disk eluting solution in a volumetric flask.

Sample Treatment

Allow a 250mL water sample to equilibrate to room temperature in a silanized amber glass bottle (or an amber polyvinyl chloride bottle). For QA/QC samples, spike with 100µg/L of each analyte. Adjust sample pH to 10.5 ± 0.2 with sodium hydoxide solution (10% w/v) or hydrochloric acid solution (10% v/v). Proceed with the extraction immediately following sample pH adjustment.

Apparatus Assembly

Assemble the Diskcover™-47mm apparatus, using the Teflon® reservior. Teflon® parts should be used to minimize the risk of interferences. If using a glassware apparatus, silanization of the apparatus may provide improved recoveries. Place the Resprep™-C8 disk on the filter support, with the WRINKLED SIDE UP.

Disk Conditioning

Add the following solvents to the disk in the sequence indicated:

Apply low vacuum (1-2 in. Hg, 3-7 kPa) immediately upon addition of the first solvent and throughout the entire conditioning sequence. Do not allow any air to pass through the disk or to reach the top surface of the disk during the conditioning process.

Note: It is preferable to add the next solvent directly to a layer of the previous solvent on the disk rather than to allow any air to contact the disk's surface.

1. Add 10 mL methanol to the disk and immediately apply low vacuum (1-2 in. Hg, 3-7 kPa). Draw through until the top surface of the methanol is just above the surface of the disk.
2. Add two 10 mL aliquots of deionized water directly to the liquid on the surface of the disk. Continue to draw through under low vacuum until the top surface of the water is just

above the surface of the disk.
3. Add 10 mL of Conditioning Solution A directly to the water on the surface of the disk. Continue to draw through under low vacuum until the top surface of the liquid is just above the surface of the disk.
4. Add two 10 mL aliquots of deionized water directly to the liquid on the surface of the disk. Continue to draw through under low vacuum until the top surface of the water is just above the surface of the disk.
5. Add 10 mL of Conditioning Solution B directly to the water on the surface of the disk. Continue to draw through under low vacuum until the top surface of the liquid is just above the surface of the disk.

Sample Addition

Pour the 250 mL sample into the apparatus, adding it directly to the layer of liquid left on the disk from the conditioning step. Increase the vacuum to approximately 10 in. Hg (35 kPa) for a flow rate of approximately 100 mL per minute. Once the sample addition has begun, the disk must not go dry until the entire sample has been processed.

Analyte Elution

Release system vacuum. Insert a silanized glass sample collection tube. Reassemble the apparatus. Add approximately 1 mL methanol to the disk, exercising care to cover the entire top surface of the disk. Immediately add 4 mL of disk eluting solution to the disk, and draw the solvent through the disk at 1-2 in. Hg (3-7 kPa) until the top surface of the liquid is just above the surface of the disk. Add an additional 4 mL of disk eluting solution directly to the liquid on the disk, and draw completely through the disk.

Final Analysis

Add 100 µL of ion-pair concentrate. Adjust final sample volume as necessary with elution solvent. Analyze 100 µL by HPLC using a Restek Pinnacle ODS (Part # 9114565) column with UV detection at 257 nm and 308 nm.

Accuracy and Precision Data from Four Determinations of Method 549.1 Analytes (Paraquat and Diquat) using Resprep™ C8 Disk Extraction and Ion-Pair HPLC Analysis with UV Detection.

	Paraquat	Diquat
Sample #1	95.5	88.6
Sample #2	97.0	89.7
Sample #3	98.4	89.2
Sample #4	93.9	85.5
Average	96.2	88.3
%RSD	1.8	1.9

Concentration = 200 ug/L of each analyte

Questions? Call Restek's Technical Service staff at 800-356-1688, ext. 4

Environmental

Applications note

cat.# 59564

Extraction of PCBs using Resprep™-C18-47 SPE disks and Resprep™-6D

Use Resprep™-C18-47 SPE disks and Resprep™-6D to extract polychlorinated biphenyls (PCBs)

Sample Pretreatment

Allow 1 liter of deionized water to equilibrate to room temperature. Adjust sample pH to less than 2 with 6N HCl. Add 5mL of methanol and mix thoroughly. For QA/QC samples, spike with 1mL of a 5ug/mL solution for a final concentration in the water of 5ppb.

Apparatus Assembly

Assemble the Diskcover™-47mm with reservoir, making sure the Teflon® o-ring is in place. Also be sure to place the C18 disk in the Diskcover™-47 wrinkled side up. Assemble the Resprep™-6D as detailed in the instruction sheet, along with the vacuum pump, vacuum line, and vacuum trap.

Disk Precleaning

Add 10mL of methylene chloride to the disk's top surface and immediately draw through under vacuum at 15 in. Hg. Continue to draw vacuum at 15 in. Hg for 5 minutes, removing all solvent.

Disk Conditioning

Add 10mL of methanol to the disk's top surface and let stand for a few minutes. Draw the methanol through the disk until the methanol's top surface is just above the disk. Do not allow any air to pass through the disk or reach the top surface of the disk. Note: It is preferable to leave extra liquid above the disk rather than allow any air to contact the disk surface.

Sample Addition

Pour the sample into the Diskcover™-47 reservoir, adding it directly to the film of methanol left on the disk from the conditioning step. Adjust the vacuum to approximately 25 in. Hg.

Disk Drying

After the entire sample has been processed, draw air through the disk under vacuum to remove any residual water from the disk.

Analyte Elution

Release system vacuum. Insert the collection rack and vessels into the glass chamber, making sure to label each vessel with the corresponding Diskcover™-47. Reassemble the Resprep™-6D. Add 5mL methylene chloride directly to the sample bottle, and gently swirl to rinse all inner surfaces of the bottle. Transfer the methlyene chloride to the disk using a glass pipette and rinse the reservoir sides in the process. Let the solvent stand for three minutes. Draw the solvent through the disk at 5 in. Hg. Repeat the bottle rinse and disk elution once more with a fresh aliquot of methlyene chloride, combining all eluates in the collection tube.

Final Analysis

Remove water from sample eluate by passing through anhydrous sodium sulfate. Concentrate to 1mL, and analyze.

For more information call technical service:
(800) 356-1688, ext. 4

Table 1

Aroclor®	peak #	congener									
	1	8	97.6	95.6	91.1	91.9	91.4	93.4	85.2	92.3	3.6
	2	18	99.0	100.4	92.4	97.0	101.5	106.0	95.2	98.8	4.1
	3	31	94.7	85.3	82.8	88.4	88.5	98.6	95.4	90.5	5.4
	4	52,69	97.3	85.9	86.2	96.1	91.3	100.5	102.0	94.2	6.0
	5	44	93.0	89.8	92.7	97.2	93.6	103.7	102.7	96.1	4.9
	1	18	112.0	109.3	113.6	94.0	96.8	102.8	89.0	102.5	8.8
	2	8	103.6	104.8	106.0	87.4	90.8	97.5	81.6	96.0	8.8
	3	31	100.6	98.7	99.9	78.7	83.0	95.0	76.3	90.3	9.8
	4	44	101.7	103.5	103.0	87.1	90.0	98.2	81.6	95.0	8.1
	5	56,92	93.5	97.3	95.6	76.4	83.0	91.7	73.8	87.3	8.8
	1	31	81.7	92.0	86.3	88.3	81.9	79.2	82.7	84.6	4.1
	2	48	79.7	89.3	87.4	96.2	83.5	85.9	82.1	86.3	5.0
	3	41	81.1	88.6	84.8	88.5	81.9	80.3	82.9	84.0	3.2
	4	70	4.1	90.3	87.6	92.8	87.7	84.4	85.0	87.4	3.0
	5	56,92	82.4	90.3	86.5	91.5	86.2	84.9	84.6	86.6	3.0
	6	110,77,154	84.8	90.9	84.5	94.0	78.8	72.4	81.0	83.8	6.7
	1	52,69	99.8	90.9	79.4	96.4	100.9	87.0	98.3	93.2	7.3
	2	95,66	95.2	89.2	79.9	91.9	99.2	80.0	52.1	83.9	14.6
	3	110,77,154	93.6	98.5	88.4	95.9	110.2	79.4	45.2	87.3	19.3
	4	118	90.1	83.2	73.2	81.6	96.0	64.2	82.4	81.5	9.7
	5	138	87.1	89.2	77.3	85.7	95.6	64.6	109.5	87.0	13.0
	1	149	87.2	97.9	91.3	85.6	79.0	82.5	88.9	87.5	5.7
	2	141	79.6	69.9	78.6	82.8	76.3	78.4	90.64	79.5	5.8
	3	187	87.2	76.1	85.9	90.3	82.3	83.3	95.4	85.8	5.7
	4	180	103.4	94.2	108.1	113.4	102.5	104.6	102.2	104.1	5.4
	5	170	79.1	73.0	84.6	88.1	78.6	81.9	67.7	79.0	6.4

RESTEK environmental

Applications note

Extraction of polynuclear aromatic hydrocarbons (PAHs) using Resprep™-18-47 SPE disks and Resprep™-6D extraction system

Sample Pretreatment

Allow 1L of deionized water to equilibrate to room temperature. Adjust sample pH to less than 2 with 6N HCl. Add 5mL of methanol and mix thoroughly. For QA/QC samples, spike with 1mL of a 2µg/mL solution for a final concentration in the water of 2ppb.

Apparatus Assembly

Assemble the Diskcover™-47 extraction disk holder with reservoir, making sure the Teflon® o-ring is in place. Also be sure to place the C18 disk in the Diskcover™-47 extraction disk holder *wrinkled side up*. Assemble the Resprep™-6D extraction system as detailed in its instruction sheet, along with the vacuum pump, vacuum line, and vacuum trap.

Disk Precleaning

Add 10mL of methylene chloride to the top surface of the disk and immediately draw it through under vacuum at 15 in. Hg. Continue to draw vacuum at 15 in. Hg for 5 minutes, removing all solvent.

Disk Conditioning

Add 10mL of methanol to the top surface of the disk and let stand for a few minutes. Draw the methanol through the disk until the methanol's top surface is just above the disk. **Do not allow any air to pass through the disk or reach the top surface of the disk.** Note: It is preferable to leave extra liquid above the disk rather than allow any air to contact the disk surface.

Sample Addition

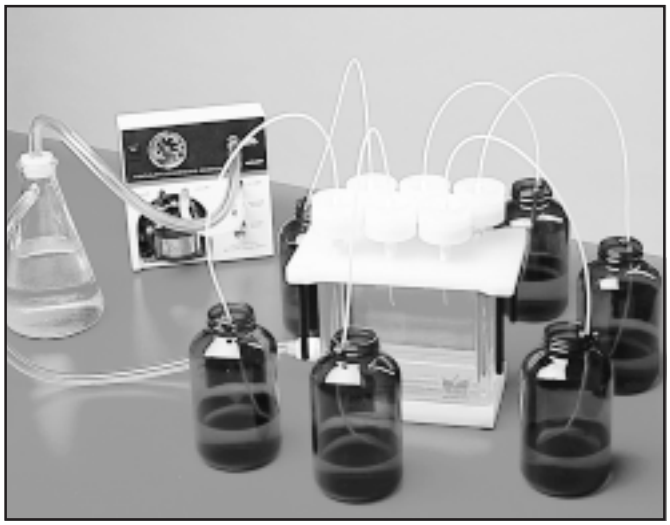
Pour the sample into the Diskcover™-47 reservoir, adding it directly to the film of methanol left on the disk from the conditioning step. Adjust the vacuum to approximately 25 in. Hg.

Disk Drying

After the entire sample has been processed, draw air through the disk under vacuum to remove any residual water from the disk.

Analyte Elution

Release system vacuum. Insert the collection rack and vessels into the glass chamber, making sure to label each vessel with the corresponding Diskcover™-47. Reassemble the Resprep™-6D extraction system. Add 5mL methylene chloride directly to the sample bottle, and gently swirl to rinse all inner surfaces of the bottle. Transfer the methlyene chloride to the disk using a glass pipette and rinse the reservoir sides in the process. Let the solvent stand for three minutes. Draw the solvent through the disk at 5 in. Hg. Repeat the bottle rinse and disk elution once more with a fresh aliquot of methlyene chloride, combining all eluates in the collection tube.



Resprep™ sample preparation products allow complete, clean sample extraction for more accurate PAH analyses.

environmental

Table I

Accuracy/precision data from seven determinations of PAHs in distilled water at 2µg/L using the Resprep™ -C18-47 glass fiber extraction disk.

Compounds	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Average	SD
naphthalene	65.7	78.8	73.7	67.7	73.5	80.8	83.0	74.7	6.5
2-methylnaphthalene	71.9	88.0	84.3	77.3	86.3	97.5	91.6	85.3	8.6
1-methylnaphthalene	75.2	90.9	86.1	81.2	87.2	99.5	96.2	88.0	8.4
biphenyl	77.1	95.0	90.5	84.1	91.5	103.2	102.1	91.9	9.3
2,6-dimethylnaphthalene	69.9	84.3	85.3	80.1	82.9	90.2	86.4	82.7	6.5
acenaphthylene	70.5	98.4	90.8	76.2	90.6	100.6	99.7	89.5	11.9
acenaphthene	83.5	102.6	94.9	90.4	97.2	104.3	106.2	97.0	8.2
2,3,6-trimethylnaphthalene	71.2	89.7	85.7	82.6	88.8	84.9	79.5	83.2	6.3
fluorene	83.8	109.0	106.8	95.6	100.9	107.4	107.3	101.5	9.1
phenanthrene	91.7	105.4	110.2	106.6	108.6	108.3	111.9	106.1	6.7
anthracene	81.5	102.0	89.3	77.7	79.4	94.2	98.6	89.0	9.7
2-methylphenanthrene	91.5	111.1	112.5	99.2	109.9	104.1	110.9	105.6	7.8
1-methylphenanthrene	98.3	112.7	112.0	104.6	108.5	107.1	110.1	107.6	5.0
3,6-dimethylphenanthrene	88.7	108.2	105.0	102.4	104.9	103.5	109.2	103.1	6.8
fluoranthene	94.3	112.9	114.6	106.7	111.0	106.6	115.2	108.8	7.3
pyrene	97.7	113.4	112.4	103.9	110.4	107.5	117.8	109.0	6.7
2,3-benzofluorene	101.1	108.3	100.7	91.1	96.3	100.2	106.8	100.6	5.9
benzo(a)anthracene	97.6	109.2	101.6	89.6	94.0	101.1	110.6	100.5	7.6
chyrsene	106.9	110.5	103.8	93.8	97.7	104.1	112.7	104.2	6.7
benzo(b)fluoranthrene	95.0	107.1	109.4	99.2	97.4	100.5	116.1	103.5	7.6
benzo(k)fluoranthrene	106.8	107.1	108.4	96.1	99.8	99.7	110.0	104.0	5.3
benzo(e)pyrene	101.3	106.2	106.6	98.8	103.4	101.1	115.9	104.8	5.7
benzo(a)pyrene	101.4	107.2	100.0	88.2	90.4	99.2	105.2	98.8	7.1
perylene	100.9	109.2	97.3	86.1	90.2	96.8	105.7	98.0	8.1
9,10-diphenylanthracene	111.4	119.7	112.0	94.6	98.0	106.0	104.9	106.7	8.6
indeno(1,2,3-cd)pyrene	110.9	119.7	113.3	102.0	105.8	110.3	115.4	111.1	5.9
dibenzo(a,h)anthracene	118.3	125.5	112.4	94.4	107.3	108.3	115.6	111.7	9.8
benzo(g,h,i)perylene	123.4	129.5	119.8	103.2	113.4	113.7	121.3	117.8	8.5
Surrogate Standards									
naphthalene-d8	65.5	83.0	73.8	65.5	75.4	80.2	79.8	74.7	7.0
acenaphthene-d10	86.2	103.6	96.4	87.8	102.6	110.7	106.1	99.1	9.3
phenanthrene-d10	101.7	115.8	122.9	110.8	118.1	129.3	122.5	117.3	9.0
chrysene-d12	125.7	136.5	122.7	105.2	124.0	150.4	170.0	133.5	21.2
perylene-d12	109.7	118.0	105.7	89.1	100.2	105.0	119.4	106.7	10.4

Petrochemical

Applications note

cat.# 59568

Restek's Rtx®-1PONA Column for the Analysis of Petroleum Products

Gasoline is a complex mixture containing hundreds of individual hydrocarbons including alkanes (paraffins), alkenes (olefins), cyclic alkanes (naphthenes), and aromatics. Using high resolution gas chromatography (HRGC), it is possible to resolve and identify over two hundred individual components in a single analysis. Once the hydrocarbons are identified and quantified, the results can be reported in various ways, including: detailed hydrocarbon analysis (DHA), hydrocarbon type analysis, and special calculations such as vapor pressure and octane number. Because the task of calibrating hundreds of peaks is extremely time consuming, committees such as the American Society of Standards and Materials (ASTM) and the Canadian General Standards Board (CGSB) have developed standardized methodology for detailed hydrocarbon analysis[1,2]. These methods specify a 100-meter column which must be reproducible if laboratories are to obtain accurate results. The Restek Rtx®-1PONA column meets or exceeds the demanding resolution and retention time requirements specified in these methods.

An example of HRGC analysis of unleaded gasoline using the Rtx®-1PONA column appears in **Figure 1**. The detailed report obtained from this analysis contains over 200 individual peaks so identification of each component can be very challenging. Since flame ionization detection is specified, identification of the individual components is practical only if the retention times and separations are comparable to those published in the ASTM or CGSB methods. In order to meet these demanding resolution and retention criteria, Restek has developed unique

To meet demanding resolution and retention criteria, Restek has developed unique quality control tests and specifications for the Rtx®-1PONA column.

Run Conditions for Figures 1 & 2

100m, 0.25mm ID 0.50µm Rtx®-1PONA
0.5µl split injection of unleaded gasoline.

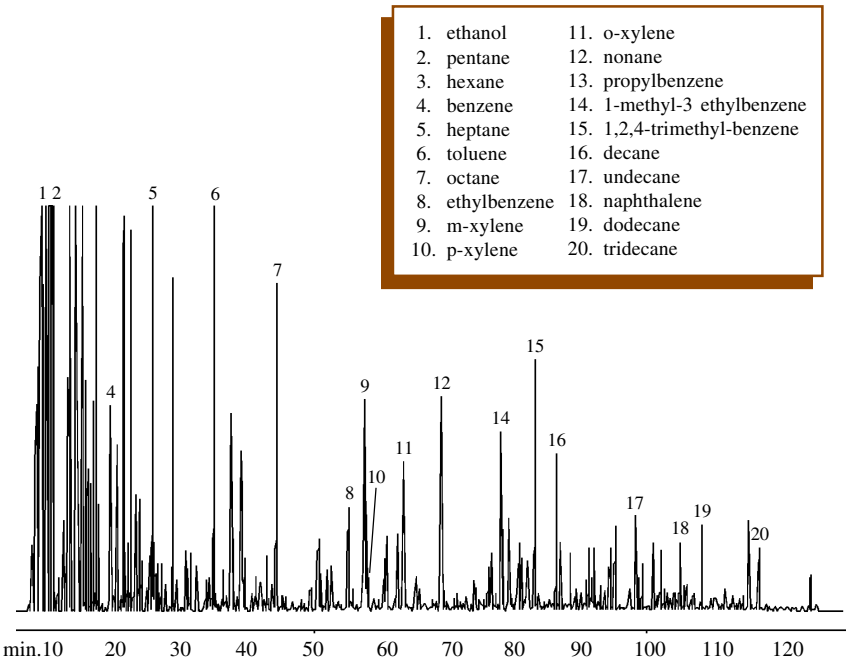
Oven temp.: 35°C (hold 13 min.) to 45°C @ 10°C/min. (hold 15 min.) to 60°C @ 1°C/min. (hold 15 min.) to 200°C @ 1.9°C/min. (hold 5 min.).
Inj./det. temp.: 250°C
Carrier gas: helium
Linear velocity: 24cm/sec. set @ 35°C
FID sensitivity: 4 x 10^{-12} AFS
Split ratio: 100:1

Product Listing

Rtx®-1PONA: (cat.# 10195)
100m, 0.25mm ID, 0.5µm

Figure 1

A 100 meter Rtx®-1PONA column resolves hundreds of individual hydrocarbons in gasoline.



quality control tests and specifications for the Rtx®-1PONA column. The measured values for retention (k), efficiency (n) and stationary phase selectivity (RI) are controlled so that each column exceeds the requirements of the ASTM and CGSB methods. These requirements include separating several "critical pairs" of closely eluting peaks of different hydrocarbon classes. **Figure 2** illustrates three of these critical separations obtained from the analysis of gasoline, without the need for cryogenic cooling.

Detailed hydrocarbon analysis provides a great deal of information regarding the chemical composition of gasoline and similar petroleum products. This information is useful in evaluating the performance of a refinery process or to calculate the chemical "value" of a particular hydrocarbon stream for chemical production or gasoline blending. It is possible to summarize the detailed chromatographic report into a hydrocarbon type analysis utilizing a computer program and a table listing the carbon number and type of each calibrated peak. **Table 1** is an example of a PONA report obtained from an unleaded gasoline using a BASIC program written for a programmable integrator. It is also possible to develop correlations for other important properties such as vapor pressure and octane number from the compositional analysis of gasoline and naphtha[3]. A 100-meter column is also specified for petrochemical analyses[4] requiring a high degree of resolution, where specialized stationary phases or column switching systems do not achieve the desired separations.

The Rtx®-1PONA column is an excellent choice for separation of complex petroleum products such as gasoline and naphtha. Each column is specially tested to exact specifications, meeting the demanding separation and retention criteria of ASTM and CGSB procedures. With HRGC, it is possible to resolve and identify hundreds of individual hydrocarbons. This information can be useful for hydrocarbon type (PONA) analysis or for estimating vapor pressure and octane number.

[1] N.G. Johanson, *Proposed ASTM Test Method for Detailed Hydrocarbon Analysis, Draft 2,* ASTM Committee D2.04.

[2] Canadian General Standards Board, *Methods of Testing Petroleum and Associated Products 3.0*, Method No. 14.3-94 (Feb 94).

[3] R.P. Walsh and J.V. Mortimer, *Hydrocarbon Processing*, p.153 September 1971.

[4] ASTM D5441 Standard Test Method for the Analysis of Methyl Tert-Butyl Ether (MTBE) by Gas Chromatography, *Annual Book of Standards*, Vol. 5.03

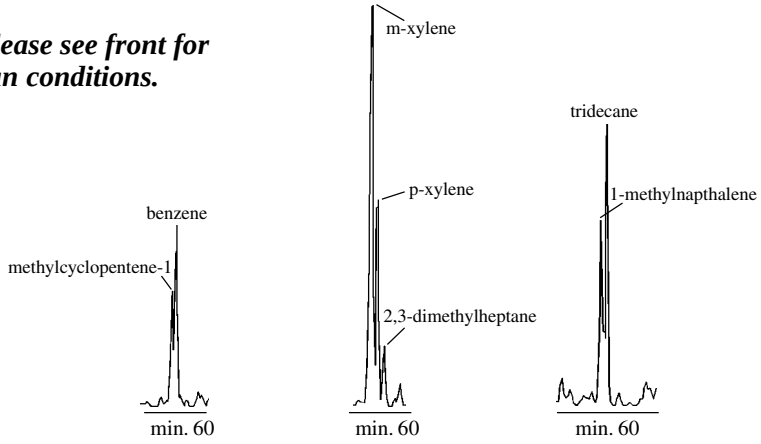
Table 1: *Hydrocarbon Type Report from Detailed Hydrocarbon Analysis*

C#	P (Wt%)	I (Wt%)	O (Wt%)	N (Wt%)	A (Wt%)	X (Wt%)
2	0	0	0	0	0	3.48
3	0.07	0	0	0	0	0.23
4	0.24	0.2	0.83	0	0	0.21
5	1.8	8.77	8.76	0.12	0	0
6	1.84	4.93	4.21	1.88	0.64	0
7	2.14	4.31	2.93	3.95	2.66	0.01
8	2.42	4.24	0.7	3.85	5.31	0.06
9	1.72	3.86	0.36	3.19	5.27	0.15
10	0.88	2.61	0.04	0.62	4.72	0.36
11	0.48	0.47	0	0.17	1.63	0.12
12	0.43	0.61	0	0.02	0.56	0.29
13	0.3	0.34	0	0	0	0
Total	12.34	30.34	17.83	13.82	20.78	4.9

Figure 2

Resolution of several critical pairs of hydrocarbons.

Please see front for run conditions.



RESTEK

ISO 9001 REGISTERED

Petrochemical

Applications note

cat.# 59570

Rtx®-1: A New Bonded Packed Column for Simulated Distillation

- Rtx®-1 bonded packed column requires minimal conditioning.
- Meets or exceeds all specifications of ASTM D2887 and D3710.
- Stable baseline to 350°C and repeatable RT's "right from the box".
- Deactivated Silcosteel® tubing and Silcoport™ packing for high inertness.
- Column lifetime superior to existing Sim Dist columns.

Simulated Distillation (Sim Dist), according to ASTM test methods D2887 or D3710 can be performed using either packed or capillary columns. Advantages of capillary columns are the columns are preconditioned so they can be used after only minimal conditioning, and the bonded stationary phases exhibit stable baselines and retention times. There are many laboratories currently using packed columns which would like to take advantage of bonded phases but do not have GC equipment that can be easily converted for use with capillary columns.

Restek's Rtx®-1 Sim Dist column is the first in a new generation of bonded packed columns having superior inertness and stability compared to conventional packed columns. These improvements are obtained by preparing the columns with Silcosteel® tubing and bonding the Rtx®-1 stationary phase to a highly deactivated Silcoport™ support. The column dimensions and packing (1/8" Silcosteel® with 10% Rtx®-1 on Silcoport™) are designed to exceed all requirements specified in ASTM Test Methods D2887 and D3710.

Bonded stationary phases require minimal conditioning and give stable baselines and retention times "right from the box."

Simulated distillation is a gas chromatographic procedure which differs from typical GC analysis requiring peak resolution and integration. The sample is analyzed using a linear temperature program so that the retention time of the hydrocarbons are proportional to their boiling points. The sample boiling range distribution is calculated by comparing the sample area and its retention time with that of an alkanes calibration standard. In order for the calibration to be valid for sample analysis, it is crucial that retention times be repeatable until the next calibration is performed. **Figure 1** is an example of the analysis of the Restek D2887 Calibration Mix (cat.# 31222) illustrating the typical pattern obtained for the alkanes under temperature programmed conditions. To demonstrate the stability of the Rtx®-1 column, a series of calibration standards were analyzed after only 30 minutes of conditioning at 350°C.

Table 1 shows the excellent retention time repeatability obtained with the column, indicating the column is suitable for sample analysis after minimal conditioning.

Column bleed is another important consideration for selecting a Sim Dist column. The baseline must be stable and free of any artifacts during the temperature program up to 350°C. Although baseline subtraction is permitted in the method, this compensation will produce errors if the baseline is not consistent. Conventional packed columns require up to 14 hours of conditioning and frequent updating of the baseline compensation run because the stationary phase is not bonded. Rtx®-1 columns, however, exhibit stable

Table I

Retention Time Repeatability for Calibration after only 30 minutes conditioning.

Hydrocarbon	Min Rt	Max Rt	Avg. RT	Stand. Dev.
C_5	0.241	0.243	0.242	0.001
C_6	0.493	0.497	0.495	0.002
C_{10}	5.746	5.765	5.752	0.005
C_{20}	18.482	18.491	18.486	0.004
C_{28}	25.093	25.103	25.098	0.004
C_{40}	32.160	32.171	32.166	0.004
C_{44}	34.316	34.328	34.326	0.007

Questions?
Call Restek's technical service staff at
800-356-1688, ext.4

and reproducible baselines with just 30 minutes of conditioning. This results in fewer baseline blanks and less frequent calibration increasing laboratory productivity.

Rtx®-1 Sim Dist 2887 Packed Columns can also be used for gasoline range simulated distillation.

Simulated distillation of gasoline range hydrocarbons according to ASTM method D3710-93 can also be performed using the Rtx®-1 Sim Dist 2887 Packed Column. **Figure 2** shows the analysis of ASTM D3710 calibration mix with the addition of n-propane, 2-methyl propane, n-butane, n-hexadecane, and n-heptadecane. To achieve baseline separation of n-propane, 2-methyl propane, and n-butane, the GC oven was cooled to -30°C with liquid nitrogen. **Figure 3** shows the analysis of a composite gasoline sample under the same run conditions. Other volatile petroleum fractions such as kerosene and jet fuel can also be analyzed with this column.

Bonded stationary phases extend column lifetime.

The Rtx®-1 stationary phase is bonded to the diatomite particles resulting in an immobilized coating which is resistant to solvents and lower in bleed than conventional packing. Since the packing is preconditioned, there is no need for extended conditioning. Extended conditioning can greatly decrease column lifetime. Since GC systems often have leaks or carrier gas that contains oxygen, it is more likely that conventional columns will be damaged during the conditioning process. **Figure 4a** shows a conventional UCW-982 column after only 170 temperature cycles, demonstrating higher bleed and more tailing than the Rtx®-1 Sim Dist column (**Figure 4b**). Although actual column lifetimes depend upon the system and type of samples analyzed, the bonded stationary phase should result in longer lifetime than its non-bonded equivalent.

Rtx®-1 Sim Dist columns have equivalent polarity to OV-101 and UCW-982.

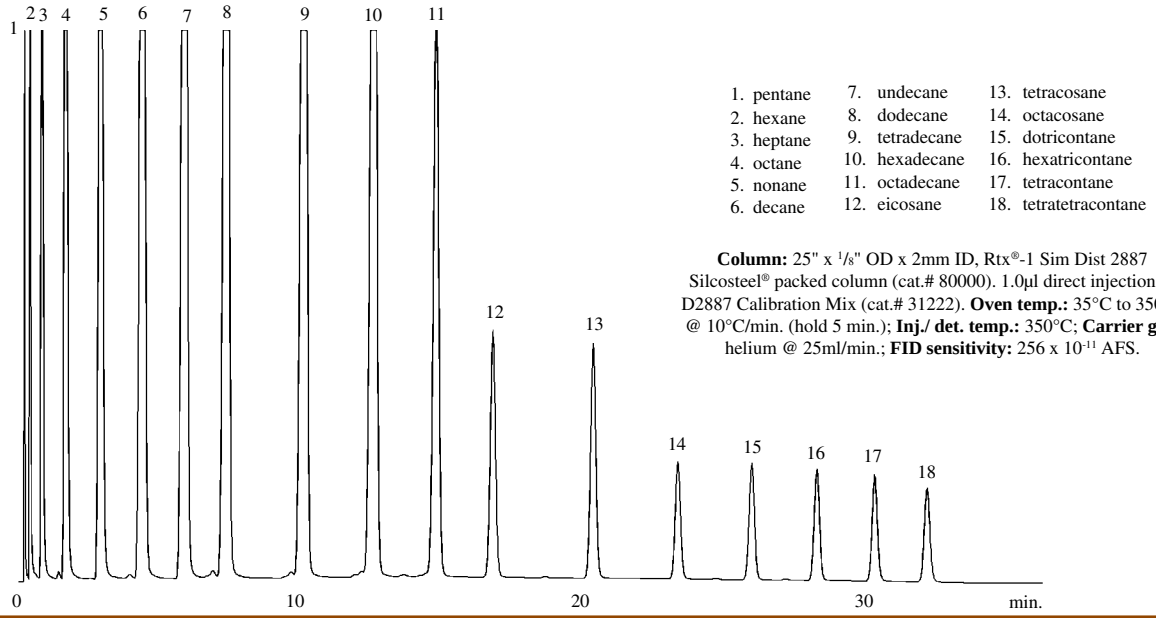
In order for a stationary phase to be acceptable for ASTM methods, the column must not exhibit selective retention for aromatic hydrocarbons compared to aliphatic hydrocarbons. This is an important test because if the polarity of a column is different, the boiling point results will demonstrate a bias, especially for highly aromatic samples. The "polarity" of the bonded Rtx®-1 column was compared with OV-101 and UCW-982, two of the most common stationary phases currently used for simulated distillation. The results of the calculated boiling points for aromatics compared to the published boiling points appear in Table II. All three silicone columns tested are essentially identical in they elute aromatics at a slightly lower temperature than the alkanes. This confirms the polarity of the Rtx®-1 column is equivalent, and the boiling range values obtained will agree with OV-101 and UCW-982 columns.

Rtx®-1 is an excellent choice for Sim Dist using packed columns.

Simulated distillation is one of the most common GC analyses performed in the petroleum laboratory. ASTM test methods D2887 and D3710 can be performed with either packed or capillary columns, but until now the benefits of bonded phases were available only to capillary users. The Rtx®-1 packed column uses a bonded stationary phase which is immobilized on Silcoport™-a specially deactivated support. The columns are prepared using Silcosteel® tubing for inertness unavailable with conventional metal tubing. Rtx®-1 bonded packed columns require minimal conditioning and give stable baselines and retention even after only 30 minutes of operation at 350°C. If your laboratory has been looking for a better Sim Dist analysis, Restek's Rtx®-1 packed columns are the answer.

Figure 1

C5 to C44 calibration analysis after only 30 minutes conditioning.

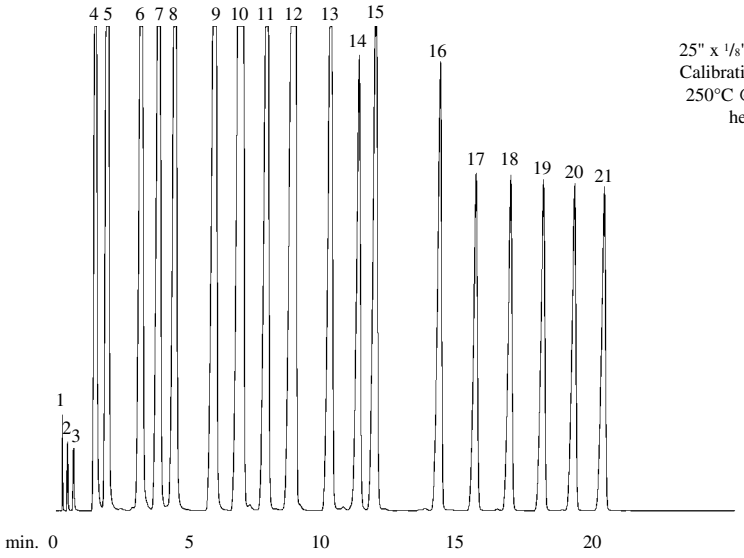


1. pentane
2. hexane
3. heptane
4. octane
5. nonane
6. decane
7. undecane
8. dodecane
9. tetradecane
10. hexadecane
11. octadecane
12. eicosane
13. tetracosane
14. octacosane
15. dotricontane
16. hexatricontane
17. tetracontane
18. tetratetracontane

Column: 25" x 1/8" OD x 2mm ID, Rtx®-1 Sim Dist 2887 Silcosteel® packed column (cat.# 80000). 1.0µl direct injection of D2887 Calibration Mix (cat.# 31222). **Oven temp.:** 35°C to 350°C @ 10°C/min. (hold 5 min.); **Inj./ det. temp.:** 350°C; **Carrier gas:** helium @ 25ml/min.; **FID sensitivity:** 256 x 10^{-11} AFS.

Figure 2

Rtx®-1 Sim Dist 2887 packed columns can also be used for ASTM D-3710 analysis..



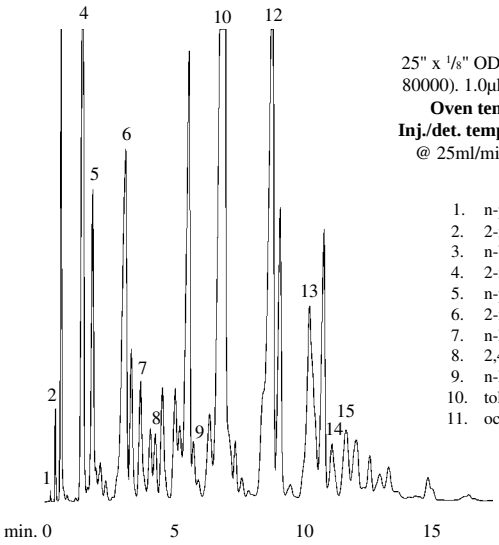
25" x 1/8" OD x 2mm ID Rtx®-1 SimDist 2887 (cat.# 80000). D-3710 Calibration Mix + C3, C4, C16, & C17 added. **Oven temp:** -30°C to 250°C @ 10°C/min.; **Inj./det. temp.:** 250°C / 300°C; **Carrier gas:** helium @ 25ml/min.**; FID sensitivity:** 256 x 10^{-11} AFS.

1. n-propane
2. 2-methyl propane
3. n-butane
4. 2-methylbutane
5. n-pentane
6. 2-methylpentane
7. n-hexane
8. 2,4-dimethylpentane
9. n-heptane
10. toluene
11. octane
12. p-xylene
13. n-propylbenzene
14. n-decane
15. n-butylbenzene
16. n-dodecane
17. n-tridecane
18. n-tetradecane
19. n-pentadecane
20. n-hexadecane
21. n-heptadecane

Other bonded phases are available. For more information, call Restek's technical service staff at **800-356-1688, ext.4.**

Figure 3

Perform simulated distillation of gasoline using the Rtx®-1 Sim Dist 2887 packed columns.



25" x 1/8" OD x 2mm ID Rtx®-1 SimDist 288 (cat.# 80000). 1.0µl direct injection of unleaded gasoline. **Oven temp:** -30°C to 250°C @ 10°C/min.; **Inj./det. temp.:** 250°C/300°C; **Carrier gas:** helium @ 25ml/min; **FID sensitivity:** 256 x 10^{-11} AFS.

1. n-propane
2. 2-methyl propane
3. n-butane
4. 2-methylbutane
5. n-pentane
6. 2-methylpentane
7. n-hexane
8. 2,4-dimethylpentane
9. n-heptane
10. toluene
11. octane
12. p-xylene
13. n-propylbenzene
14. n-decane
15. n-butylbenzene
16. n-dodecane
17. n-tridecane
18. n-tetradecane
19. n-pentadecane
20. n-hexadecane
21. n-heptadecane

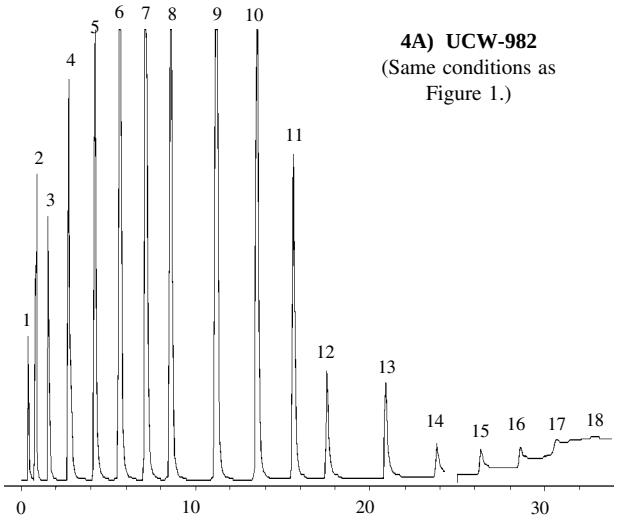
Table II

Comparison of bonded and conventional packed column indicates no polarity differences.

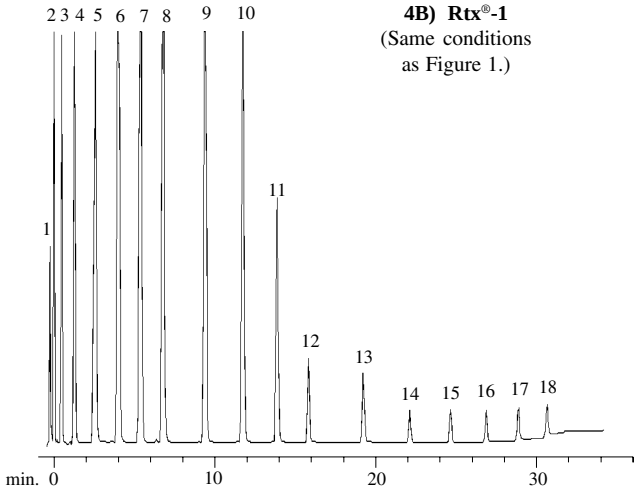
Aromatic Hydrocarbon	Published BP[1] (°C)			
		Rtx®-1	UCW-982	OV-101
benzene	80	81.3	82	80.3
p-xylene	139	138.6	140.2	137.7
naphthalene	218	204.6	206.9	204.3
acenaphthylene	280	252.7	255.6	252.2
anthracene	342	304.1	307.2	303.4
chrysene	447	385.6	389.2	384.9
dibenzo(a,h)anthracene	524	452.3	455.7	450.4

Figure 4

Bonded packed columns exhibit lower bleed and longer lifetimes after 170 temperature cycles.



4A) UCW-982
(Same conditions as Figure 1.)



4B) Rtx®-1
(Same conditions as Figure 1.)

Product List

Rtx®-1 Sim Dist 2887 25" x 1/8" OD x 2mm ID Silcosteel® packed column cat.# 80000

ASTM Petrochemical Mixtures Available

Calibration and column test mixtures are available for ASTM Methods D2887 and D3710. These standards are made with the same quality and care as our environmental standards. Stock products of single ampul and cost-effective 10-packs are available for immediate shipment. Each standard is supplied with a data sheet indicating the exact concentration and a sample chromatogram.

D2887 Column Test Mix

Contain 1% (w/v) ea. of n-hexadecane and n-octadecane dissolved in n-octane

Packaged 1ml/ampul
Cat.# **31221** - single
Cat.# **31321** - 10pk.

D3710 Calibration Mix

Compound	Concentration (% w/w)
2-methylbutane	10
n-pentane	8
2-methylpentane	6
n-hexane	6
2,4-dimethylpentane	6
n-heptane	10
toluene	12
n-octane	5
p-xylene	14
n-propylbenzene	5
n-decane	4
n-butylbenzene	4
n-dodecane	4
n-tridecane	2
n-tetradecane	2
n-pentadecane	2

Packaged 1ml/ampul
Cat.# **31223** - single
Cat.# **31323** - 10pk.

D2887 Calibration Mix

Compound	Concentration (% w/w)
n-decane	12
n-dodecane	12
n-dotriacontane	1
n-eicosane	2
n-heptane	6
n-hexadecane	10
n-hexane	6
n-hexatriacontane	1
n-nonane	8
n-octacosane	1
n-octadecane	5
n-octane	8
n-tetracontane	2
n-tetratetracontane	1
n-undecane	12

Packaged 1ml/ampul
Cat.# **31222** - single
Cat.# **31322** - 10pk.

RESTEK

ISO 9001 REGISTERED

Capillary Columns

Applications note

cat.# 59571

The New Rtx®-Wax Column

Restek's new Rtx®-Wax column has:

- a 20°C minimum operating temperature.
- thermal stability with guaranteed low bleed to 250°C.
- the capability to resolve xylene isomers for BTEX analysis.
- excellent inertness for aldehyde analysis.

Restek's Rtx®-Wax columns are made with a new Crossbond® polyethylene glycol (PEG) stationary phase. This new innovation in polymer technology has produced the most inert and efficient PEG columns currently available. The extended operating temperature range allows the analysis of compounds with a wide volatility range, while the Crossbond® technology ensures low bleed at temperatures as high as 250°C.

The selectivity of the Rtx®-Wax is comparable to other bonded Carbowax® columns*, yielding excellent resolution of compounds ranging from intermediate to high polarity. This exceptional inertness and efficiency allows versatility in difficult analyses such as isomeric separations, aldehydes, and alcohols.

Reduced Minimum Operating Temperature

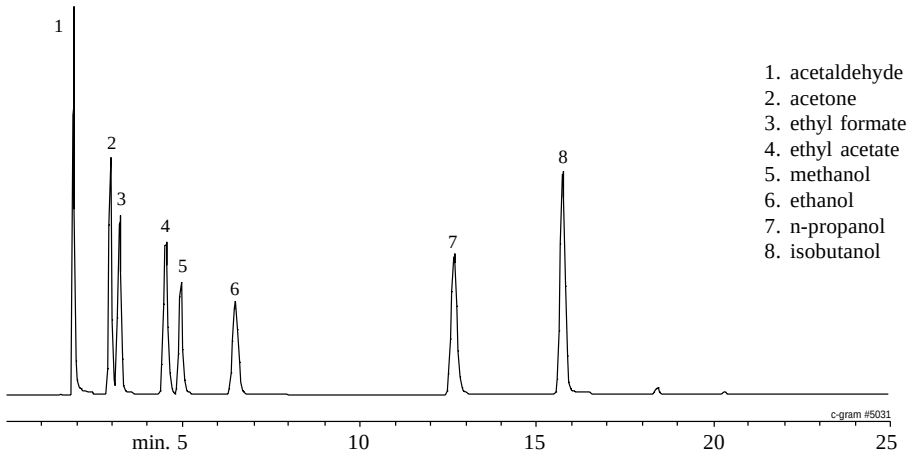
Many PEG columns undergo a solid-liquid phase transition at temperatures below 40°C that results in a loss of efficiency, reduced sample capacity, and poor retention time reproducibility[1]. The new Rtx®-Wax column can effectively operate at 20°C without displaying any adverse effects. This is especially advantageous for applications involving purge & trap and headspace analyses where volatile components must be cold-trapped onto the column. Figures 1 and 2 demonstrate the difference in PEG column performance at 20°C. **Figure 1** is a chromatogram of eight volatile components on the Rtx®-Wax column. **Figure 2** (on the next page) shows the same analysis and conditions on a Stabilwax® column. The Rtx®-Wax column provides better column efficiency and resolution of the analytes demonstrating its superior suitability for low temperature analyses.

Questions?
Call our technical service staff at
800-356-1688, ext. 4

** Selectivity data available upon request.*

Figure 1

The Rtx®-Wax maintains column efficiency at 20°C allowing separation of volatile components.



30m, 0.53mm ID, 1.0µm Rtx-Wax (cat.# 12455). 0.1µl split injection of volatile solvents.
Oven temp.: 20°C (hold 10 min.) to 100°C @ 5°C/min.; **Inj./det. temp.:** 200°C;
Carrier gas: helium; **Linear velocity:** 40cm/sec. set @ 20°C; **FID sensitivity:** 8 x 10^{-11} AFS; **Split ratio:** 10:1

Thermal Stability to 250°C

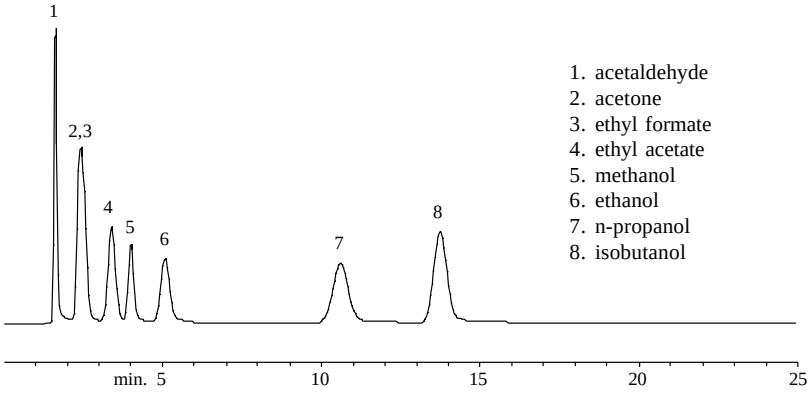
Crosslinking or bonding of the PEG stationary phase minimizes bleed to ensure accurate identification and quantitation of higher-boiling components. As a result, minimal detector contamination and extended column lifetime are observed. Only a small baseline rise is evident at the maximum operating temperature for the stationary phase. In comparison to similar PEGs from other manufacturers, the Rtx®-Wax exhibits the lowest bleed profile at 250°C (see **Figure 3**).

Resolution of BTEX isomers

The new Rtx®-Wax column offers the same selectivity as other Carbowax® columns for isomers of substituted aromatics. This is useful for BTEX analyses that require the specific quantitation of the individual xylene isomers. **Figure 4** illustrates that all components in the BTEX analysis (including meta-, para-, and ortho-xylene) are completely resolved in just 13 minutes.

Figure 2

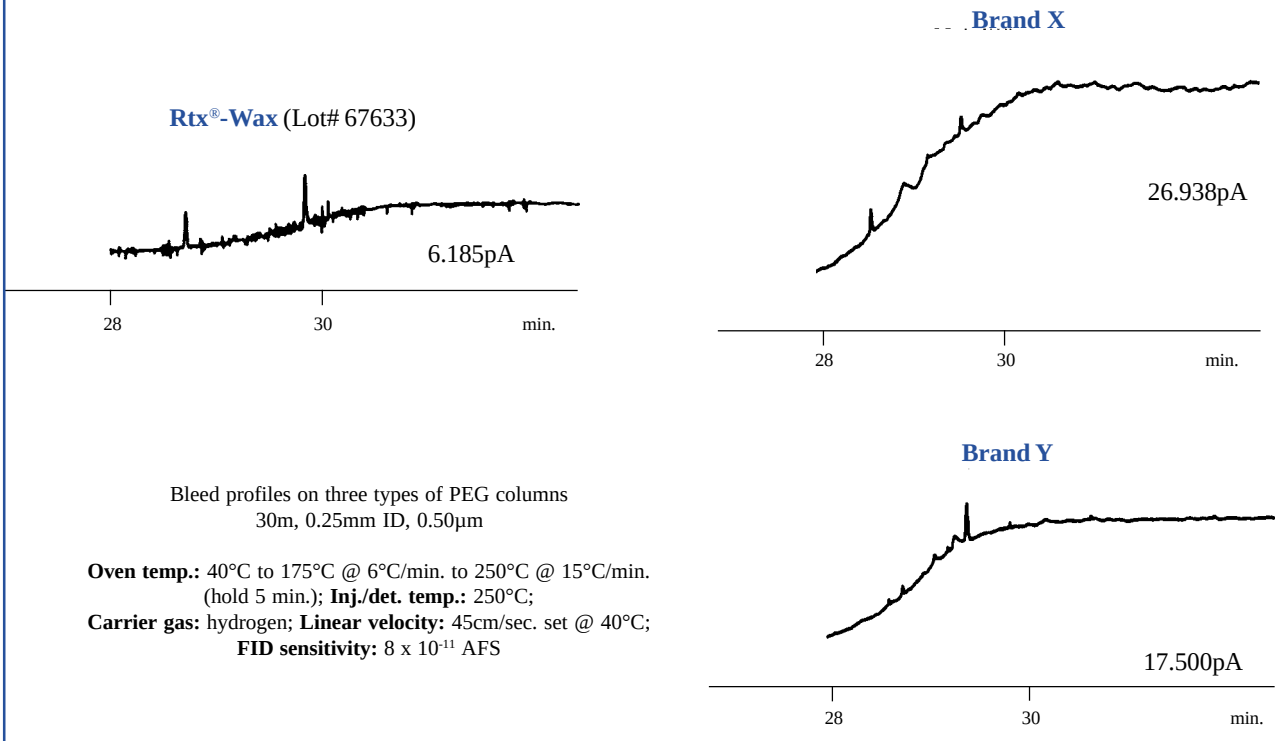
The Stabilwax® column demonstrates a phase transition below 40°C, resulting in decreased efficiency and separation power.



30m, 053mm ID, 1.0µm Stabilwax® (cat.# 10655). 0.1µl split injection of volatile solvents.
On-column conc.: 60ng; **Oven temp.:** 20°C (hold 10 min.) to 100°C @ 5°C/min.;
Inj./det. temp.: 200°C; **Carrier gas:** helium; **Linear velocity:** 40cm/sec. set @ 20°C;
FID sensitivity: 8 x 10^{-11} AFS; **Split ratio:** 10:1

Figure 3

Rtx®-Wax exhibits lowest bleed profile at 250°C.



Bleed profiles on three types of PEG columns
30m, 0.25mm ID, 0.50µm

Oven temp.: 40°C to 175°C @ 6°C/min. to 250°C @ 15°C/min. (hold 5 min.); **Inj./det. temp.:** 250°C;
Carrier gas: hydrogen; **Linear velocity:** 45cm/sec. set @ 40°C;
FID sensitivity: 8 x 10^{-11} AFS

Excellent Inertness for Aldehydes

Most PEG columns can effectively analyze alcohols, esters, and acids, but some exhibit peak tailing with aldehydes. Because the stationary phase undergoes an extensive purification procedure, peak tailing in the analysis of a series of aldehydes is non-existent on the Rtx®-Wax column as shown in **Figure 5**.

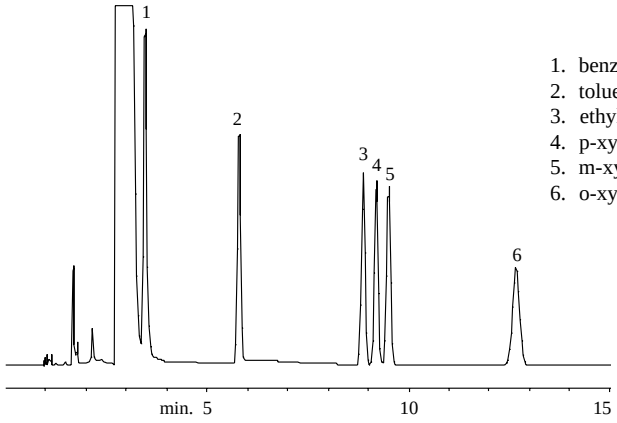
In comparison with other available Carbowax® columns, the Crossbond® Rtx®-Wax column provides an extended operational temperature range, excellent efficiency, selectivity, and superior inertness. These advantages make it the logical choice for all Carbowax® applications.

References

[1] Sandra, P.; David, F.; Turner, K.A.; McNair, H.M.; Brownstein, A.D. "Observations with High-Molecular-Weight Polyethylene Glycol Stationary Phases in Capillary Gas Chromatography," *Journal of Chromatography*, 1989.

Figure 4

The Rtx®-Wax column efficiently resolves all xylene isomers in BTEX analysis.



1. benzene
2. toluene
3. ethylbenzene
4. p-xylene
5. m-xylene
6. o-xylene

30m, 0.53mm ID, 1.0µm Rtx®-Wax (cat.# 12455)
0.25µl direct injection of a BTEX sample. On-column conc.: 50ppm
Oven temp.: 35°C to 75°C @ 2°C/min. (hold 5 min.);
Inj./det. temp.: 200°C; **Carrier gas:** helium;
Linear velocity: 26cm/sec. set @ 35°C; **FID sensitivity:** 8 x 10^{-11} AFS

Figure 5

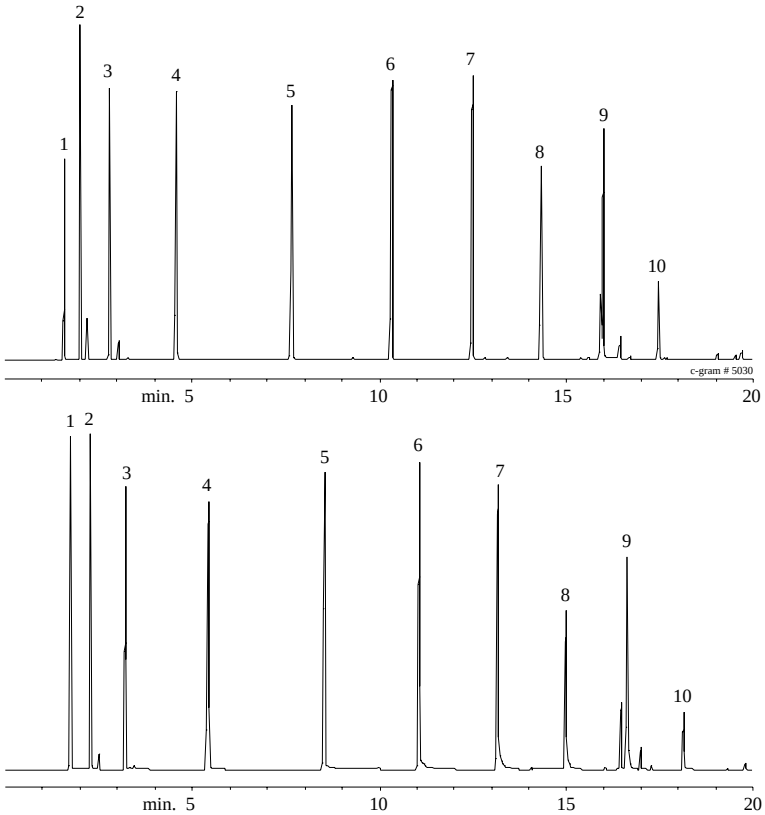
Rtx®-Wax exhibits excellent symmetrical peak shape for aldehydes compared to the Stabilwax® column.

Rtx®-Wax (cat.# 12438)
30m, 0.25mm ID, 0.50µm

1. ethanal
2. propanal
3. butanal
4. pentanal
5. hexanal
6. heptanal
7. octanal
8. nonanal
9. decanal
10. undecanal

Sample: split injection of C2-C11 aldehydes mixture **On-column conc.:** 250ng.
Oven temp.: 40°C (hold 5 min.) to 200°C @ 10°C/min.; **Inj./det. temp.:** 200°C;
Carrier gas: hydrogen; **Linear velocity:** 35cm/sec. set @ 40°C; **FID sensitivity:** 8 x 10^{-11} AFS; **Split ratio:** 100:1

Stabilwax® (cat.# 10638)
30m, 0.25mm ID, 0.50µm



Product Listing

	mm ID	df (µ)	temp. range (°C)	15–meter	30–meter	60–meter
Rtx®-WAX Fused Silica Capillary Columns	0.25	0.10 0.25 0.50	20-250 20-250 20-250	12405 12420 12435	12408 12423 12438	—— 12426 12441
	0.32	0.10 0.25 0.50 1.00	20-250 20-250 20-250 20-240/250	12406 12421 12436 12451	12409 12424 12439 12454	—— 12427 12442 12457
	0.53	0.25 0.50 1.00	20-250 20-250 20-240/250	12422 12437 12452	12425 12440 12455	—— 12443 12458
	mm ID	**df (µ)**	**temp. range (°C)**	**10–meter**	**20–meter**	
	0.10	0.10 0.20	20-250 20-240/250	41601 41603	41602 41604	—— ——

Questions? Call our technical service staff at **800-356-1688, ext. 4**

RESTEK
micro & packed columns

Applicationsnote

Analyze Sulfur Compounds at ppb Levels, Using an Rt-XLSulfur Micropacked GC Column or an Rtx[fi]-1 Thick Film Capillary GC Column

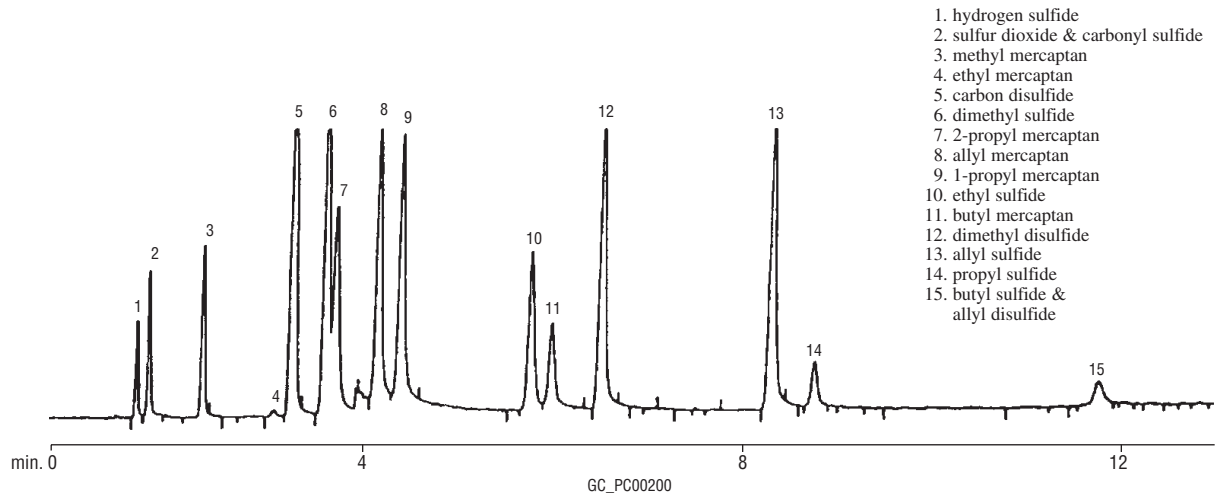
Sulfur compounds in petroleum streams can have detrimental effects on the performance and longevity of the catalysts used in hydrocarbon processing. Furthermore, the toxicity and odor associated with sulfurs is of significant environmental importance. In short, to protect both processing equipment and the environment, ability to quantify sulfur compounds to ppb levels is imperative.

Gas chromatography is the method of choice for the analysis of sulfur compounds. Both packed and capillary GC columns have been successfully used for this application. Although gas chromatographic analysis of the sulfur compounds in petroleum streams is important, this often is a difficult application. With packed columns, the choice of column tubing is critical for accurate determination of sulfur compounds, particularly at low concentrations. Analyses on glass, Teflon®, or stainless steel columns all present distinct problems. Glass columns exhibit poor inertness and lack ruggedness for field or process control use, and results are subject to variability because of column-to-column variation in ID. Teflon® tubing, although more robust than glass, is plagued by three significant problems: 1) shrinkage as the column cools causes back diffusion of oxygen and water into the packing material which, if not addressed, can cause retention times to vary by as much as 15%; 2) oxygen and water diffuse through the tubing wall, significantly decreasing column longevity and creating reproducibility problems; 3) a maximum column temperature limit of only 210ºC makes it impossible to quickly elute high molecular weight sulfur compounds. Without specialized surface passivation, stainless steel columns simply do not offer the inertness needed to monitor active sulfur compounds at ppb levels.

One of the proven approaches for analyzing sulfur compounds by GC is to use a thick film, 100% polydimethylsiloxane Rtx®-1 capillary column. Figure 1 illustrates the analysis of sulfur compounds on a 60-meter x 0.53mm ID x 7µm Rtx®-1 column. The thick film is needed to resolve the volatile sulfur compounds, but makes for long retention times for higher molecular weight

Figure 1

Sulfur compounds on a thick film Rtx®-1 capillary column.

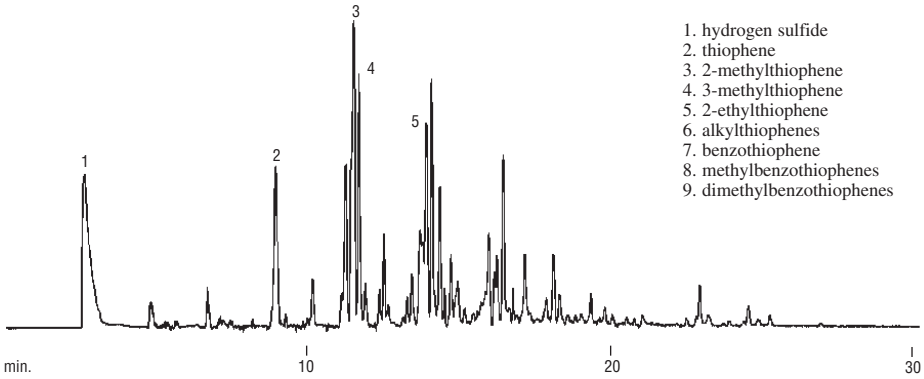


60m, 0.53mm ID, 7.0µm Rtx®-1 (cat.# 10193)
100µL direct injection of sulfur compounds, approximately 10ppm each.

Oven temp.: 50°C to 200°C @ 15°C/min.; **Inj. / det. temp.:** 50°C / 230°C;
Carrier gas: helium; **Linear velocity:** 30cm/sec. set @ 50°C; **Det.:** FPD

Figure 2

Higher weight sulfur compounds on a 30-meter Rtx®-1 column with a 4.0µm phase film.

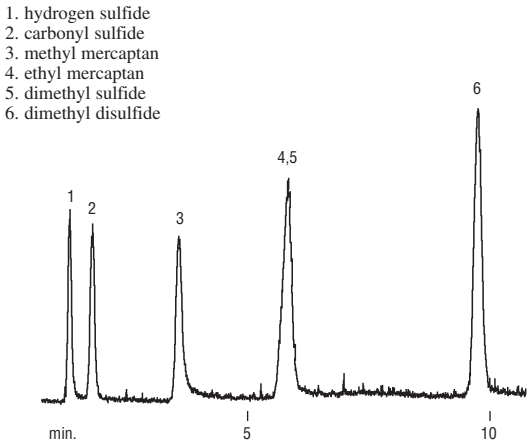


30m, 0.32mm ID, 4.0µm Rtx®-1 (cat.# 10198). 1.0µL split injection of naphtha containing 500ppm total sulfur compounds.

Oven temp.: 35°C to 275°C @ 10°C/min. (hold 5 min.);
Inj./det. temp.: 275°C; **Det.:** atomic emission detector (181nm);
Carrier gas: helium; **Linear velocity:** 24cm/sec. (0.8mL/min.); **Split ratio:** 10:1

Figure 3

An Rt-XLSulfur™ micropacked column exhibits excellent inertness for low ppbv-levels of sulfur compounds.

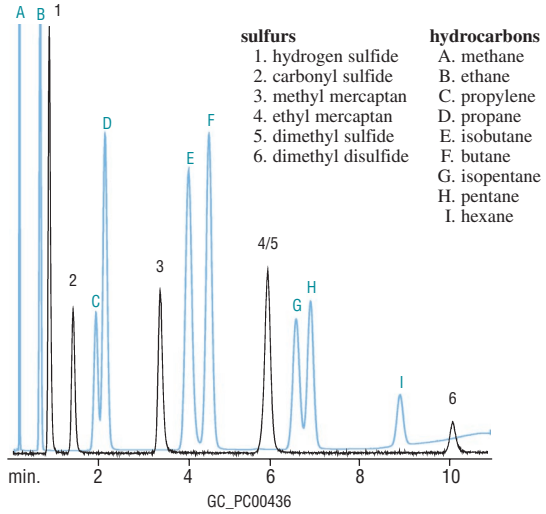


1m, 0.75mm ID Rt-XLSulfur™ micropacked column (cat.# 19806)

Conc.: 1mL of 50ppbv each sulfur compound; **Oven temp.:** 60°C to 230°C @ 15°C/min.; **Carrier gas:** helium; **Flow rate:** 9mL/min.; **Det.:** sulfur chemiluminescence detector (SCD)

Figure 4

Sulfur compounds resolved from C1-C6 hydrocarbons, using an Rt-XLSulfur™ micropacked column.



1m, 0.75mm ID Rt-XLSulfur™ micropacked column (cat.# 19806)

Conc.: 50ppb each analyte; **Oven temp.:** 60°C to 230°C @ 15°C/min.; **Carrier gas:** helium; **Flow rate:** 9mL/min.; **Det.:** SCD/FID

Sulfur standard courtesy of DCG Partnership 1 Ltd., Pearland, TX.

sulfur compounds. Alternatively, a 30-meter, 0.32mm ID, 4µm Rtx®-1 column can be used to analyze higher molecular weight sulfur compounds, such as thiophenes (Figure 2).

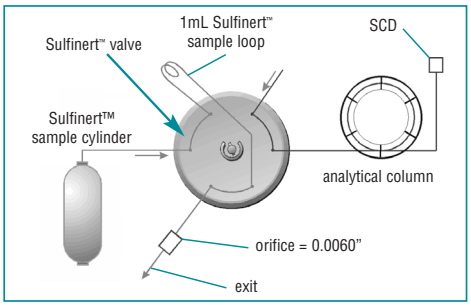
Another excellent approach for analyzing low molecular weight sulfur compounds is the use of micropacked columns. The Rt-XLSulfur™ micropacked column contains a specially deactivated divinylbenzene porous polymer in stainless steel tubing, deactivated through Restek's state-of-the-art Sulfinert™ passivation process. The inertness of both the packing material and the tubing ensure a column that is capable of analyzing active sulfur compounds to 10ppb. Moreover, the Rt-XLSulfur™ micropacked column displays minimal bleed, well within limits necessary for ppb-level sulfur analysis, after a brief conditioning period (<30 minutes). The maximum temperature limit, 310°C, allows rapid elution of the higher molecular weight analytes. This column achieves the critical separation of hydrogen sulfide (H_2S), carbonyl sulfide (COS), and sulfur dioxide (SO_2), as defined in the International Society of Beverage Technologists (ISBT) Procedure 14.0. Figure 3 shows the highly volatile H_2S and COS separated using a 1-meter, 0.75mm ID Rt-XLSulfur™ micropacked column. Additionally, these volatile sulfur compounds are well-retained and well-resolved from the hydrocarbons that could interfere with quantification on some sulfur-specific detectors (Figure 4).

Note that to achieve this high level of sensitivity, every component of the sample pathway must be inert: the porous polymer, the column tubing, the column end fittings, and, additionally, the sample loop and/or inlet sleeve. Sample pathways in the analyses shown in Figures 1 through 4 were passivated using Restek's Sulfinert™ deactivation process. Figure 5 shows a schematic diagram of a system designed to analyze volatile and reactive sulfur compounds. From the Sulfinert™-treated sample cylinder used to collect and store the sample, to the Sulfinert™-treated valve and sample loop used to transfer the sample to the GC system, to either the inert capillary or packed column, Restek offers a complete line of products to ensure consistent and reliable analysis of sulfur compounds.

For more information about the Sulfinert™ passivation technique, request a copy of Restek's brochure on Sulfinert™ coatings (Lit. cat.# 59203).

Figure 5

Analytical system designed to detect losses from a 17ppbv standard.



Product Listing

Rt-XLSulfur™ Micropacked Columns

Other column dimensions can be prepared on a custom basis. Please inquire. Purchase installation kit separately.

OD	ID (mm)	1-Meter	2-Meter
1/16"	1.0mm	19804	19805
0.95mm	0.75mm	19806	19807

Installation Kits

	for 0.75mm ID col.	for 1mm ID col.	for 2mm ID col.
For valve applications	21062	21065	21067
For split applications	21063	—	—
For all Agilent GCs	21064	—	—
For direct injections	—	21066	—

Rtx®-1 Capillary Columns (Fused Silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	15m	30m	60m	75m	105m
0.32mm	3.00	-60 to 280/300°C	10181	10184	10187		10190
	4.00	-60 to 280/300°C		10198			
	5.00	-60 to 260/280°C	10176	10178	10180		
0.45mm	2.55	-60 to 270/290°C				10992	
0.53mm	3.00	-60 to 270/290°C	10182	10185	10188		10189
	5.00	-60 to 270/290°C	10177	10179	10183		10194
	7.00	-60 to 240/260°C	10191	10192	10193		

The maximum temperatures listed are for 15- and 30-meter lengths. Longer lengths may have a slightly reduced maximum temperature.

Sulfinert™ Welded 304 Grade Stainless Steel Tubing

Available in lengths from 5—400 feet. Call for details.

ID	OD	cat.#
0.011" (0.28mm)	0.022" (0.56mm)	22500
0.021" (0.53mm)	0.029" (0.74mm)	22501
0.010" (0.25mm)	1/16" (1.59mm)	22502
0.020" (0.51mm)	1/16" (1.59mm)	22503
0.030" (0.76mm)	1/16" (1.59mm)	22504
0.040" (1.02mm)	1/16" (1.59mm)	22505
0.085" (2.16mm)	1/8" (3.18mm)*	22506
0.210" (5.33mm)	1/4" (6.35mm)*	22507

**0.020" wall thickness*

Sulfinert™ Seamless 316 Grade Stainless Steel Tubing

Available in lengths from 5—400 feet. Call for details.

ID	OD	cat.#
0.055" (1.40mm)	1/8" (3.18mm)**	22508
0.180" (4.57mm)	1/4" (6.35mm)**	22509

***0.035" wall thickness*

Sulfinert™-Treated Sample Cylinders

- Sizes from 75cc to 2250cc.
- Durable 316 stainless steel.
- 1/4-inch female NPT threaded ends.
- D.O.T. rated to 1800psi at room temperature.

Size	qty.	cat.#
75cc	ea.	24130
150cc	ea.	24131
300cc	ea.	24132
500cc	ea.	24133
1000cc	ea.	24134
2250cc	ea.	21394

Sulfinert™ Sample Cylinder Valves

- Maximum operating pressure: 5000psig.
- Temperature range for KEL-F® stem tip: -20°F to 250°F (-29°C to 121°C).

Description	qty.	cat.#
(A) 1/4" NPT Exit, KEL-F® Stem Tip	ea.	24127
(B) 1/4" Compression Exit, KEL-F® Stem Tip	ea.	24128
(C) 1/4" Female NPT Outlet (built-in rupture disc)	ea.	21395

Sulfinert™-Treated Gas Sampling Valves and Sample Loops

- Ideal for samples containing low-concentration sulfur compounds.
- Sample loop sizes from 5µL to 5cc.

"W Type" Sulfinert™ Gas Sampling Valves 1/16" fittings, 0.40mm port diameter

Description	qty.	cat.#
Sulfinert™ Gas Sampling Valve; 4-Port	ea.	20584
Sulfinert™ Gas Sampling Valve; 6-Port	ea.	20585
Sulfinert™ Gas Sampling Valve; 10-Port	ea.	20586

Replacement Rotors

Description	qty.	cat.#
Replacement Rotor for 4-Port Sulfinert™ Gas Sampling Valve	ea.	20587
Replacement Rotor for 6-Port Sulfinert™ Gas Sampling Valve	ea.	20588
Replacement Rotor for 10-Port Sulfinert™ Gas Sampling Valve	ea.	20589

Sulfinert™ Gas Sample Loops for "W Type" valves 1/16" fittings

Sizes	qty.	cat.#
5µL	ea.	22840
10µL	ea.	22841
20µL	ea.	22842
25µL	ea.	22843
50µL	ea.	22844
100µL	ea.	22845
250µL	ea.	22846
500µL	ea.	22847
1cc	ea.	22848
2cc	ea.	22849
5cc	ea.	22850

Sulfinert™-Treated Fittings

These are example products—a full line of 1/16", 1/8", and 1/4" fittings is available. Please refer to our catalog.

	Size	qty.	cat.#
 union	1/16"	ea.	22520
	1/8"	ea.	22521
	1/4"	ea.	22522
 tee	1/16"	ea.	22526
	1/8"	ea.	22527
	1/4"	ea.	22528
 reducing union	1/8" to 1/16"	ea.	22523
	1/4" to 1/16"	ea.	22524
	1/4" to 1/8"	ea.	22525
 elbow	1/16"	ea.	22529
	1/8"	ea.	22530
	1/4"	ea.	22531

For information about having system components custom Sulfinert™-treated, contact our Technical Service group, 800-356-1688 or 814-353-1300, ext. 4, or contact your Restek representative.

Restek Trademarks: Crossbond, Rtx, Rt-XLSulfur, Sulfinert, and the Restek logo.
Other Trademarks: KEL-F (3M Co.), Teflon (E.I. du Pont de Nemours & Co., Inc.)

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Clinical Forensic

Applications note

cat.# 59574

Fire Debris Analysis

Capillary gas chromatography is the method of choice for analyzing suspected arson samples from fire debris. Because of the complex composition of most accelerants used in arson cases, it is crucial that positive identification be made of the material used to start the fire. The American Society for Testing Materials (ASTM) has developed standard test procedures for analyzing these samples. The information presented in this article references ASTM E1387, "Standard Test Method for Flammable or Combustible Liquid Residues in Extracts from Samples of Fire Debris by Gas Chromatography".

As with all analytical procedures, sample collection, preservation, chain of custody, and sample preparation play crucial roles in the process. Samples may be extracted using several different techniques[1] (beyond the scope of this article) for introduction into the gas chromatograph.

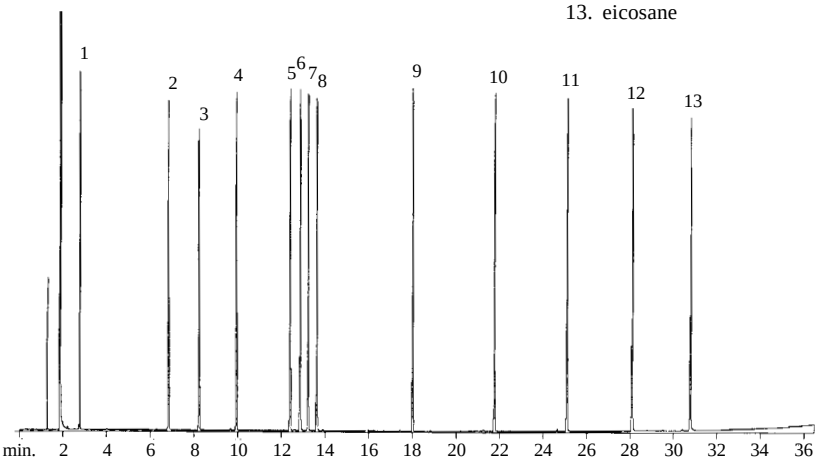
Appropriate capillary column selection is the first decision the analyst must make. The ASTM standard allows the use of any capillary column and conditions, provided that a Resolution Test Mix is completely resolved into the individual components. This resolution test mix consists of equal weights of the even numbered n-alkanes from C6 to C20, plus several aromatic compounds. The aromatics specified are: p-xylene, toluene, 2-ethyltoluene, 3-ethyltoluene, and 1,2,4-trimethylbenzene.

Several different stationary phases and column configurations can provide the resolution needed. Typically, laboratories can use 30-meter columns coated with either Rtx®-1 (100% dimethyl polysiloxane) or Rtx®-5 (5% diphenyl 95% dimethyl polysiloxane). Film thicknesses can vary from 1.0 to 1.5µm. Choice of column ID should depend upon sample capacity and the detection system employed. The standard allows for the use of either FID or MS detectors. If MS detection is employed, use a 0.25mm ID column to minimize carrier gas flow. If FID detection is employed, use a 0.53mm ID to maximize column capacity. Use of 0.53mm ID columns can minimize expensive duplicate analyses or dilutions if the concentration of accelerants are very high. **Figure 1** shows the complete resolution of all 13 components in the column resolution check mix on a 30m, 0.53mm ID, 1.5µm Rtx®-1 column with an FID detector. The linear velocity and temperature program chosen allow the entire analysis to be completed in approximately 16 minutes.

Figure 1

Resolution Check Mix demonstrates proper column performance.

1. hexane
2. toluene
3. octane
4. p-xylene
5. 3-ethyltoluene
6. 2-ethyltoluene
7. 1,2,4-trimethylbenzene
8. decane
9. dodecane
10. tetradecane
11. hexadecane
12. octadecane
13. eicosane



30m, 0.53mm ID, 1.50µm Rtx®-1 (cat.# 10170).
1.0µl split injection of E1387-90 Column Resolution Check Mix (cat.# 31224).
Oven temp.: 40°C (hold 3 min.) to 75°C @ 15°C/min. to 275°C @ 20°C/min. (hold 5 min.); **Inj./det. temp.:** 250°C/285°C; **Carrier gas:** hydrogen; **Linear velocity:** 50cm/sec. set @ 40°C; **FID sensitivity:** 4.10 x 10^{-9} AFS **Split ratio:** 30:1

After establishing the correct conditions to obtain complete resolution of the test mix components, the analyst must then calibrate the instrument. In fire debris analysis, this involves purchase and preparation of common accelerants used to ignite fires with subsequent injection into the GC. Quantitation of unknown samples is not performed. The analyst must provide positive identification to the field investigators of any accelerants found in the samples collected. To do this, the analyst must be able to recognize typical chromatographic patterns of each accelerant. **Figure 2** shows the chromatographic pattern obtained from an injection of an unleaded gasoline standard.

To further complicate this analysis, many factors will change the chromatographic pattern obtained from fire debris. The first is weathering of the material from the heat of the fire along with dilution of water used to extinguish the blaze. This weathering can dramatically change the chromatographic pattern of the material. Typically, lower boiling materials are lost by the heat, leaving the higher boiling compounds remaining. This type of weathering can be simulated in the laboratory by evaporating the material under controlled conditions. The advantage of performing the evaporation in the laboratory is that the exact amount of weight loss compared to the original starting material can be measured and controlled. **Figure 3** shows the analysis of a 99% weathered unleaded gasoline. The gasoline has been weathered to a 99% weight loss and an exact concentration calibration standard was prepared with the remaining material. By analyzing known weathered products, the analyst can more readily recognize the type of original starting material.

There are five basic classes of complex petroleum distillates normally identifiable in arson samples. A sixth class of accelerants (Class 0) includes single compounds sometimes used and identified. **Table I** shows the complete list of classes, typical chromatographic range of each material (based upon hydrocarbon elution), and examples of each type.

Figure 2

Unleaded Gasoline Standard (Unweathered)

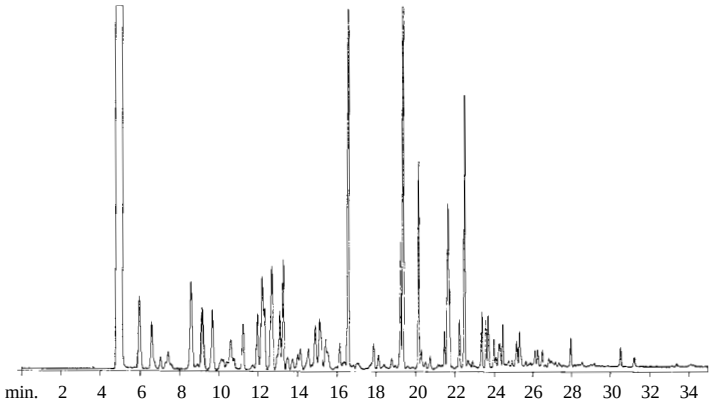
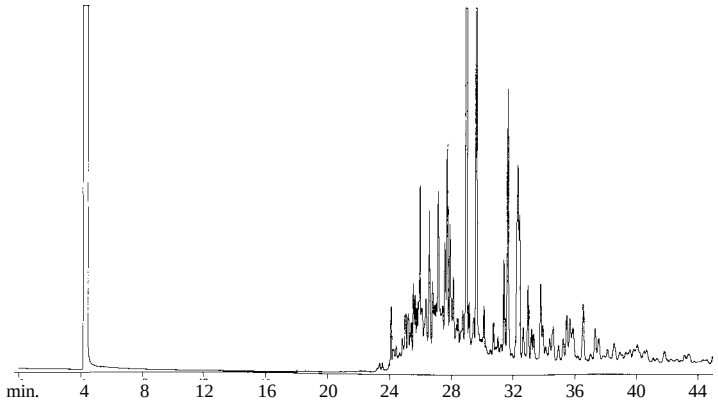


Figure 3

Unleaded Gasoline (99% Weathered)



Conditions for Figures 2 & 3: 30m, 0.53mm ID, 1.50µm Rtx®-1 (cat.# 10170). **Oven temp.:** 40°C (hold 3 min.) to 75°C @ 15°C/min. to 275°C @ 20°C/min. (hold 5 min.); **Inj./det. temp.:** 250°C/285°C; **Carrier gas:** hydrogen; **Linear velocity:** 50cm/sec. set @ 40°C; **FID sensitivity:** 4.10 x 10^{-9} AFS **Split ratio:** 30:1

Table I - Typical Hydrocarbons

Class #		Range	Examples
1	Light Petroleum	C4-C8	Pocket lighter fuel, petroleum ethers, some rubber cement solvents
2	Gasoline	C4-C12	Gasoline (ALL), some camping fuels
3	Medium Petroleum Distillates (MPD)	C8-C12	Mineral spirits, paint thinners, some torch fuels, some charcoal fuels, some charcoal starters
4	Kerosene	C9-C16	Kerosene, No. 1 Fuel Oil, Jet-A Fuel Oil, Jet-A Fuel, some charcoal starters, some torch fuels
5	Heavy Petroleum Distillates (HPD)	C10-C23	No. 2 Fuel Oil, Diesel Fuel #2
0	Unclassified	Variable	Alcohols, acetone, toluene, some lamp oils, camping fuels, lacquer thinners

There are additional variables which can make identification of the petroleum residue more difficult. Included would be co-extracted volatiles and pyrolyzates from the fire debris. The extent of these co-extracted interferences would, in part, be dependent upon the sample preparation method used. The ASTM method does provide minimum requirements for class identification for each type of accelerant, but in the end the experience of the analyst is crucial.

Adequate chromatographic resolution can be obtained on several different capillary columns. Typically, the best resolution can be obtained on an Rtx®-1 (100% dimethyl polysiloxane) stationary phase. Column configuration should be based upon the detection system being used and sample capacity. Calibration with weathered petrochemical standards also plays an important part in identification of accelerants extracted from fire debris samples. ❖

References

1) ASTM Standard Practices for Fire Debris Extraction:
   - ASTM E1412 Passive Headspace Concentration
   - ASTM E1413 Dynamic Headspace Concentration
   - ASTM E1385 Steam Distillation Concentration
   - ASTM E1386 Solvent Extraction Concentration
   - ASTM E1388 Sampling of Headspace Vapors
   - ASTM E1389 Cleanup by Acid Stripping

Rtx®-1 Column Listing
30m, 0.53mm ID, 1.5µm cat.# 10170
30m, 0.32mm ID, 1.0µm cat.# 10154
30m, 0.25mm ID, 1.0µm cat.# 10153

Weathered Petrochemical Analytical Reference Materials

May be used for:

- Underground Storage Tank Monitoring
- ASTM fire debris analysis

Laboratories monitoring underground storage tanks often find it difficult to determine the type of petrochemical detected during an analysis. The main reason for this is the petroleum product has weathered from exposure to air, water, sunlight, and microbial action. All of these factors can lead to misidentification of the original product.

A similar situation occurs for forensic analysts investigating potential arson cases. When arson is suspected, samples are taken from the site and analyzed using ASTM E1387 methods. In these methods, quantitation is not performed but identification of the accelerant (if any) is crucial. Again, weathering of the petrochemical can drastically change the chromatographic profile when compared to non-weathered material.

Restek is now offering as stock products, weathered petrochemical products to meet many of these difficult situations. All of these standards are prepared from commercially acquired materials. The material is then weathered in the laboratory based upon a specific weight loss from the original weight of starting material. While we cannot duplicate all environmental or arson factors, these standards may be useful in identification of the type of petrochemical detected.

ASTM E1387 Fire Debris Analysis

Adequate column resolution is addressed in this protocol. Any capillary column can be used provided resolution of all analytes can be achieved. To demonstrate performance, a column resolution check mix must be analyzed prior to any sample analysis. Listed below is the required column performance mixture.

E1387 Column Resolution Check Mix

Contains the compounds listed at 2000µg/ml each in methylene chloride. Packaged 1ml per ampul.

decane	octadecane
dodecane	octane
eicosane	tetradecane
2-ethyltoluene	toluene
3-ethyltoluene	1,2,4-trimethylbenzene
hexadecane	p-xylene
hexane	

cat.#: 31224 ea.
31224-500 ea. w/data pk.
31224-510 5-pk.
31224-520 5-pk. w/data pk.
31324 10-pk. w/data pk.

Unleaded Gasoline ASTM Class 2 Accelerant	**Single cat.#**	**Single w/data pack cat.#**	**5 pack cat.#**	**5 pack w/data pack cat.#**	**10 pack cat.#**
Unleaded Gasoline Standard (unweathered)	30096	30096-500	30096-510	30096-520	30196
Unleaded Gasoline Standard (25% weathered)	30097	30097-500	30097-510	30097-520	30197
Unleaded Gasoline Standard (50% weathered)	30098	30098-500	30098-510	30098-520	30198
Unleaded Gasoline Standard (75% weathered)	30099	30099-500	30099-510	30099-520	30199
Unleaded Gasoline Standard (99% weathered)	30436	30436-500	30436-510	30436-520	30536
Weathered Gasoline Kit (cat.# 30100 or 30100-500 w/data pack) Contains one ampul (1ml) of unweathered standard (30096), 25% (30097), 50% (30098), and 75% (30099) weathered standards.					
Weathered Gasoline Kit #2 (cat.# 30437 or 30437-500 w/data pack) Contains one ampul (1ml) of unweathered standard (30096), 25% (30097), 50% (30098), 75% (30099), and 99% (30436) weathered standards.					

Mineral Spirits ASTM Class 3 Accelerant	**Single cat.#**	**Single w/data pack cat.#**	**5 pack cat.#**	**5 pack w/data pack cat.#**	**10 pack cat.#**
Mineral Spirits Standard (unweathered)	31225	31225-500	31225-510	31225-520	31325
Mineral Spirits Standard (25% weathered)	31226	31226-500	31226-510	31226-520	31326
Mineral Spirits Standard (50% weathered)	31227	31227-500	31227-510	31227-520	31327
Mineral Spirits Standard (75% weathered)	31228	31228-500	31228-510	31228-520	31328
Weathered Mineral Spirits Kit (cat.# 31237 or 31237-500 w/data pack) Contains one ampul (1ml) of unweathered standard (31225), 25% (31226), 50% (31227), and 75% (31228) weathered standards.					

Kerosene ASTM Class 4 Accelerant	**Single cat.#**	**Single w/data pack cat.#**	**5 pack cat.#**	**5 pack w/data pack cat.#**	**10 pack cat.#**
Kerosene Standard (unweathered)	31229	31229-500	31229-510	31229-520	31329
Kerosene Standard (25% weathered)	31230	31230-500	31230-510	31230-520	31330
Kerosene Standard (50% weathered)	31231	31231-500	31231-510	31231-520	31331
Kerosene Standard (75% weathered)	31232	31232-500	31232-510	31232-520	31332
Weathered Kerosene Kit (cat.# 31238 or 31238-500 w/data pack) Contains one ampul (1ml) of unweathered standard (31229), 25% (31230), 50% (31231), and 75% (31232) weathered standards.					

Diesel Fuel #2 ASTM Class 5 Accelerant	**Single cat.#**	**Single w/data pack cat.#**	**5 pack cat.#**	**5 pack w/data pack cat.#**	**10 pack cat.#**
Diesel Fuel #2 Standard (unweathered)	31233	31233-500	31233-510	31233-520	31333
Diesel Fuel #2 Standard (25% weathered)	31234	31234-500	31234-510	31234-520	31334
Diesel Fuel #2 Standard (50% weathered)	31235	31235-500	31235-510	31235-520	31335
Diesel Fuel #2 Standard (75% weathered)	31236	31236-500	31236-510	31236-520	31336
Weathered Diesel Fuel #2 Kit (cat.# 31239 or 31239-500 w/data pack) Contains one ampul (1ml) of unweathered standard (31233), 25% (31234), 50% (31235), and 75% (31236) weathered standards.					

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Clinical Forensic

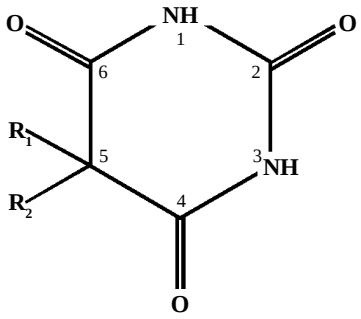
Applications note

cat.# 59575

Barbiturate Analysis

Figure 1

Basic Structure for Barbiturates



Barbiturates are a class of compounds that are central nervous system depressants. They are categorized as sedatives or hypnotics and are primarily used in the treatment of anxiety, insomnia, and convulsive disorders. Physical effects of the barbiturates range from mild sedation to coma. Barbiturates are based on a pyrimidine ring structure. Substitution at the 2, 4, and 6 positions gives the basic structure for the oxybarbiturates (**Figure 1**). Replacement of the oxygen at position 2 with sulfur results in the formation of thiobarbiturates. Barbiturates can be ranked according to their onset of activity, duration of action and degree of hypnotic potency. These pharmacological effects are influenced by the types of functional groups attached at position 5. The inclusion of alkyl or aryl groups, the number of carbons in the alkyl side chains, and the degree of branching will affect activity and toxicity.

Extended administration or abuse of barbiturates can lead to physical and psychological dependence. Tolerance to the effects of barbiturates on the central nervous system can be built up with continued exposure to the drug. While tolerance to the intoxicating effects of barbiturates may increase with use, there is very little increase in tolerance to the toxic side effects of high doses. As a result, the therapeutic index for barbiturates is lower than for other sedative/hypnotic drugs like the benzodiazepines. The barbiturates also have an additive effect when administered with other central nervous system depressants. The combination of the low therapeutic index and the additive effects of other CNS depressants makes monitoring for barbiturates an important aspect of drug overdose screening.

Barbiturates can be analyzed in either their underivatized or derivatized forms by gas chromatography. Derivatization of the barbiturates is most commonly performed by methylation of the amido nitrogens in positions 1 and 3. Methylating reagents like tetramethylammonium hydroxide (TMAH) and trimethylanilinium hydroxide (TMPAH) can be used for on-column derivatization of the barbiturates. While derivatization can improve the peak shape and response, extraneous peak formation can interfere with some analyses. Proper injection port set-up is important in obtaining reproducible results with on-column derivatization. Methylation of barbiturates is catalyzed by the addition of heat to the reaction mixture. After sample injection, the residence time of the barbiturates and the derivatizing reagent inside the injection port is very short. Since contact of the sample with the heated surface area inside the injection port liner needs to be maximized, liners that are packed with wool or that contain flow disrupting elements, like the Cyclosplitter® sleeves are recommended. In addition, injection port temperatures should be maintained in

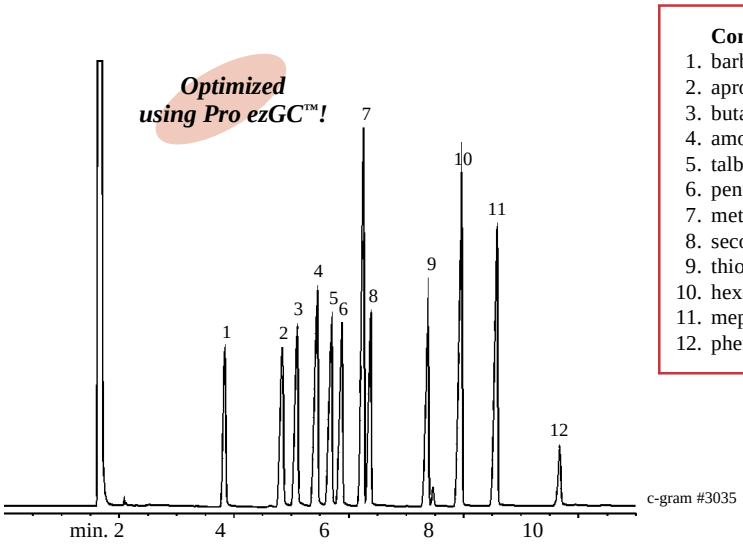
excess of 250°C in order to efficiently complete the derivatization process.

Analysis of barbiturates can also be performed on underivatized compounds. However, underivatized barbiturates have a tendency to produce overloaded or tailing peaks. Maintain injection port liners, guard columns, and analytical columns regularly to achieve good peak shape and adequate resolution. **Figure 2** shows the separation of a set of underivatized barbiturates using an Rtx®-35 column. Lower polarity stationary phases like the Rtx®-5 can be used to separate the barbiturates, but intermediate polarity stationary phases tend to provide better peak shape and improved resolution.

Barbiturates are an important part of drug screening. Extra care should be taken when analyzing barbiturates in either the derivatized or underivatized form. Intermediate polarity columns combined with well maintained injection port liners and guard columns will contribute to better peak shape and resolution.

Figure 2

Underivatized barbiturates on an Rtx®-35.



Compounds
1. barbital
2. aprobarbital
3. butalbital
4. amobarbital
5. talbutal
6. pentobarbital
7. methohexital
8. secobarbital
9. thiopental
10. hexobarbital
11. mephobarbital
12. phenobarbital

30m, 0.32mm ID, 0.50µm Rtx®-35 (cat.# 10439). 1.0µl split injection of barbiturates.
Oven temp.: 210°C (hold 2 min.) to 300°C @ 7°C/min. (hold 2 min.);
Inj./det. temp.: 300°C; **Carrier gas:** helium; **Linear velocity:** 35cm/sec. set @ 210°C;
FID sensitivity: 5.12 x 10^{-10} AFS **Split vent:** 30:1

Clinical Forensic Applications note

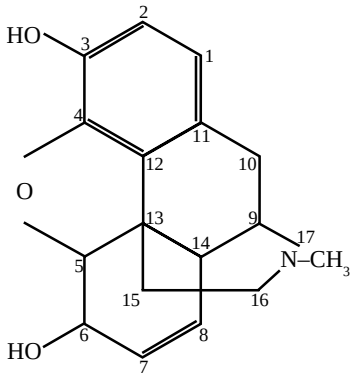
cat.# 59576

Opiate Analysis

Opiates or opioids are terms that classify a group of compounds with morphine-like actions. Their pharmacological properties include analgesia or pain relief, drowsiness and respiratory depression. **Figure 1** shows the structure for morphine. Substitutions at the 3, 6, and 17 positions produce compounds with varying degrees of potency and pharmacological activity. The National Institute for Drug Abuse

Figure 1

Morphine Structure



(NIDA) has targeted opiates as a class to be monitored in urine for detection of drug abuse. Testing guidelines have been established with a limit of detection of 0.3µg/ml for morphine. Screening of opiates is commonly done by using enzyme immunoassays. Enzyme immunoassays have the ability to cross react with a number of structurally similar opiates including codeine, hydromorphone, hydrocodone, levorphanol, and oxycodone. In order to differentiate between all of the possible substances being detected by enzyme immunoassay, confirmational analysis by GC/MS should be performed.

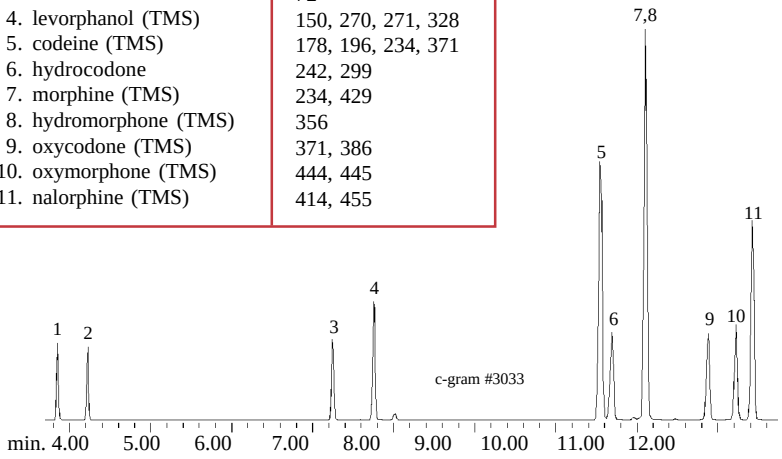
Chromatographic performance of the opiates is significantly affected by small changes in their chemical structure. The presence of hydroxyl groups at the 3 and 6 positions produce compounds that are more polar and reactive. Compounds with reactive hydroxyl groups in their chemical structure can suffer from adsorption and peak tailing, leading to diminished response in chromatographic systems that contain active sites. Sample preparation of sensitive compounds, like opiates, should take place in silanized glassware and samples should be stored in deactivated sample vials. Derivatization of reactive hydroxyl groups can improve chromatographic performance and detection limits and prevent sample loss on glassware and sample vials. Both trimethylsilyl and fluoroacyl derivatives of the opiates yield end products that are less polar and/or more volatile than the underivatized compound.

For this analysis, trimethylsilyl derivatives were prepared using BSTFA with 1% TMCS. Derivatizing the reactive hydroxyl group with a less polar trimethylsilyl group eliminates the tailing peaks commonly seen with compounds like morphine. **Figure 2** shows the analysis of a selection of opiates on an Rtx®-5 column. Compounds that have been derivatized prior to analysis are designated as TMS in the peak list. The TMS derivatized

Compounds	Ions Monitored
1. meperidine	71, 246
2. alphaprodine	172, 187
3. methadone	72
4. levorphanol (TMS)	150, 270, 271, 328
5. codeine (TMS)	178, 196, 234, 371
6. hydrocodone	242, 299
7. morphine (TMS)	234, 429
8. hydromorphone (TMS)	356
9. oxycodone (TMS)	371, 386
10. oxymorphone (TMS)	444, 445
11. nalorphine (TMS)	414, 455

Figure 2

Opiates analysis on an Rtx®-5 column.



30m, 0.25mm ID, 0.25µm Rtx®-5 (cat.# 10223). 2.0µl split injection of opiates.
Oven temp.: 200°C to 325°C @ 7°C/min.; **Inj./det. temp.:** 250°C/300°C;
Carrier gas: helium; **Linear velocity:** 30cm/sec. set @ 200°C;
Split ratio: 50:1 **Ionization:** EI **Mode:** SIM

opiates chromatograph well on a low polarity (Rtx®-5) column with good resolution and peak shape.

Sensitivity and specificity in confirming the presence of opiates in different samples can be enhanced by selectively choosing certain ions to monitor. Identification based upon the presence of distinctive, high mass ions is preferred, especially when analyzing derivatized compounds. Trimethylsilyl derivatives will add 72 amu for every hydroxyl group derivatized.

Effective protocols for opiate analysis include extensive sample preparation and optimized instrument parameters. Derivative formation and the use of deactivated glassware, sample vials, and inlet liners will ensure maximum recoveries and response. Optimized detector parameters using selected ions for detection will aid in the identification of different compounds.

Product Listing:
Rtx®-5
30m, 0.25mm ID, 0.25µm (cat.# 10223)

	Description	Concentration	cat.#
Pharmaceutical standards All standards are diluted in methanol. Packaged 1ml per ampul.	codeine	1000µg/ml	34000
	hydrocodone	1000µg/ml	34002
	hydromorphone	1000µg/ml	34063
	levorphanol	1000µg/ml	34003
	meperidine	1000µg/ml	34004
	methadone	1000µg/ml	34005
	morphine	1000µg/ml	34006
	oxycodone	1000µg/ml	34007
	oxymorphone	1000µg/ml	34065

For a complete list of Restek's Quantitative Drug Standards, please request a copy of our General Catalog.

#59577C

RESTEK pharmaceutical

Applicationsnote

GC Analysis of Organic Volatile Impurities According to USP <467> Supplement Two of USP 25-NF 20, effective January, 2002

A new test for the gas chromatographic (GC) analysis of Organic Volatile Impurities (OVI) in pharmaceutical products was published in the Third Supplement to the US Pharmacopoeia (USP) XXII-NF XVII, which became effective November 15, 1990. Since its original appearance in the USP, this testing protocol has undergone many revisions and additions.[1-6] The most recent change was published as USP 25, effective January 1, 2002.[13] The biggest prior change was to the limit test concentrations, which now match European Pharmacopoeia (EP) concentrations and ICH guidelines for the five USP <467>-regulated solvents (Table I).[8, 9] The January 2002 revision makes no significant changes.

USP has officially removed the limit test requirements for benzene from any article specified to be tested by <467> for organic volatile impurities, except where a specific limit for benzene is in the individual monograph.[10] The revision was needed because Methods I and V were unable to detect benzene at 2ppm. Method IV, the only method that detects benzene at 2ppm, became official in Supplement Two of USP 24-NF 19.[11]

Figure 1 shows an analysis using USP <467> Method I on a G27 analytical column with a phenylmethyl guard column. Note that the sample preparation used in this analysis deviates from the method-specified 1:50 dilution in distilled water. A 1:10 dilution in distilled water was used to obtain a detectable amount of benzene by direct injection.

USP also has clarified that a 5m phenylmethyl guard column is not needed for the Method IV headspace analysis.[10] **Figure 2** shows an analysis using Method IV at the revised concentrations, the method-specified sample preparation procedure, a G43 analytical column, and no guard column.

Table I

Limit Test Concentrations for USP <467>

benzene*	2ppm	methylene chloride	600ppm
chloroform	60ppm	trichloroethene	80ppm
1,4-dioxane	380ppm		

** Testing for benzene only required when specified in the individual monograph.*

Table II

USP <467> Methods and corresponding chromatographic systems

Method I

G27 with 5m phenylmethyl guard column (5% phenyl/95% methyl polysiloxane)
30m, 0.53mm ID, 5.0µm, (Rtx®-G27 column, cat.# 10279-126)
Sample Introduction: direct aqueous injection

Method IV

G43 (6% cyanopropylphenyl/94% dimethylpolysiloxane)
30m, 0.53mm ID, 3.0µm, (Rtx®-G43 column, cat.# 16085)
Sample Introduction: static headspace

Method V

G43 with 5m phenylmethyl guard column
(6% cyanopropylphenyl/94%dimethylpolysiloxane)
30m, 0.53mm ID, 3.0µm (Rtx®-G43 column, cat.# 16085-126)
Sample Introduction: direct aqueous injection

Method VI

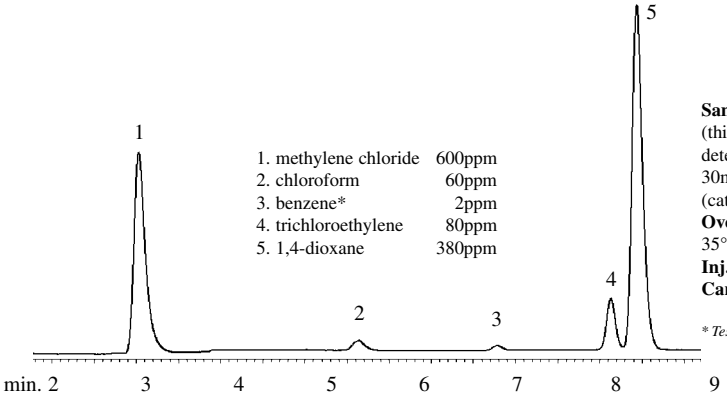
Choice of 9 columns, depending on monograph
Sample Introduction: direct aqueous injection

Method for Coated Tablets

0.2% polyethylene glycol, MW 1500 (G39) on graphitized carbon (S7)
(0.2% Carbowax® 1500 on 80/100 CarboBlack™ C packed column, cat.# 80122)
Sample Introduction: static headspace

Figure 1

A minor modification of the dilution concentration for Method I allows analysis of 2ppm benzene, using an Rtx®-G27 column.



Sample Preparation: 1:10 dilution of cat.# 36007 in distilled water (this deviation from the 1:50 dilution in the method was needed to obtain a detectable benzene peak).
30m, 0.53mm ID, 5.0µm Rtx®-G27 with 5m phenylmethyl Integra-Guard™ (cat.# 10279-126).
Oven temp.: 35°C (hold 5 min.) to 175°C @ 8°C/min., to 260°C @ 35°C/min. (hold 16 min.); **Inj. port:** Uniliner® direct injection sleeve 70°C; **Inj. size:** 1µL; **Det. temp.:** 260°C; **FID sensitivity:** 1 x 10^{-12} AFS; **Carrier gas:** helium, 4.1psi constant pressure, 35cm/sec. set @ 35°C.

** Testing for benzene only required when specified in the individual monograph.*

pharmaceutical

Figure 3 shows an analysis using USP 24 <467> Method V, a G43 analytical column with a phenylmethyl guard column and, once again, a 1:10 dilution in order to obtain a detectable benzene peak.

USP made changes in 1997 to overcome the difficulties resulting from unregulated solvents coeluting with regulated solvents, and thereby causing over-representation of the latter concentrations using GC/flame ionization detection (FID) methods.[12] GC/mass spectrometry (MS) or a second, validated column having a different stationary phase may be used to confirm the presence of the coeluting unregulated solvent and report the correct concentration of regulated solvent. **Figures 4, 5, and 6** show the different elution orders for commonly-used pharmaceutical processing solvents on G27, G43, and Stabilwax® columns. The latter are useful secondary columns for confirmational analysis.

We will continue to review changes to pharmaceutical OVI testing. Restek reference materials listed on page 4 of this applications note meet the most recent USP updates. **For more information regarding these applications, please call Restek technical service at 800-356-1688 or 814-353-1300, ext. 4, or your local Restek representative.**

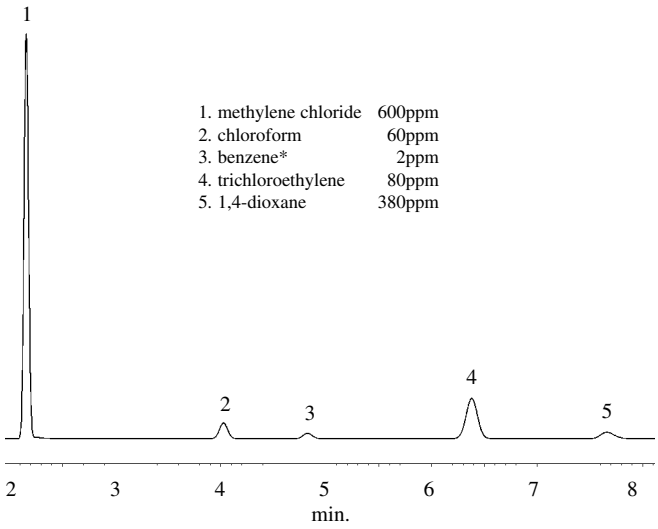
References

1. M.S. Bergren and D.W. Foust, "Comments on USP General Chapter, Organic Volatile Impurities <467>, and Associated Monograph Proposals," *Pharmacopoeial Forum*, May/June 1991, Vol. 17, No. 3, pp. 1963-1968.
2. J.A. Krasowski, H. Dinh, T.J. O'Hanlon, R.F. Lindauer, "Comments on Organic Volatile Impurities, Method I, <467>," *Pharmacopoeial Forum*, May/June 1991, Vol. 17, No. 3, pp. 1969-1972.
3. Pharmacopoeial Forum, March/April 1991, Vol. 17, No. 2, p. 1653.
4. Fifth Supplement, USP-NF, Organic Volatile Impurities <467>, Nov. 15, 1991, pp. 2706-2708.
5. "Organic Volatile Impurities <467>," *Pharmacopoeial Forum*, May-June 1993, Vol. 19, No. 3, pp. 5335-5337.
6. Pharmacopoeial Forum, September/October 1992, Vol. 18, No. 5, p. 4028.
7. USP 24/NF 19, <467> Organic Volatile Impurities, (1877-1878).
8. "ICH Harmonized Tripartite Guideline, Impurities: Guideline for Residual Solvents," *The Fourth International Conference on Harmonization*, July 17, 1997.
9. European Pharmacopoeia, Supplement 1999, pp. 14-15, 208.
10. Pharmacopoeial Forum, November - December 1999, Vol. 25, Number 6, (9223 - 9224).
11. Supplement Two, USP 24/NF 19, August 1, 2000.
12. Sixth Supplement, USP-NF, Organic Volatile Impurities <467>, May 15, 1997, pp. 3766-3768.
13. USP 25/NF 20, <467> Organic Volatile Impurities, January 1, 2002.

These references are not available from Restek.

Figure 2

The Rtx®-G43 column provides the resolution and detection limits needed for <467> revised limit test concentrations in USP Method IV. (no guard column)

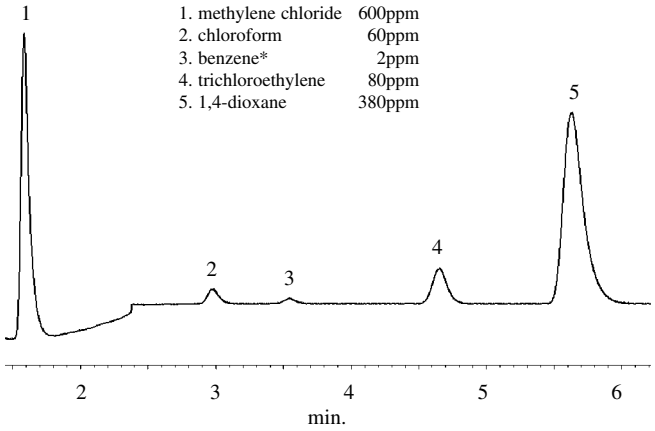


Sample Preparation: 100µL of cat.# 36007 in 5mL distilled water, 1g sodium sulfate in a 20mL headspace vial.
30m, 0.53mm ID, 3.0µm Rtx®-G43 (cat.# 16085)
Oven temp.: 40°C (hold 20 min.) to 240°C @ 35°C/min. (hold 20 min.);
Inj. temp: 140°C, 1mm split sleeve (cat.# 20916);
Det. temp.: 260°C;
FID sensitivity: 1.25 x 10^{-11} AFS;
Carrier gas: helium, 3.5psi constant pressure, 35cm/sec. set @ 40°C;
Split ratio: 2:1; ThermoQuest HS 2000 Headspace Autosampler Vial 80°C, 60 min. shaker on.

** Testing for benzene only required when specified in the individual monograph.*

Figure 3

Achieve analysis of 2ppm benzene for Method V, using 1:10 dilution and an Rtx®-G43 column.



Sample Preparation: 1:10 dilution of cat.# 36007 in distilled water (this deviation from the 1:50 dilution in the method was needed to obtain a detectable benzene peak).
30m, 0.53mm ID, 3.0µm Rtx®-G43 with 5m phenylmethyl Integra-Guard™ (cat.# 16085-126). ThermoQuest Trace 2000 Series. Uniliner® direct injection sleeve.
Oven temp.: 40°C (hold 20 min.) to 240°C @ 35°C/min. (hold 20 min.);
Inj. temp.: 140°C;
FID sensitivity: 260°C, 1 x 10^{-11} AFS;
Carrier gas: 4.1psi helium @ 35°C/sec.,
Det. temp.: 260°C

** Testing for benzene only required when specified in the individual monograph.*

Figure 4

An Rtx®-G27 column resolves many solvents commonly used in pharmaceutical processing.

30m, 0.53mm ID, 5.0µm Rtx®-G27 with 5m phenylmethyl Integra-Guard™ (cat.# 10279-126).
Headspace injection of 24 common residual solvents for pharmaceutical processing. Prepared to equal about 500ppm in the bulk pharmaceutical. Samples shaken and heated at 90°C for 15 minutes, 1mL headspace injection.
Oven temp.: 35°C (hold 10 min.) to 100°C @ 5°C/min., to 240°C @ 25°C/min. (hold 5 min.);
Inj./det. temp.: 220°C/240°C; **FID sensitivity:** 1.05 x 10^{-11} AFS; **Carrier gas:** helium, 35cm/sec. set @ 35°C; **Split ratio:** 2:1.

1. ethylene oxide
2. methanol
3. diethyl ether
4. 1,1-dichloroethene
5. acetone
6. isopropanol
7. acetonitrile
8. methylene chloride
9. *n*-hexane
10. *n*-propanol
11. methyl ethyl ketone
12. ethyl acetate
13. tetrahydrofuran
14. chloroform
15. 1,1,1-trichloroethane
16. carbon tetrachloride
17. benzene*
18. 1,2-dichloroethane
19. heptane
20. trichloroethylene
21. *n*-butanol
22. 1,4-dioxane
23. pyridine
24. toluene

** Testing for benzene only required when specified in the individual monograph.*

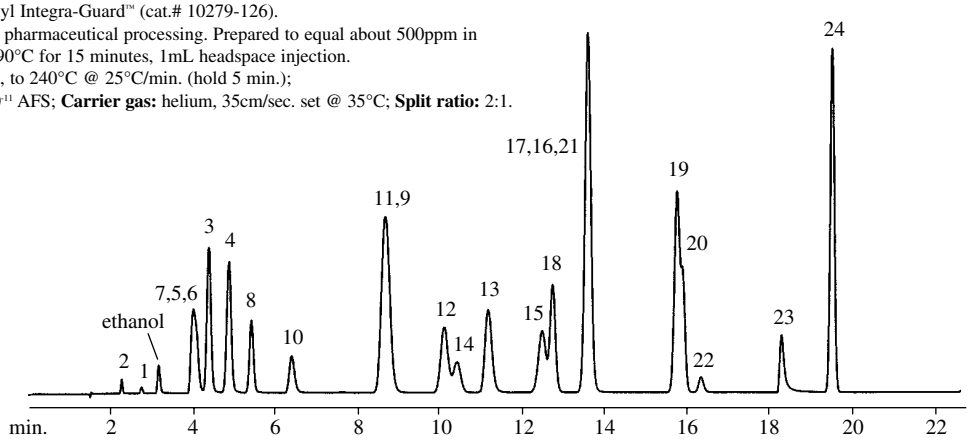


Figure 5

An Rtx®-G43 column shows excellent resolution of commonly-used pharmaceutical processing solvents. European Pharmacopoeia Class 1 and Class 2 compounds at the regulation limit concentration.

1. methanol
2. 1,1-dichloroethene
3. acetonitrile
4. methylene chloride (dichloromethane)
5. hexane (C6)
6. *cis*-1,2-dichloroethene
7. nitromethane
8. chloroform
9. cyclohexane
10. 1,1,1-trichloroethane
11. carbon tetrachloride
12. benzene*
13. 1,2-dimethoxyethane
14. 1,2-dichloroethane
15. trichloroethylene (1,1,2-trichlorethene)
16. methylcyclohexane
17. 1,4-dioxane
18. pyridine
19. toluene
20. 2-hexanone
21. chlorobenzene
22. DMF
23. ethylbenzene
24. *m*-xylene
25. *p*-xylene
26. *o*-xylene
27. N,N-dimethylacetamide
28. 1,2,3,4-tetrahydronaphthalene

** Testing for benzene only required when specified in the individual monograph.*

30m x .53mm ID x 3.0mm Rtx®-G43 (cat.# 16085-126).
Headspace injection of 28 Class 1 and 2 residual solvents for pharmaceutical processing. Prepared at the regulatory limit concentration. Samples shaken and heated at 80°C for 15 minutes, 1mL headspace injection.
Oven temp.: 40°C (hold 20 min.) to 240°C @ 10°C/min. (hold 20 min.);
Inj./det. temp.: 200°C/250°C; **FID sensitivity:** 1.1 x 10^{-11} AFS;
Carrier gas: hydrogen @ 35cm/sec.; **Split ratio:** 2:1.

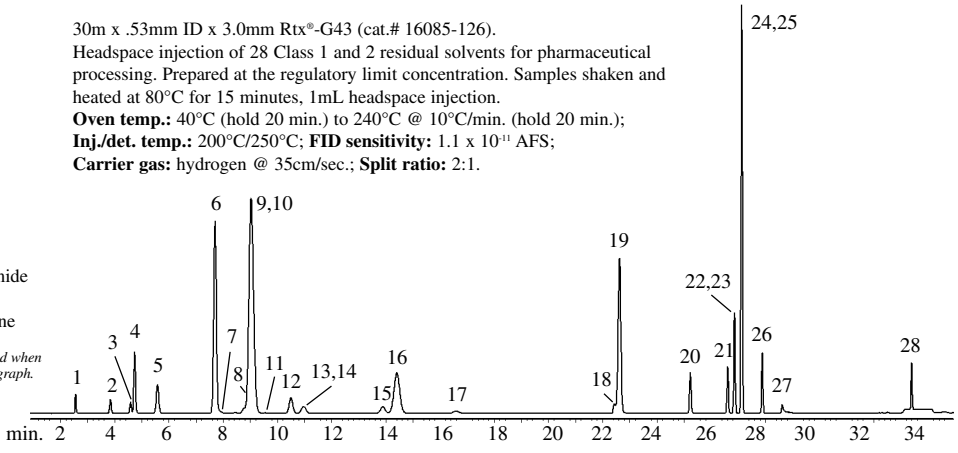


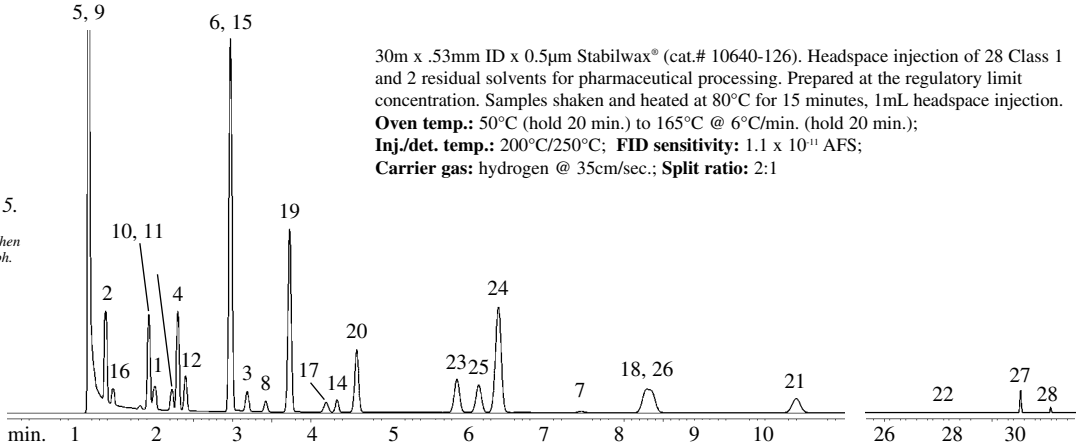
Figure 6

A Stabilwax® column makes an excellent confirmation column for commonly-used pharmaceutical processing solvents. European Pharmacopoeia Class 1 and Class 2 compounds at the regulation limit concentration.

Same peak list as Figure 5.

** Testing for benzene only required when specified in the individual monograph.*

30m x .53mm ID x 0.5µm Stabilwax® (cat.# 10640-126). Headspace injection of 28 Class 1 and 2 residual solvents for pharmaceutical processing. Prepared at the regulatory limit concentration. Samples shaken and heated at 80°C for 15 minutes, 1mL headspace injection.
Oven temp.: 50°C (hold 20 min.) to 165°C @ 6°C/min. (hold 20 min.);
Inj./det. temp.: 200°C/250°C; **FID sensitivity:** 1.1 x 10^{-11} AFS;
Carrier gas: hydrogen @ 35cm/sec.; **Split ratio:** 2:1



Product Listing

These mixes meet requirements of USP 24/NF 19, effective 1/1/2000.

USP <467> Calibration Mix #6

chloroform	60µg/mL	methylene chloride	600
1,4-dioxane	380	trichloroethylene	80

Prepared in methanol, 1mL/ampul

each	10-pk.
36008	36108

USP <467> Calibration Mix #7

chloroform	60µg/mL	methylene chloride	600
1,4-dioxane	380	trichloroethylene	80

Prepared in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36009	36109

USP <467> Calibration Mix #4

benzene	2µg/mL	methylene chloride	600
chloroform	60	trichloroethene	80
1,4-dioxane	380		

Prepared in methanol, 1mL/ampul

each	10-pk.
36006	36106

USP <467> Calibration Mix #5

benzene	2µg/mL	methylene chloride	600
chloroform	60	trichloroethene	80
1,4-dioxane	380		

Prepared in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36007	36107

These mixes meet requirements of USP 23/NF 18, effective 1/1/1995–12/31/1999.

USP <467> Calibration Mix #2

benzene	100µg/mL	methylene chloride	500
chloroform	50	trichloroethene	100
1,4-dioxane	100		

Prepared in methanol, 1mL/ampul

each	10-pk.
36002	36102

USP <467> Calibration Mix #3

benzene	100µg/mL	methylene chloride	500
chloroform	50	trichloroethene	100
1,4-dioxane	100		

Prepared in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36004	36104

USP <467> Method—ethylene oxide standard

500µg/mL in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36005	36105

Rtx®-G27 column with 5m phenylmethyl Integra-Guard™

(5% phenyl/95% methyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	5.00	-60 to 270/290°C	10279-126

Rtx®-G43 column with 5m phenylmethyl Integra-Guard™

(6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	3.00	-20 to 240°C	16085-126

Rtx®-G43 column, USP <467> Method IV

(6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	3.00	-20 to 240°C	16085

Stabilwax® columns

ID	df (µm)	Temp. Limits	30-Meter
0.32mm	0.25	40 to 250/260°C	10624
0.53mm	0.50	40 to 250/260°C	10640

CarboBlack™ packed column

Description	Length	OD	ID	cat.#
0.2% Carbowax® 1500 on 80/100 CarboBlack™ C	2m	1/8"	2mm	80122-*

**Please call for cat.# suffix for your specific GC column configuration.*

Custom OVI mixtures are available on request.

RESTEK ISO 9001 REGISTERED

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food & flavor

Applications note

Fast, Selective Triglyceride Analysis

Triglycerides are naturally occurring esters of fatty acids and glycerols. They are widely analyzed in the food industry for natural oil and fat characterization as well as fat adulteration. The triglycerides usually are converted to their methyl esters (FAMEs) to determine the fatty acid composition and percent saturated vs. unsaturated fat. Triglyceride analyses have become very important in recent years since health conscious consumers are concerned with minimizing their dietary intake of saturated fats to reduce the risk of heart disease.

Fatty acid groups in the triglyceride molecule can be classified as saturated [myristic acid (C14:0), palmitic acid (C16:0), or stearic acid (C18:0)], monounsaturated [oleic acid (C18:1)], or polyunsaturated [linoleic acid (C18:2) or linolenic acid (C18:3)]. Typically, triglycerides are characterized by degree of unsaturation. For example, a triglyceride molecule containing the groups stearic acid, oleic acid, and linoleic acid (denoted SOL) would have a greater degree of unsaturation than that of tripalmitin (denoted PPP). Table I shows the nomenclature of fatty acids and triglycerides.[1]

Capillary GC columns are the preferred analytical tool for triglyceride analysis because they yield shorter analysis times, higher efficiency, and better quantitation than packed column GC, HPLC, or SFC. The Rtx®-65TG column is truly an improvement over classical packed columns because triglycerides with the same carbon number but different degrees of unsaturation can be well separated. Also, minimal sample preparation is required for capillary GC analysis. The sample is liquefied by warming and diluting to 50pg/µL with dichloromethane or diisopropyl ether[2,3]. Additional sample clean up is required if significant amounts of monoglycerides, diglycerides, and fatty acids are known to be present in the sample[2].

Column Selection for Triglyceride Analysis

The high molecular weights of triglycerides require capillary columns with high thermal stability. Low bleed columns also are extremely important for accurate triglyceride quantitation. Triglyceride polarity increases with the degree of unsaturation in the fatty acid (i.e., the number of double bonds present). The triglyceride with the most double bonds has the highest polarity and the longest retention time. Therefore, a high temperature is required to elute the higher polarity triglycerides and maintain a short analysis time. Lower response of high molecular weight triglycerides has been observed and originally was attributed to thermal decomposition of triglycerides in the injection port. However, the decreased response also can be caused by both high molecular weight discrimination in the

Table I

Fatty acid and triglyceride nomenclature (after Geeraert and Sandra)

Fatty Acids

Abbreviation	Common Name	IUPAC Name	Short Form
La	lauric acid	dodecanoic acid	C 12:0
M	myristic acid	tetradecanoic acid	C 14:0
P	palmitic acid	hexadecanoic acid	C 16:0
S	stearic acid	octadecanoic acid	C 18:0
A	arachidic acid	eicosanoic acid	C 20:0
Be	behenic acid	dodosanoic acid	C 22:0
Lg	lignoceric acid	tetracosanoic acid	C 24:0
O	oleic acid	*cis*-9-octadecenoic acid	C 18:1
L	linoleic acid	*cis, cis*-9,12-octadecadienoic acid	C 18:2
Ln	linolenic acid	*cis, cis, cis*-9,12,15-octadecatrienoic acid	C 18:3
Ga	gadoleic acid	*cis*-11-eicosenoic acid	C 20:1

Glycerides

Abbreviation	Common Name	Carbon Number	# of Unsaturated Fatty Acids
PPP	tripalmitin	48	0
PLO	palmito-linoleo-olein	52	2

injection port and increased solute band broadening in the stationary phase.

Triglycerides are separated according to carbon number or molecular weight on non-polar, methyl silicone columns (AOAC and AOCS Methods use this type of column). However, no resolution is achieved for differences in unsaturation within the unsaturated fatty acids. Therefore, triglycerides such as POP, SOS, and POS would all appear as a single peak. Triglyceride separation by degree of unsaturation, as well as carbon number requires a highly polar stationary phase. While polar stationary phases such as 50% phenyl/50% methyl offer the necessary selectivity, they traditionally have suffered from relatively low thermal stability. Phenyl/methyl polysiloxanes generally exhibit lower maximum operating temperatures when compared with methyl silicones.

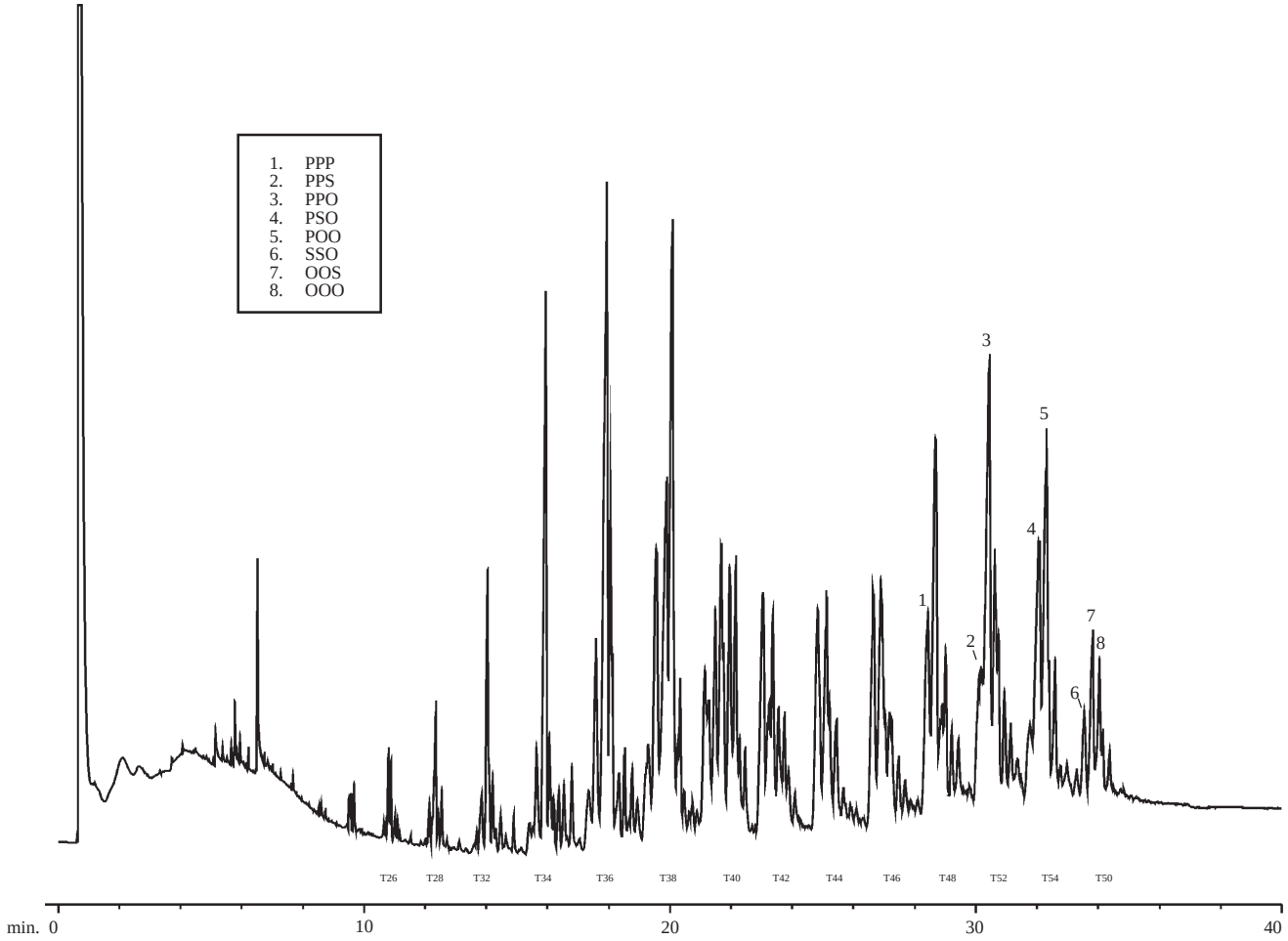
Separate Triglycerides by Carbon Number and Degree of Unsaturation Using an Rtx®-65TG Column

The chemists at Restek have combined innovative polymer synthesis with advanced deactivation techniques to create a highly polar, uniquely selective stationary phase with the extended thermal stability required for triglyceride analysis. This produces columns that last longer and exhibit less bleed at temperatures as high as 370°C. The Rtx®-65TG column (65% phenyl/35% methyl polysiloxane) is selective for resolving triglycerides according to degree of unsaturation as well as carbon number.

Figure 1 shows butter fat triglycerides run on a 30m, 0.25mm, 0.10µm Rtx®-65TG column using a septum-equipped programmable injector (SPI). The SPI injector reduces molecular weight

Figure 1

The Rtx®-65TG column exhibits excellent resolution of butter fat triglycerides in under 35 minutes at 365°C.



30m, 0.25mm ID, 0.10µm Rtx®-65TG (cat.# 17008)
0.2µL direct injection of 1% butter fat in isooctane.

Oven temp.:	80°C (hold 1 min.) to 240°C @ 30°C/min. to 365°C @ 4°C/min. (hold 5 min.)
Det. temp.:	380°C
Carrier gas:	hydrogen
Linear velocity:	70cm/sec.
FID sensitivity:	16 x 10^{-11} AFS
SPI injector:	high-performance capillary insert 60°C, 300°C/min. to 400°C (hold 5 min.)

food & flavor

discrimination and thermal degradation, which is common with normal split/splitless injectors. The column shows excellent separation and peak symmetry of the triglycerides by both degree of unsaturation and carbon number. Fine structure resolution of T50 (PPS/PPO), T52 (PSS/PSO/POO), and T54 (SSS/SSO/SOO/OOO) triglycerides is obtained with minimal column bleed at 365°C. The optimized analysis time is less than 35 minutes. Figure 2 shows the analysis of canola, corn, and olive oils on a 15m, 0.25mm ID, 0.10µm Rtx®-65TG column. The unsaturates are all well separated in less than 8 minutes with minimal column bleed at 365°C.

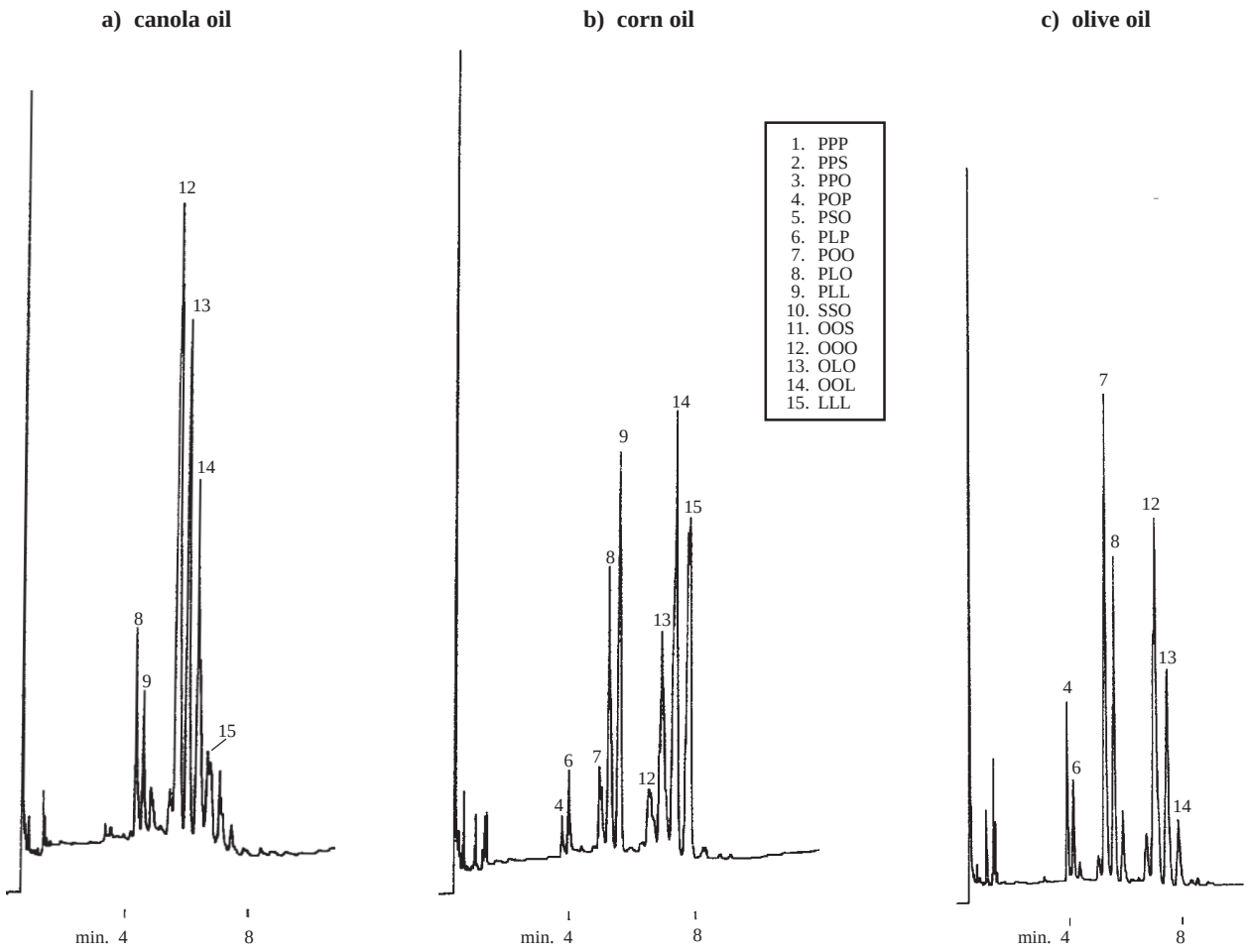
The Rtx®-65TG column is ideal for triglyceride analysis. Separation by degree of unsaturation as well as carbon number is obtained in under 35 minutes with minimal column bleed at 365°C. Because Rtx®-65 columns are individually tested with a temperature-programmed triglyceride test mixture, low bleed and high efficiency are guaranteed. Rtx®-65TG columns are available in both 15- and 30-meter lengths in 0.25, 0.32, and 0.53mm IDs with a 0.10µm film thickness.

References:

1. Geeraert and Sandra, "Capillary GC of Triglycerides in Fats and Oils Using a High Temperature Phenyl Methyl Silicone Stationary Phase," *Journal of HRC & CC, Vol. 8, Aug 1985, pp. 415-419.*
2. Association of Analytical Chemists, Official Methods of Analysis of the AOAC, 17th ed., Method 986.19.
3. American Oil Chemists Society, Official Methods and Recommended Practices (1994), Method Ce 5-86.

Figure 2

Obtain fine structure resolution by degree of unsaturation with common oils on a 15-meter Rtx®-65TG column.



15m, 0.25mm ID, 0.10µm Rtx®-65TG (cat.# 17005)
0.8µL split injection of triglycerides.

Oven temp.:
a) 355°C (hold 1 min.) to 365°C @ 1°C/min.
b) 350°C (hold 0.5 min.) to 365°C @ 1°C/min.
c) 350°C (hold 1 min.) to 365°C @ 0.5°C/min.

Inj. & det. temp.: 365°C
Carrier gas: hydrogen
Linear velocity: 50cm/sec.
FID sensitivity: 8 x 10^{-11} AFS
Split ratio: 40:1

Product Listing

Rtx®-65TG Columns

ID	df (µm)	Temp. Limits	15m	30m
0.25mm	0.10	40 to 370°C	17005	17008
0.32mm	0.10	40 to 370°C	17006	17009
0.53mm	0.10	40 to 360/370°C	17007	17010

MXT®-65TG Columns

ID	df (µm)	Temp. Limits	15m	30m
0.25mm	0.10	20 to 370°C	77005	77008
0.53mm	0.10	20 to 370°C	77007	77010

Inlet Liners

PTV Liners for Agilent GCs	ID/OD & Length (mm)	ea.	cat.# 5-pk.	10-pk.
Straight Glass Inlet Liner	2.0 ID 3.0 OD x 71	—	—	21157
Baffled Glass Inlet Liner	1.5 ID 3.0 OD x 71	—	—	21704
Glass Inlet Liner with Wool*	2.0 ID 3.0 OD x 71	—	—	21156

SPI Liners for Varian GCs	ID/OD & length (mm)	ea.	cat.# 5-pk.	25-pk.
0.5mm SPI	0.53 ID 4.6 OD x 54	20775	20776	20777
0.8mm SPI	0.80 ID 4.6 OD x 54	20778	20779	20780
SPI with Buffer	2.4 ID 4.6 OD x 54	20850	20851	20852

**Liner is packed with fused silica wool. To order glass wool instead, add the suffix "-202" to the liner's catalog number.*

17A PTV Liners for Shimadzu GCs	ID/OD & Length (mm)	ea.	cat.# 5-pk.	25-pk.
17A PTV Sleeve*	1.6 ID 4.0 OD x 95	21705	21706	21707

PTV Liners for Perkin-Elmer GCs	ID/OD & Length (mm)	ea.	cat.# 5-pk.	25-pk.
PTV Press-Tight®	1.0 ID 2.0 OD x 88	20733	20734	20735
PTV Injector	1.0 ID 2.0 OD x 88	20742	20743	20744

Siltek™ Inlet Liners

For Siltek™-deactivated inlet liners, add the corresponding suffix number to your liner catalog number.

qty.	Siltek™	Siltek™ with Siltek™ wool	Siltek™ with CarboFrit™
each	-214.1	-213.1	-216.1
5-pk.	-214.5	-213.5	-216.5
25-pk.	-214.25	-213.25	-216.25

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Applications note

High-Resolution Analyses of Fatty Acid Methyl Esters by Gas Chromatography

Fatty acid methyl esters (FAMEs) analysis is an important tool both in characterizing fats and oils and in determining the total fat content in foods. Fats can be extracted from a matrix, using a non-polar solvent, and saponified to produce salts of the free fatty acids. After derivatizing the free acids to form the methyl esters, the mixture readily can be analyzed by gas chromatography (GC), due to the volatility and thermal stability of the FAMEs. Gas chromatography has become an important technique in fats and oils analysis because accurate results can be obtained for complex, as well as simple, sample matrices.

FAMEs analyses were among the first applications for gas chromatography, so many of the GC methods originally written for analysis of fats and oils described packed column technology. Capillary columns offer significant advantages, however, including more efficient separations. When analyzing fats and oils with complex fatty acid profiles, such as the *cis* and *trans* forms of polyunsaturated fatty acids, higher efficiencies are needed to resolve the individual components. Capillary columns with Carbowax®-type (polyethylene glycol) stationary phases typically are used for analyses of saturated and unsaturated fatty acid methyl esters, and biscyanopropyl phases are used to resolve *cis* and *trans* isomers of polyunsaturated components.

Creating FAMEs

Lipids are normally extracted from matrices using a non-polar solvent, such as ether, and saponified to produce the free fatty acid salts. The fatty acid salts then are derivatized to form the fatty acid methyl esters, to increase volatility, improve peak symmetry, decrease sample activity, and thus provide more accurate analytical data. The official methods of AOAC International[1] and the American Oil Chemists Society (AOCS)[2] both contain procedures for the derivatization reaction, as does the European Pharmacopoeia.[3] In general, the glycerides are saponified by refluxing with methanolic sodium hydroxide. The esterification is effected with a reagent such as boron trifluoride in methanol and the FAMEs are extracted with a non-polar solvent (e.g., heptane) for analysis by GC.

Several groups of researchers have proposed simplified procedures for creating the methyl esters. For example, lipids can be transmethylated *in situ*. This option combines all of the conventional steps, except for the drying and post-reaction work-up, into one step.[4] For some samples, trimethyl-sulfonium hydroxide (TMSH), an alternative derivatization reagent, can be used for transesterification. A major advantage of this approach is that the derivatization can be performed in a single, fast reaction step.

Analyzing Polyunsaturated FAMEs

Stabilwax® and Rtx®-Wax columns provide excellent resolution of FAMEs derived from either plant or animal sources. Polyunsaturated FAMEs typically are analyzed on one of these Carbowax®-type capillary columns; analysis times of 35-50 minutes generally are required to fully resolve the C21:5 FAME from the C23:0 internal standard, and the C24:0 FAME from C22:6.

The FAMEWAX® polyethylene glycol stationary phase is specially tested with a polyunsaturated FAMEs mix to ensure resolution of the omega-3 and omega-6 fatty acids of interest, including those specified above. In addition, FAMEs such as methyl eicosapaenoate (C20:5) and methyl docosahexaenoate (C22:6), found in nutraceutical ingredients and products such as marine oils, also are resolved. FAMEWAX® columns offer excellent resolution of polyunsaturated FAMEs with significantly reduced analysis times, compared to traditional Carbowax® stationary phases. In fact, analysis times of less than 10 minutes are possible! Figure 1 shows an

Figure 1—A FAMEWAX® capillary column provides fast, efficient separations of marine oil-based FAMEs.

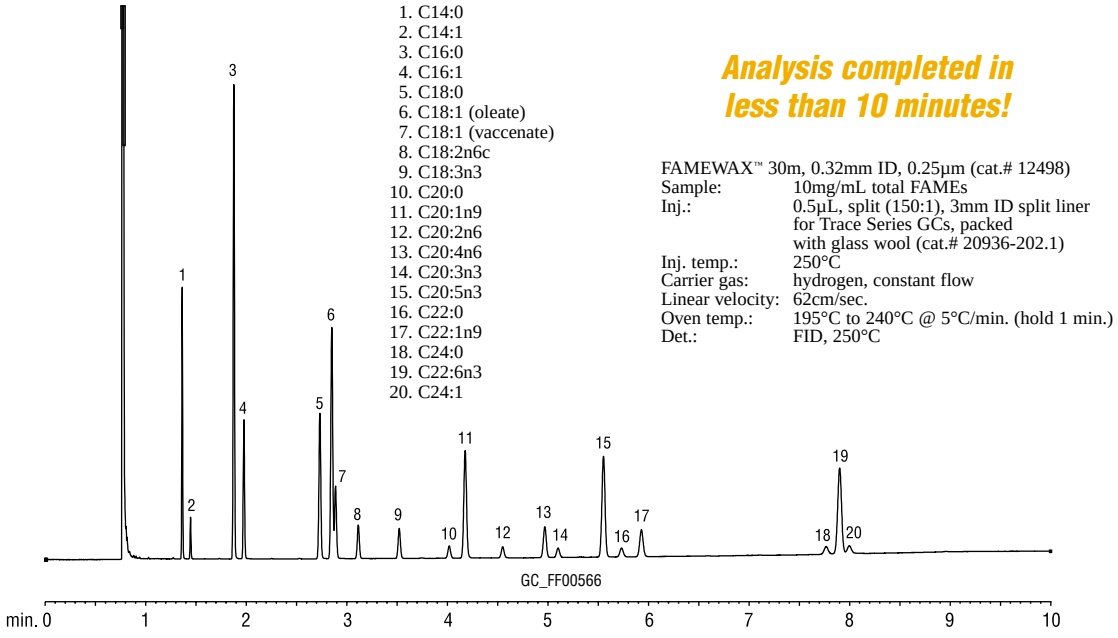


Figure 2—Rapid, efficient analysis of FAMEs derived from a marine oil capsule, using a FAMEWAX® column.

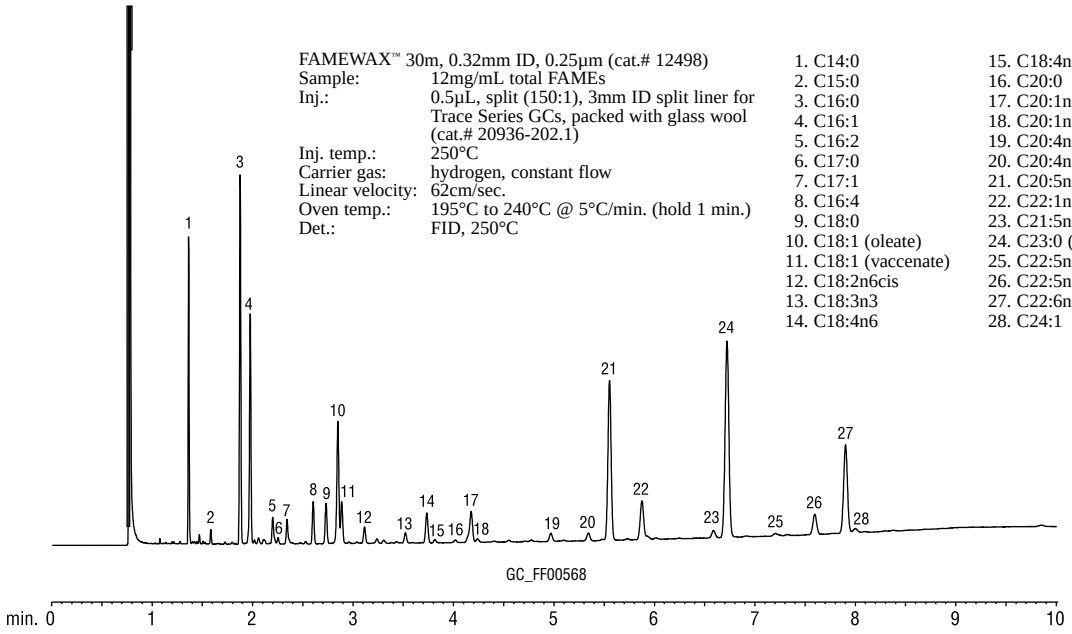
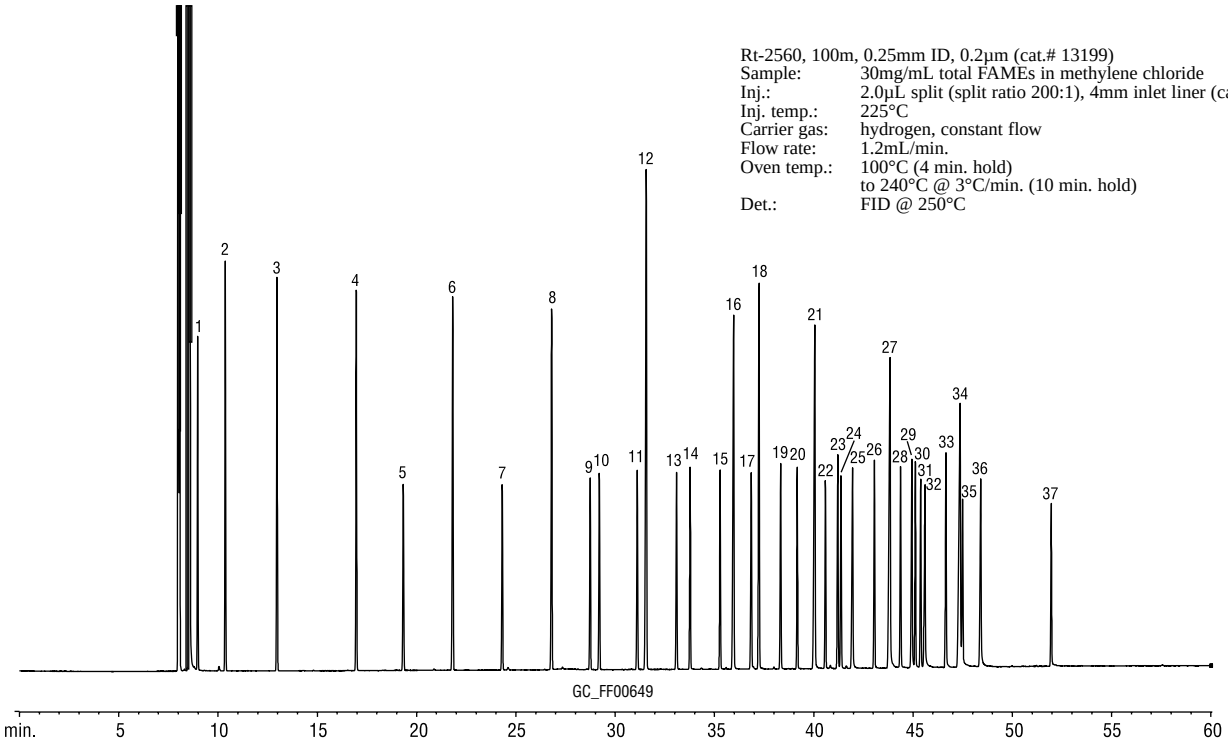


Figure 3—Effective analysis of a 37 FAME mix on an Rt-2560 capillary column.



Compound	% in Mix
1. C4:0 methyl butyrate	4.0
2. C6:0 methyl hexanoate	4.0
3. C8:0 methyl octanoate	4.0
4. C10:0 methyl decanoate	4.0
5. C11:0 methyl undecanoate	2.0
6. C12:0 methyl laurate	4.0
7. C13:0 methyl tridecanoate	2.0
8. C14:0 methyl myristate	4.0
9. C14:1 methyl myristoleate (*cis*-9)	2.0
10. C15:0 methyl pentadecanoate	2.0
11. C15:1 methyl pentadecenoate (*cis*-10)	2.0
12. C16:0 methyl palmitate	6.0
13. C16:1 methyl palmitoleate (*cis*-9)	2.0
14. C17:0 methyl heptadecanoate	2.0
15. C17:1 methyl heptadecenoate (*cis*-10)	2.0
16. C18:0 methyl stearate	4.0
17. C18:1 methyl elaidate (*trans*-9)	2.0
18. C18:1 methyl oleate (*cis*-9)	4.0
19. C18:2 methyl linoleaidate (*trans*-9,12)	2.0
20. C18:2 methyl linoleate (*cis*-9,12)	2.0
21. C18:3 methyl γ-linolenate (*cis*-6,9,12)	2.0
22. C20:0 methyl arachidate	4.0
23. C20:1 methyl eicosenoate (*cis*-11)	2.0
24. C18:3 methyl linolenate (*cis*-9,12,15)	2.0
25. C21:0 methyl heneicosanoate	2.0
26. C20:2 methyl eicosadienoate (*cis*-11,14)	2.0
27. C20:3 methyl eicosatrienoate (*cis*-11,14,17)	2.0
28. C22:0 methyl behenate	4.0
29. C22:1 methyl erucate (*cis*-13)	2.0
30. C20:3 methyl eicosatrienoate (*cis*-11,14,17)	2.0
31. C20:4 methyl arachidonate (*cis*-5,8,11,14)	2.0
32. C23:0 methyl tricosanoate	2.0
33. C22:2 methyl docosadienoate (*cis*-13,16)	2.0
34. C20:5 methyl eicosapentaenoate (*cis*-5,8,11,14,17)	2.0
35. C24:0 methyl lignocerate	4.0
36. C24:1 methyl nervonate (*cis*-15)	2.0
37. C22:6 methyl docosahexaenoate (*cis*-4,7,10,13,16,19)	2.0

analysis of a marine-oil FAME standard; a marine oil sample is shown in Figure 2. Both analyses are characterized by fast, effective resolution and sharp, symmetric peaks.

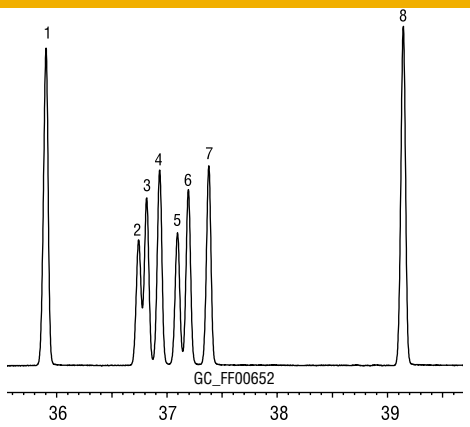
Resolving *cis* and *trans* Isomers

Individual *cis* and *trans* isomers are resolved on a 100-meter Rt-2560 column, making this the column of choice for analyzing partially hydrogenated fats. The highly polar biscyanopropyl phase gives the selectivity needed for resolving FAMEs isomers, such as the *cis* and *trans* forms of C18:1. The *trans* isomers elute before the *cis* isomers on this phase, opposite of the elution order on Carbowax®-based phases such as FAMEWAX™ or Rtx®-Wax. Figure 3 shows the chromatographic separation of 37 FAMEs typically encountered in vegetable, animal, and marine fats and oils, using an Rt-2560 column.

AOAC method 996.06[1] specifies the determination of total fat content based on the fatty acid content, after conversion to the methyl esters. This is the specified method for determining total fat content for nutritional labeling purposes. After quantifying the total FAMEs present in the derivatized sample, the amount of fat (as triglycerides) in the sample is calculated, based on initial sample weight. The 100-meter Rt-2560 column meets the requirements of this procedure. This column also allows quantification of the total *trans* content.

To calibrate the GC system for assays of this type, use a FAME mixture such as our 37-component Food Industry FAME Mix (Figure 3) or our 28-component NLEA FAME Mix (Figure 4). Both standards include a gravimetric certificate of analysis to help ensure accurate quantification. To ensure correct identifications of the individual *cis* and *trans* isomers of C18:1, use our *cis/trans* Isomer Mix, as shown in Figure 5.

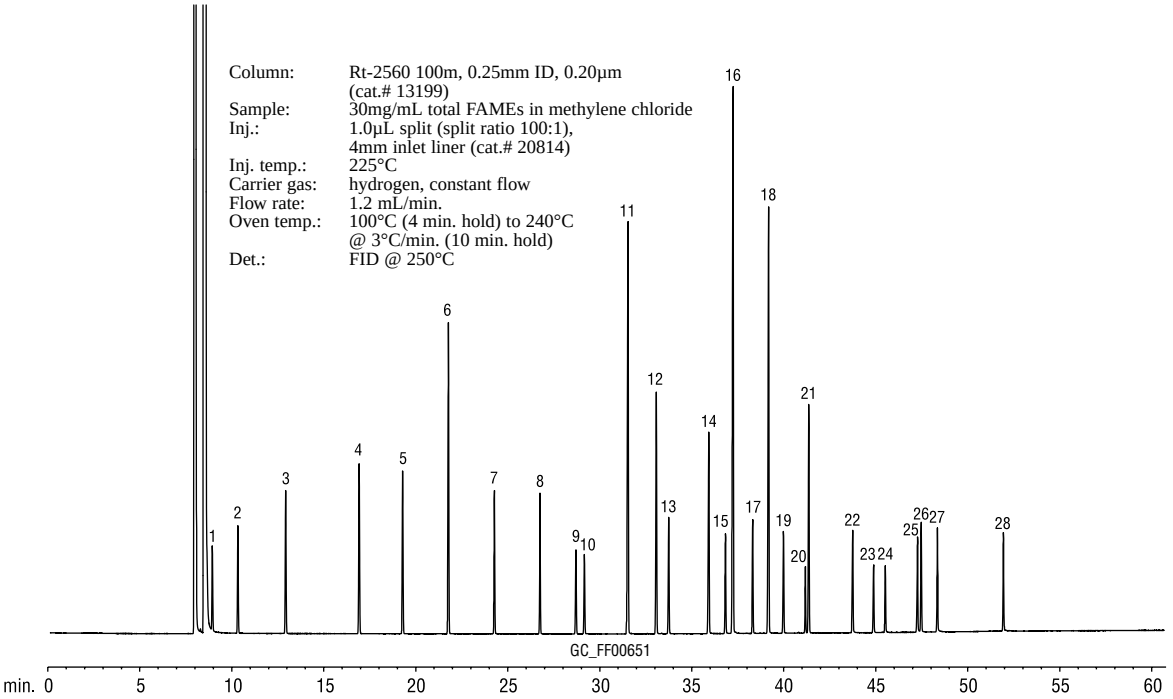
Figure 5—Resolve *cis* and *trans* isomers of unsaturated FAMEs on an Rt-2560 column.



Compound	% in Mix
1. C18:0 methyl stearate	20.0
2. C18:1 methyl petroselaidate (*trans*-6)	8.0
3. C18:1 methyl elaidate (*trans*-9)	10.0
4. C18:1 methyl transvaccenate (*trans*-11)	12.0
5. C18:1 methyl petroselinate (*cis*-6)	8.0
6. C18:1 methyl oleate (*cis*-9)	10.0
7. C18:1 methyl vaccenate (*cis*-11)	12.0
8. C18:2 methyl linoleate (*cis*-9,12)	20.0

Rt-2560, 100m, 0.25mm ID, 0.2µm (cat.# 13199)
Sample: 10mg/mL total FAMEs in methylene chloride
Inj.: 1.0µL split (split ratio 20:1), 4mm inlet liner (cat.# 20814)
Inj. temp.: 225°C
Carrier gas: hydrogen, constant flow
Flow rate: 1.2mL/min.
Oven temp.: 100°C (4 min. hold) to 240°C @ 3°C/min. (10 min. hold)
Det.: FID @ 250°C

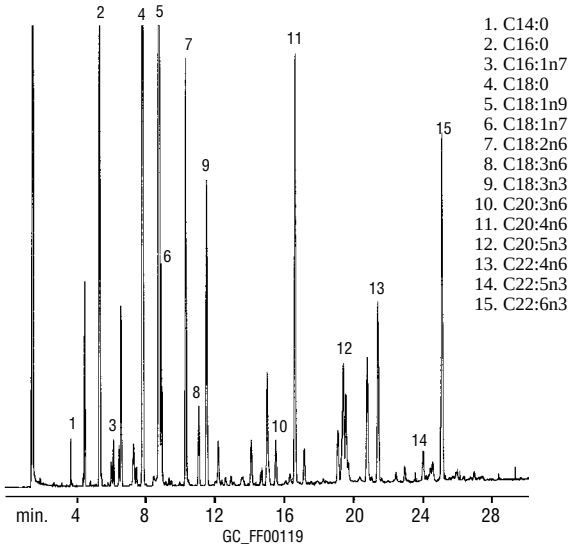
Figure 4—Use the NLEA FAME Mix to standardize Fat-by-Fatty Acid Composition methods, such as AOAC 996.06.



1. C4:0 methyl butyrate
2. C6:0 methyl heanoate
3. C8:0 methyl octanoate
4. C10:0 methyl decanoate
5. C11:0 methyl undecanoate
6. C12:0 methyl laurate
7. C13:0 methyl tridecanoate
8. C14:0 methyl myristate
9. C14:1 methyl myristoleate (*cis*-9)
10. C15:0 methyl pentadecanoate
11. C16:0 methyl palmitate
12. C16:1 methyl palmitoleate (*cis*-9)
13. C17:0 methyl heptadecanoate
14. C18:0 methyl stearate
15. C18:1 methyl elaidate (*trans*-9)
16. C18:1 methyl oleate (*cis*-9)
17. C18:2 methyl linoelaidate (*trans*-9,12)
18. C18:2 methyl linoleate (*cis*-9,12)
19. C20:0 methyl arachidate
20. C20:1 methyl eicosenoate (*cis*-11)
21. C18:3 methyl linolenate (*cis*-9,12,15)
22. C22:0 methyl behenate
23. C22:1 methyl erucate (*cis*-13)
24. C23:0 methyl tricosanoate
25. C24:0 methyl lignocerate
26. C20:5 methyl eicosapentaenoate (*cis*-5,8,11,14,17)
27. C24:1 methyl mervonate (*cis*-15)
28. C22:6 methyl docosahexaenoate (*cis*-4,7,10,13,16,19)

Rtx®-2330, a 90% biscyanopropyl phase, also resolves *cis* and *trans* FAME isomers. These columns are slightly less polar than Rt-2560 columns. Figure 6 shows the analysis of an animal-based fat, using an Rtx®-2330 column. As on Rt-2560 columns, the *trans* forms of the FAMEs elute before the *cis* forms.

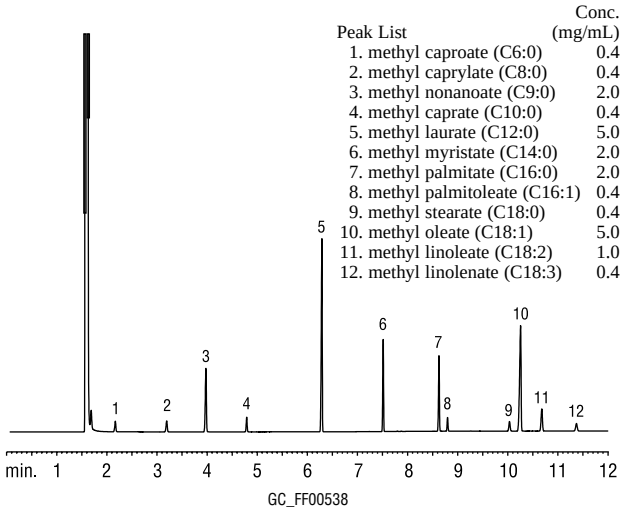
Figure 6—Analysis of an animal-based fat, using an Rtx®-2330 capillary column.



30m, 0.32mm ID, 0.20µm Rtx®-2330 (cat.# 10724)
0.1µL split injection of PUFA 2 mix.

Oven temp.:	160°C to 250°C @ 2°C/min. (hold 10 min.)
Inj. & det. temp.:	260°C
Carrier gas:	hydrogen
Linear velocity:	40cm/sec.
FID sensitivity:	8 x 10^{-11} AFS
Split ratio:	20:1

Figure 7—Saw palmetto FAME standard resolved on an Rtx®-Wax column.



30m, 0.25mm, 0.25µm Rtx®-Wax (cat.# 12423)
1µL injection of saw palmetto standard
Conc.: see peak list

Oven temp.:	120°C (hold 3 min.) to 220°C at 20°C/min. (hold 12 min.)
Inj./det. temp.:	250°C/300°C
Carrier gas:	helium
Linear velocity:	1mL/min. 34 cm/sec.
Split ratio:	100:1

Analyzing Botanical Products

Gas chromatography can be used to analyze the marker compounds in some botanical products, such as saw palmetto. In this product, the marker compounds are the fatty acids. The Institute for Nutraceutical Advancement (INA) has published a method for the analysis of the fatty acid content in saw palmetto by gas chromatography. The analysis is performed after converting the acids to the methyl esters. Both Rtx®-Wax and Stabilwax® capillary columns provide the efficiency and selectivity needed to perform this analysis, and allow accurate identification of the individual fatty acids (Figures 7 and 8).

Summary

Capillary gas chromatography is an essential tool in analyses of fats, oils, and fat-containing products. GC is especially useful for determining total fat content, *trans* fat content, and total omega-3 polyunsaturated fatty acid content in foods. The choice of capillary column depends on the information required. For polyunsaturated FAMEs analysis, a FAMEWAX® column allows fast, accurate quantification. A more polar Rt-2560 column is the column of choice when determining the total fat content, or the amount of *trans* fat, in an ingredient or end product.

Whatever your fatty acid analysis requirements, Restek can provide the consistent-performance analytical columns and reference materials that will help you to accurately characterize your materials.

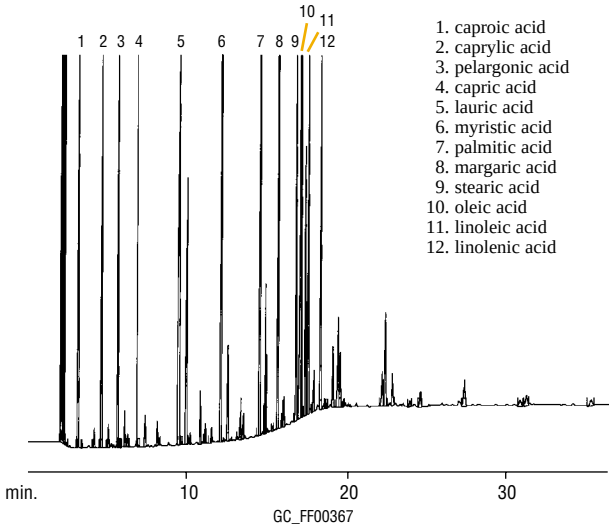
References

1. *Official Methods of Analysis*, 17th edition, AOAC International, 2000.
2. *Official Methods and Recommended Practices of the AOCS*, 5th edition, American Oil Chemists Society.
3. *European Pharmacopoeia*, 4th edition, method 2001:1352.
4. Liu, K.-S., *JAOCS* 71 (11): 1179 (1994).
5. Miller, K.D. *et. Al.*, *JHRC* 16: 161 (1993).

Also Available from Restek

Selection Guide for Polar WAX GC Column Phases (free on request, lit. cat.# 59890).

Figure 8—Fatty acids in saw palmetto, a Stabilwax® column after conversion to methyl esters.



50m, 0.25mm ID, 0.25µm Stabilwax® (cat.# 10626-105).
1µL split injection.

Oven temp.:	110°C (hold 1 min.) to 240°C @ 8°C/min. (hold 25 min.)
Inj./FID temp.:	230°C/250°C
Carrier gas:	hydrogen @ 2.5mL/min.
Split flow:	37.5mL/min.
Septum purge:	3mL/min.

Chromatogram provided by The Institute for Nutraceutical Advancement (INA)

GC Columns for FAMEs Analyses

Ordering Information | FAMEWAX™ (Fused Silica)

(Crossbond® polyethylene glycol)

ID	df (µm)	temp. limits	30-Meter
0.25mm	0.25	20 to 250°C	12497
0.32mm	0.25	20 to 250°C	12498
0.53mm	0.50	20 to 250°C	12499

Ordering Information | Stabilwax® (Fused Silica)

(Crossbond® Carbowax®—provides oxidation resistance)

ID	df (µm)	temp. limits	15-Meter	30-Meter	30-Meter 6/pk.	60-Meter
0.25mm	0.10	40 to 250°C	10605	10608		10611
	0.25	40 to 250°C	10620	10623		10626
	0.50	40 to 250°C	10635	10638		10641
0.32mm	0.10	40 to 250°C	10606	10609		10612
	0.25	40 to 250°C	10621	10624		10627
	0.50	40 to 250°C	10636	10639		10642
0.53mm	0.10	40 to 250°C	10607	10610		10613
	0.25	40 to 250°C	10622	10625		10628
	0.50	40 to 250°C	10637	10640		10643
	1.00	40 to 240/250°C	10652	10655	10655-600	10658

Ordering Information | Rtx®-Wax (Fused Silica)

(Crossbond® polyethelene glycol)

ID	df (µm)	temp. limits**	15-Meter	30-Meter	60-Meter
0.25mm	0.10	20 to 250°C	12405	12408	
	0.25	20 to 250°C	12420	12423	12426
	0.50	20 to 250°C	12435	12438	12441
0.32mm	0.10	20 to 250°C	12406	12409	
	0.25	20 to 250°C	12421	12424	12427
	0.50	20 to 250°C	12436	12439	12442
	1.00	20 to 240/250°C	12451	12454	12457
0.53mm	0.25	20 to 250°C	12422	12425	
	0.50	20 to 250°C	12437	12440	12443
	1.00	20 to 240/250°C	12452	12455	12458
ID	**df (µm)**	**temp. limits**	**10-Meter**	**20-Meter**	
0.10mm	0.10	20 to 250°C	41601	41602	
	0.20	20 to 240/250°C	41603	41604	

Ordering Information | Rt-2560 (Fused Silica)

ID	df (µm)	temp. limits	100-Meter
0.25mm	0.20	20 to 250°C	13199

Ordering Information | Rtx®-2330* (Fused Silica)

(90% biscyanopropyl/10% phenylcyanopropyl)

ID	df (µm)	temp. limits**	15-Meter	30-Meter	60-Meter	105-Meter
0.25mm	0.10	0 to 260/275°C	10705	10708	10711	10714
	0.20	0 to 260/275°C	10720	10723	10726	10729
0.32mm	0.10	0 to 260/275°C	10706	10709	10712	10715
	0.20	0 to 260/275°C	10721	10724	10727	10730
0.53mm	0.10	0 to 260/275°C	10707	10710	10713	
	0.20	0 to 260/275°C	10722	10725	10728	
ID	**df (µm)**	**temp. limits**	**10-Meter**	**20-Meter**	**40-Meter**	
0.18mm	0.10	0 to 260/275°C	40701	40702	40703	

**Not solvent rinsable.*
***The maximum temperatures listed are for 15- and 30-meter lengths. Longer lengths may have a slightly reduced maximum temperature.*

Vials and Limited Volume Inserts

Convenience Kits: Includes Vials*, Caps, & Septa

Description	100-pk.	1000-pk.
2mL Clear Vial, deactivated**, PTFE/Natural Rubber Seal	24671	24672
2mL Amber Vial, deactivated**, PTFE/Natural Rubber Seal	24673	24674
2.0mL Clear Vial, untreated, PTFE/Natural Rubber Seal	21196	21197
2.0mL Amber Vial, untreated, PTFE/Natural Rubber Seal	21198	21199
2.0mL Clear Vial, untreated, PTFE/Silicone Seal	24646	24647
2.0mL Amber Vial, untreated, PTFE/Silicone Seal	24648	24649

**Crimp top vials, 2.0mL, 12 x 32mm, 11mm crimp finish.*
***Silcote™ CL7 deactivation.*

Limited Volume Inserts for 2mL, Crimp-Top & Short-Cap, Screw-Thread Vials

Description	100-pk.	1000-pk.
50µL Glass, Polypropylene, Bottom Spring	24513	21782
250µL Glass, BM Insert w/ Bottom Spring	21776	21777
250µL Glass, BM Insert w/ Glass Flange (Step™ Design)	24516	21779
350µL Glass, Flat Bottom Insert	21780	24517
250µL Polypropylene, Bottom Spring	24518	—
250µL Polypropylene, Top Flange	24519	—
250µL Polypropylene, No Spring	24520	—

BM = Big Mouth

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Analytical Reference Materials for FAMEs Analyses

Marine Oil FAME Mix (20 components)

Chain	Description	% by Weight
C14:0	methyl myristate	6.0
C14:1	methyl myristoleate	1.0
C16:0	methyl palmitate	16.0
C16:1	methyl palmitoleate	5.0
C18:0	methyl stearate	8.0
C18:1	methyl oleate	13.0
C18:1	methyl vaccenate	4.0
C18:2	methyl linoleate	2.0
C18:3	methyl linolenate	2.0
C20:0	methyl arachidate	1.0
C20:1	methyl 11-eicosenoate	9.0
C20:2	methyl 11-14-eicosadienoate	1.0
C20:4	methyl arachidonate	3.0
C20:3	methyl 11-14-17-eicosatrienoate	1.0
C20:5	methyl eicosapentaenoate	10.0
C22:0	methyl behenate	1.0
C22:1	methyl erucate	3.0
C22:6	methyl docosahexaenoate	12.0
C24:0	methyl lingnocerate	1.0
C24:1	methyl nervonate	1.0

100mg
35066

Food Industry FAME Mix (37 components)

Chain	% by Weight	Chain	% by Weight
C4:0	4.0	C18:2(all-*cis*-9,12)	2.0
C6:0	4.0	C18:3(all-*cis*-6,9,12)	2.0
C8:0	4.0	C18:3(all-*cis*-9,12,15)	2.0
C10:0	4.0	C20:0	4.0
C11:0	2.0	C20:1(*cis*-11)	2.0
C12:0	4.0	C20:2(all-*cis*-11,14,)	2.0
C13:	2.0	C20:3 (all-*cis*-8,11,14)	2.0
C14:0	4.0	C20:3(all-*cis*-11,14,17)	2.0
C14:1(*cis*-9)	2.0	C20:4(all-*cis*-5,8,11,14)	2.0
C15:0	2.0	C20:5(all-*cis*-5,8,11,14,17)	2.0
C15:1(*cis*-10)	2.0	C21:0	2.0
C16:0	6.0	C22:0	4.0
C16;1(*cis*-9)	2.0	C22:1(*cis*-13)	2.0
C17:0	2.0	C22:2(all-*cis*-13,16)	2.0
C17:1(*cis*-10)	2.0	22:6(all-*cis*-4,7,10,13,16,19)	2.0
C18:0	4.0	C23:0	2.0
C18:1(*trans*-9)	2.0	C24:0	4.0
C18:1(*cis*-9)	4.0	C24:1(*cis*-15)	2.0
C18:2(all-*trans*-9,12)	2.0		

30mg/mL total in methylene chloride, 1mL/ampul

ea.
35077

NLEA FAME Mix (28 components)

Chain	% by Weight	Chain	% by Weight
C4:0	1.5	C18:1(*trans*-9)	2.5
C6:0	1.5	C18:1(*cis*-9)	15.0
C8:0	2.0	C18:2(all-*trans*-9,12)	2.5
C10:0	2.5	C18:2(all-*cis*-9,12)	10.0
C11:0	2.5	C18:3(all-*cis*-9,12,15)	5.0
C12:0	5.0	C20:0	2.5
C13:	2.5	C20:1(*cis*-11)	1.5
C14:0	2.5	C20:5(all-*cis*-5,8,11,14,17)	2.5
C14:1(*cis*-9)	1.5	C22:0	2.5
C15:0	1.5	C22:1(*cis*-13)	1.5
C16:0	10.0	C22:6(all-*cis*-4,7,10,13,16,19)	2.5
C16;1(*cis*-9)	5.0	C23:0	1.5
C17:0	2.5	C24:0	2.5
C18:0	5.0	C24:1(*cis*-15)	2.5

30mg/mL total in methylene chloride, 1mL/ampul

ea.
35078

cis/*trans* FAME Mix (8 components)

Description	% by Weight
methyl elaidate (C18:1 *trans*-9)	10.0
methyl linoleate (C18:2 *cis*-9,12)	20.0
methyl oleate (C18:1 *cis*-9)	10.0
methyl petroselinate (C18:1*cis*-6)	8.0
methyl petroselaidate (C18:1*trans*-6)	8.0
methyl stearate (C18:0)	20.0
methyl transvaccenate (C18:1 *trans*-11)	12.0
methyl vaccenate (C18:1 *cis*-11)	12.0

10mg/mL total in methylene chloride, 1mL/ampul

ea.
35079

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Applications note

Using Computer Modeling to Optimize FAME Analysis

Background

Gas chromatography (GC) is an effective means of characterizing fatty acids in food as well as other matrices. The Association of Official Chemists (AOAC) and the American Oil Chemists Society (AOCS) provide several methods for GC analysis of fatty acid methyl esters (FAMEs). AOAC Method 963.22 provides general guidelines and conditions for analyzing a wide range of saturated and unsaturated FAMEs, from C8:0 to C24:0, using packed column GC[1]. If capillary GC is used as an alternative method, specifications can be met or exceeded easily. AOAC Official Method 991.39[1] and AOCS Official Method Ce 1b-89[2] describe the analysis of polyunsaturated FAMEs in fish oils using capillary GC[2]. These methods list conditions for separating all of the FAMEs in complex fish oils on polyethylene glycol (PEG) and cyanopropyl stationary phases. By properly optimizing analytical conditions, you can improve resolution and decrease analysis time.

Unfortunately, the typical optimization process can be extremely time consuming and frustrating. Furthermore, many analysts identify unsaturated fatty acids, including polyunsaturated fatty acids (PUFAs) by equivalent chain length (ECL) values rather than by retention time[3]. Changing conditions may alter the elution order, and require peak re-identification. Trial and error with different column configurations and parameters, along with the necessary re-identifications, can waste additional analyst and instrument time. A more efficient approach to optimizing analytical parameters is to use computer modeling software. Programs such as *Pro ezGC*™ use thermodynamic retention indices (TRIs) to model GC analyses and provide optimized conditions in minutes. Additionally, this program can recalculate the ECL values for the new set of run conditions. *Pro ezGC*™ can provide optimized FAME analyses that meet or exceed the resolution and ECL specifications stated in the official methods.

How Well Does Computer Modeling Work?

To illustrate the accuracy and efficiency of the modeling software, a mixture of 21 saturated FAMEs were analyzed on a 60m, 0.25mm ID, 0.25µm Rtx®-Wax column (cat.# 12426). The FAMEs ranged from methyl butanoate (C4:0) to methyl tetracosanoate (C24:0). TRIs were generated by analyzing the mixture with two different temperature programs. The first program was a relatively slow ramp, while the second was a fast ramp. The resulting retention times for each component were entered into the program, which automatically calculated the TRIs. The software then was able to evaluate a wide range of run conditions and predict the resulting retention times under each set of conditions. Once the predictions are made, the software selects the set of conditions that provides the best separation in the fastest analysis time. To demonstrate the accuracy of this

Table I

Experimental retention times versus predicted retention times.

FAMEs MSD Data
60m, 0.25mm ID, 0.25µm Rtx®-Wax column (cat.# 12426)

Oven temp.:
45°C @ 6°C/min. to 265°C (hold 7 min.)

Carrier gas:
hydrogen (constant pressure)

Linear velocity:
53.5cm/sec. @ 45°C

Dead time:
1.980 min. @ 45°C

	Component	Exp. tR (min.)	Calc. tR (min.)	Exp. -Calc. Error (min.)	(Exp. -Calc.)/ Exp. % Error
1.	me butanoate	3.918	3.924	-0.006	-0.1
2.	me pentanoate	5.315	5.327	-0.012	-0.2
3.	me hexanoate	7.158	7.171	-0.013	-0.2
4.	me heptanoate	9.293	9.299	-0.006	-0.1
5.	me octanoate	11.573	11.577	-0.004	-0.0
6.	me nonanoate	13.855	13.854	0.001	0.0
7.	me decanoate	16.083	16.075	0.008	0.0
8.	me undecanoate	18.228	18.205	0.023	0.1
9.	me dodecanoate	20.282	20.253	0.029	0.1
10.	me tridecanoate	22.247	22.191	0.056	0.3
11.	me tetradecanoate	24.127	24.078	0.049	0.2
12.	me pentadecanoate	25.927	25.855	0.072	0.3
13.	me hexadecanoate	27.653	27.567	0.086	0.3
14.	me heptadecanoate	29.310	29.238	0.072	0.2
15.	me octadecanoate	30.902	30.856	0.046	0.1
16.	me nonadecanoate	32.432	32.382	0.050	0.2
17.	me eicosanoate	33.907	33.824	0.083	0.2
18.	me heneicosanoate	35.330	35.205	0.125	0.4
19.	me docosanoate	36.702	36.542	0.160	0.4
20.	me tricosanoate	38.120	37.872	0.248	0.6
21.	me tetracosanoate	39.710	39.396	0.314	0.8
			Avg. error:	*0.070*	*0.2*

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software, the FAMEs were analyzed using an optimized temperature program chosen by the software. Table I shows the percent error between the predicted and the actual retention times. The program's average prediction error was only 0.2%, with a maximum error less than 1%!

Modeling Saturated and Unsaturated FAMEs in Cocoa Butter

AOAC Method 963.22, entitled "Methyl Esters of Fatty Acid in Oils and Fats," describes the GC analysis of FAMEs ranging from 8 to 24 hydrocarbons in chain length. Although the method describes GC using packed columns, capillary columns coated with Carbowax® PEG 20M can easily exceed the required specifications. The minimum separation requirements are: 1) a resolution value (R) of at least 1.25 between methyl stearate (C18:0) and methyl oleate (C18:1n9); and 2) baseline resolution of methyl linolenate (C18:3n3), methyl arachidate (C20:0), and methyl gadoleate (C20:1). Methyl stearate should elute 15 minutes after the solvent peak, and the analytical column should have at least 2000 theoretical plates.

GC operating conditions were entered into the computer program to obtain the optimum analysis of a reference standard of cocoa butter FAMEs. Then, a very broad range of temperature conditions including the initial temperature, the program rate, and the final temperature were entered into the software. Based upon these selection criteria, over 1000 possible temperature programs were evaluated by the software in only a few minutes. The solutions were automatically ranked according to the best separations and fastest analysis times. In this instance, the default resolution factor was set at 1.5 to provide baseline resolution. Although this value is typical, it may be increased or decreased to meet the unique needs of a specific analysis.

The software predicted that all of the cocoa butter FAMEs could be separated adequately in less than 10 minutes on a 30m, 0.25mm ID, 0.25µm Famewax™ column (cat.# 12497). Using this optimized capillary method, total analysis is completed in less time than methyl stearate elutes under the original AOAC recommended conditions! This capillary column provides approximately 2700 plates-per-meter, which exceeds the efficiency requirement specified in AOAC Method 963.22. The new analytical conditions not only provide baseline resolution of the required components, but of all components (R>1.5), thereby easily exceeding the method's resolution requirement. Figure 1 shows the predicted chromatogram provided by the software and the actual analysis of the cocoa butter FAMEs standard reference mixture. The actual chromatogram shows excellent correlation to the chromatogram predicted by the software.

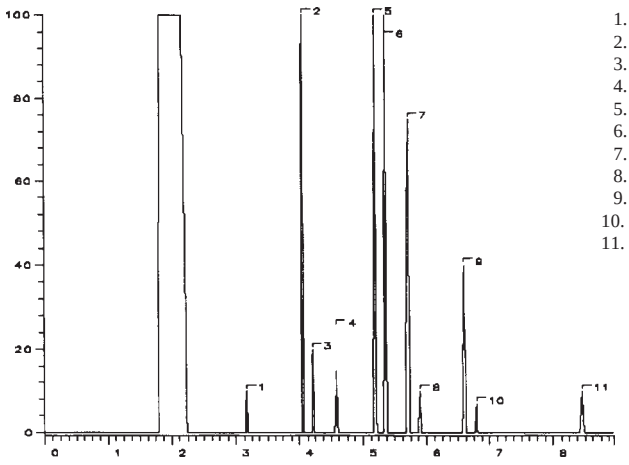
Reducing Analysis Time of Menhaden Oil PUFAs

GC analysis of menhaden oil is described in AOAC Method 991.39, entitled "Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters," and in AOCS Official Method Ce 1b-89, entitled "Fatty Acid Composition by GLC." These methods illustrate chromatograms of polyunsaturated fatty acids (PUFAs) on Carbowax®-20M capillary columns. Any column providing the same elution pattern of FAMEs and baseline

Figure 1

Cocoa Butter FAME Reference Standard

Predicted

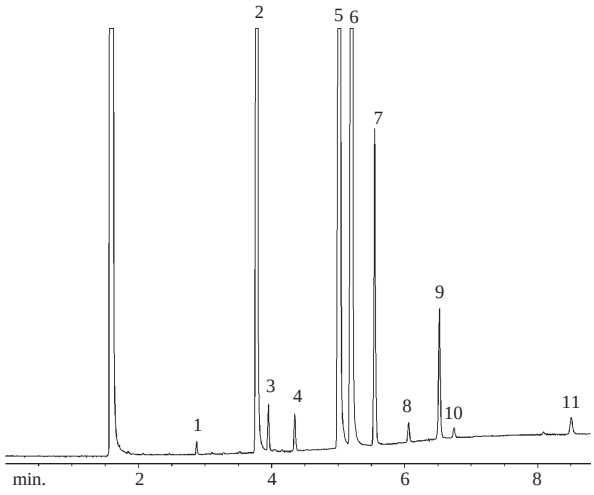


1. C14:0
2. C16:0
3. C16:1n7
4. C17:0
5. C18:0
6. C18:1n9
7. C18:2n6
8. C18:3n3
9. C20:0
10. C20:1n9
11. C22:0

30m, 0.25mm ID, 0.25µm Famewax™ (cat.# 12497).

Oven temp.:	200°C to 250°C @ 8°C/min. (hold 3 min.)
Carrier gas:	hydrogen
Linear velocity:	31cm/sec.

Actual



30m, 0.25mm ID, 0.25µm Famewax™ (cat.# 12497).
1.0µL split injection of #14 FAME Standard (cat.# 35035).

Oven temp.:	200°C to 250°C @ 8°C/min. (hold 3 min.)
Inj./det. temp.:	250°C
Det. sensitivity:	8 x 10^{-11} AFS
Carrier gas:	hydrogen
Linear velocity:	31.4cm/sec. @ 200°C
Split ratio:	45:1

separation of C21:5n3, C23:0, and C22:4n6 can be used for these methods. The peak height of the internal standard, methyl tricosanoate (C23:0), must be greater than 50% of the peak heights of methyl eicosapentaenoate (EPA) and docosahexanoate (DHA)[1]. In addition, Method Ce 1b-89 specifies that methyl tetracoanoate (C24:0) must be baseline resolved from DHA (C22:6n3)[2].

Pro ezGC™ software and a Famewax™ column were used to optimize the analysis of menhaden oil. Using the GC conditions suggested in these methods results in baseline resolution of all critical components, but with an analysis time of more than 45 minutes. The computer program was able to determine a faster, more efficient analysis time that still resulted in the required separations. A program with a single temperature ramp rate and a higher column flow rate allowed baseline separation of all critical components in only 30 minutes. Figure 2 is the actual optimized analysis of menhaden oil. Analysis time was reduced by 23 minutes, a greater than 50% savings in analysis time!

Calculating ECL Values for Polyunsaturated FAMEs Under Optimized Conditions

Since there are many mono-, di-, and polyunsaturated FAMEs in a menhaden oil sample, identification by ECL is useful. ECLs are similar to linear temperature-program indices, but are calculated relative to saturated FAMEs instead of hydrocarbons. Although the elution order did not change with the optimized chromatogram of menhaden oil, there could still be slight changes in the ECL values due to changes in retention of the individual FAMEs. To ensure the accuracy of the identifications, the *Pro ezGC*™ software can recalculate the ECL values from the actual retention times of the analyses. After entering a few marker retention times, such as the saturated FAMEs in the oil, the program calculates ECLs for all of the components in the sample. Table II illustrates the retention times and ECL values for the FAMEs in menhaden oil using the optimized conditions.

Summary

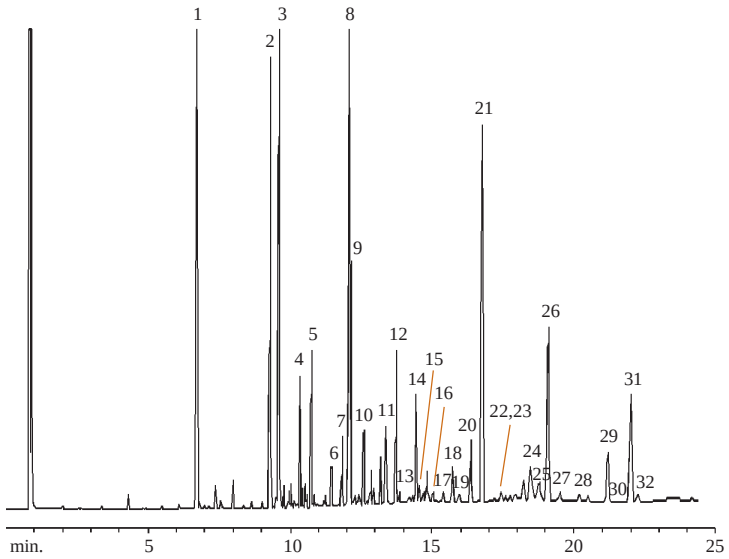
Computer modeling on the basis of TRIs is a powerful, accurate, and effective tool for optimizing the analysis of FAMEs. The predicted analysis can be illustrated clearly in the form of a list of components and retention times or by a simulated chromatogram. Comparisons between actual retention times of C4-C24 saturated FAMEs and predicted vs. actual chromatograms of cocoa butter FAMEs demonstrate the precision and accuracy of computer modeling. In addition, ECL values can be calculated for the actual column and conditions used.

References

1. *Association of Analytical Chemists, International AOAC Official Methods of Analysis. 15th Ed.: 3rd Supplement, 1992, pp 140-142.*
2. *American Oil Chemists Society, Official Methods and Recommended Practices of the American Oil Chemists Society, 1994. 8.*
3. *Christie, W.W., Gas Chromatography and Lipids, 1989, pp 92-96.*

Figure 2

Menhaden Oil PUFA Analysis (Actual Chromatogram)



1. C14:0	12. C18:4n3	23. C22:1n9
2. C16:0	13. C20:0	24. C22:2n6
3. C16:1n7	14. C20:1n9	25. C21:5n3
4. C16:2n4	15. C20:1n7	26. C23:0
5. C16:3n4	16. C20:2n6	27. C22:4n6
6. C16:4n1	17. C20:3n6	28. C22:5n6
7. C18:0	18. C20:4n6	29. C22:5n3
8. C18:1n9	19. C20:3n3	30. C24:0
9. C18:1n7	20. C20:4n3	31. C22:6n3
10. C18:2n6	21. C20:5n3	32. C24:1n9
11. C18:3n3	22. C22:0	

30m, 0.25mm ID, 0.25µm Famewax™ (cat.# 12497).
0.8µL split injection of menhaden oil PUFA with C23:0 (IS).
On-column concentration 100-150ng.

Oven temp.:	120°C to 220°C @ 7°C/min. (hold 20 min.).
Inj. & det. temp.:	220°C
Carrier gas:	hydrogen
Linear velocity:	60 cm/sec. set @ 120°C
FID sensitivity:	8 x 10^{-11} AFS
Split ratio:	50:1

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Table II

ECL values for FAMEs in Menhaden Oil

Retention Indices & Corrected Calculated Retention Times

Column:
30m, 0.25mm ID, 0.25µm Famewax™ column (cat.# 12497)

Oven temp.:
120°C to 220°C @ 7°C/min. (hold 11 min.)

Carrier gas:
hydrogen (constant pressure)

Linear velocity:
60 cm/sec. @ 120°C

Dead time:
0.833 min. @ 120°C

Component	Calc. tR (min.)	ECLs	Component	Calc. tR (min.)	ECLs
1. C14:0	6.630	14.00	17. C20:3n6	15.375	20.85
2. C16:0	9.230	16.00	18. C20:4n6	15.685	21.07
3. C16:1n7	9.539	16.27	19. C20:3n3	15.928	21.24
4. C16:2n4	10.260	16.87	20. C20:4n3	16.342	21.52
5. C16:3n4	10.669	17.19	21. C20:5n3	16.714	21.77
6. C16:4n1	11.358	17.70	22. C22:0	17.076	22.00
7. C18:0	11.775	18.00	23. C22:1n9	17.393	22.16
8. C18:1n9	12.023	18.22	24. C22:2n6	18.286	22.60
9. C18:1n7	12.098	18.29	25. C21:5n3	18.752	22.83
10. C18:2n6	12.567	18.69	26. C23:0	19.124	23.00
11. C18:3n3	13.347	19.33	27. C22:4n6	19.419	23.13
12. C18:4n3	13.702	19.60	28. C22:5n6	20.186	23.45
13. C20:0	14.224	20.00	29. C22:5n3	21.035	23.80
14. C20:1n9	14.437	20.16	30. C24:0	21.551	24.00
15. C20:1n7	14.536	20.24	31. C22:6n3	21.848	24.11
16. C20:2n6	15.019	20.60	32. C24:1n9	22.142	24.23

Product Listing

FAMEWAX™ Columns

ID	df (µm)	Temp. Limits	30m
0.25mm	0.25	20 to 250°C	11120
0.32mm	0.25	20 to 250°C	11136
0.53mm	0.50	20 to 250°C	11137

Methods Development Software

Description	cat.#
Pro ezGC™ (V1.0 for Windows®)	21487
Master Library Set	21460

RESTEK ISO 9001 REGISTERED

Petrochemical

Applications note

cat.# 59587

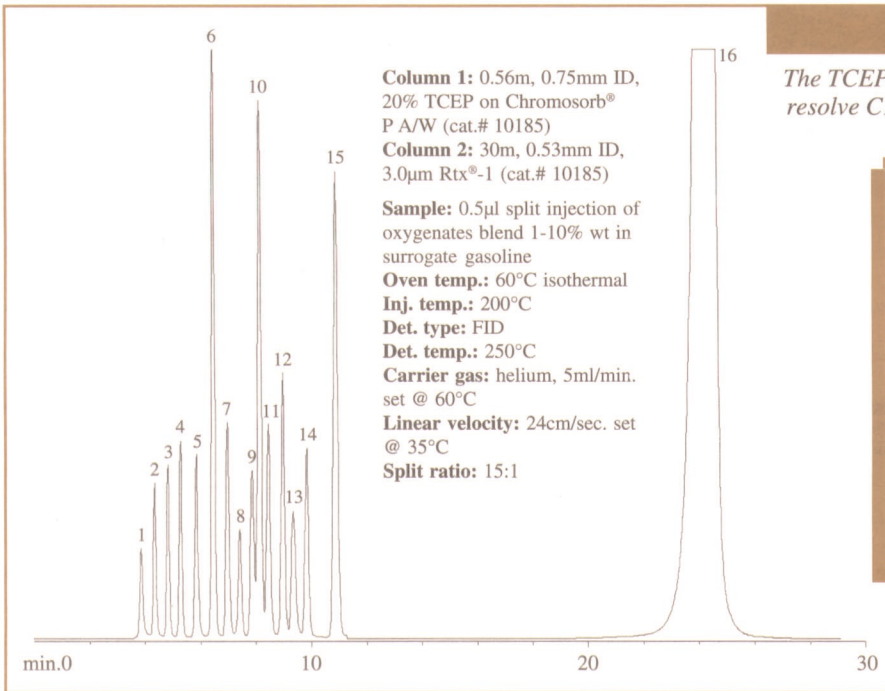
Analyzing Oxygenates in Gasoline

The EPA issued a mandate for the use of oxygenate enhancers as gasoline additives in 1989. Congress passed *The Clean Air Act*, further solidifying the implementation of reformulated oxygenated gasoline, in 1990. Oxygenates increase the octane rating of gasoline but reduce harmful emissions into the atmosphere. The phase-in period for reformulated oxygenated fuel began in 1989 and continued until 1995. Requirements for oxygenated fuel depend on geographic location with strict regulations in non-attainment areas.[1]

There are two GC methods which can be used for the measurement of the individual alcohols and ethers in gasoline. These two methods are the single-column OFID method[2,3] and the dual column ASTM method D4815-93.[4] Restek now offers columns, calibration standards, and specially deactivated tubing for the analysis of alcohols and ethers in gasoline according to both ASTM and EPA methodology.

ASTM and EPA Method Column Selection

Oxygenate additives in gasoline could potentially consist of several ethers and/or alcohols with either methyl tert-butylether (MTBE), ethyl tert-butylether (ETBE), or ethanol being major constituents. ASTM Test Method D4815-93 specifies the use of two columns: a micro-packed pre-column of 1,2,3-tris-2-cyanoethoxy-propane (TCEP), and an analytical capillary column of methyl silicone (Rtx®-1 or MXT®-1). These columns are configured with a 10-port valve to accomplish the heartcutting and backflushing necessary in order to resolve oxygenates from hydrocarbons present in gasoline. The sample is first directed to the TCEP column. This column has high retention for polar oxygenates, while the more volatile hydrocarbons are vented. The valve is then actuated, backflushing the remaining sample to the Rtx®-1 or MXT®-1 column where separation of oxygenates occurs. After the elution of the last oxygenate (tert-amyl methyl ether), the valve is redirected and remaining heavy hydrocarbons are backflushed from the Rtx®-1 column as a single peak. A separation example of all the specified alcohols and ethers appears in **Figure 1**.



Column 1: 0.56m, 0.75mm ID, 20% TCEP on Chromosorb® P A/W (cat.# 10185)
Column 2: 30m, 0.53mm ID, 3.0µm Rtx®-1 (cat.# 10185)

Sample: 0.5µl split injection of oxygenates blend 1-10% wt in surrogate gasoline
Oven temp.: 60°C isothermal
Inj. temp.: 200°C
Det. type: FID
Det. temp.: 250°C
Carrier gas: helium, 5ml/min. set @ 60°C
Linear velocity: 24cm/sec. set @ 35°C
Split ratio: 15:1

Figure 1

The TCEP and Rtx®-1 columns, connected in series, resolve C1-C4 alcohols, MTBE, ETBE, and TAME.

1. methanol
2. ethanol
3. iso-propanol
4. tert-butanol
5. *n*-propanol
6. methyl-tert-butyl ether (MTBE)
7. sec-butanol
8. di-isopropyl ether (DIPE)
9. iso-butanol
10. ethyl-tert-butyl ether (ETBE)
11. tert-amyl ether
12. dimethoxy ethane (DME)
13. *n*-butanol
14. benzene
15. tert-amyl methyl ether (TAME)
16. total heavy hydrocarbons

Fused silica lined stainless steel improves peak shapes for alcohols.

In order to achieve optimum peak width in this valve system, small diameter sample transfer tubing is recommended to minimize band broadening and resolution loss. Because alcohols can adsorb on both the stainless steel transfer line tubing and TCEP pre-column stainless steel surface, Restek recommends using fused silica lined stainless steel (Silcosteel®) for transfer lines and the TCEP pre-column. Silcosteel® tubing provides the inertness of fused silica tubing, resulting in excellent peak shape for the oxygenates (**Figure 1**). The Restek TCEP Micropacked column is prepared using 0.75mm ID tubing, which gives a more reproducible retention time than columns prepared from smaller ID tubing. This column also produces a slightly longer and more reproducible valve time (i.e.: 0.28 minutes), which helps when initially setting this critical parameter. For methods using the oxygen specific OFID, a 60-meter Rtx®-1 column will resolve the oxygenated components.

High purity standards are available for a 5-point calibration curve.

Restek now offers calibration standards that meet the method requirements found in all three oxygenates test methods, including ASTM D4815, ASTM D5599, and the EPA OFID. Restek's QA tests guarantee 99%+ individual compound purity required by ASTM and EPA procedures. After preparation using a NIST traceable balance, each solution undergoes extensive GC testing to assure that calibration mixes meet strict standards of accuracy. A data pack containing purity data and complete quality assurance documentation is available for each of these standards. All three methods specify using multi-point calibration with a minimum of five points for all 12 oxygenates. Quantitation of the individual oxygenates is performed using an internal standard such as 1,2-dimethoxyethane, present at 5 WT% level in all standards. The recommended concentration range of alcohols is determined by the concentrations typically expected in reformulated gasoline, whereas the ethers are between 0.1 to 20 WT%. In addition, the total mass of oxygen content (including the internal standard), in each calibration mixture, must not exceed 30%. Restek's oxygenate in gasoline calibration standards meet or exceed all ASTM and EPA method specifications.

To meet the requirements of ASTM Test Method D 4815-93, an analyst must consider the sample handling system, calibration standards, and choice of columns. By implementing a low volume valve and small inner diameter Silcosteel® transfer lines, optimum resolution of oxygenates can be achieved. In addition, by using a Silcosteel® 0.75mm ID TCEP pre-column and the Rtx®-1 or MXT®-1 analytical column, optimum resolution can be attained. For the OFID procedures, Restek offers a low-bleed, 60-meter Rtx®-1 or MXT®-1 methyl silicone column.

We offer the columns, calibration standards, and specially deactivated tubing specifically for the analysis of alcohols and ethers in gasoline according to ASTM and EPA methodology.

Product List

For ASTM D4815-93:

Rtx®-1
30m, 0.53mm ID, 3.0µm
cat.# 10185
or
MXT®-1 (Silcosteel®)*
30m, 0.53mm ID, 3.0µm
cat.# 70185
and
TCEP (Micropacked)
0.56m, 0.75mm ID
cat.# 19040

Valve Transfer Line:

Silcosteel® Fused Silica Lined Stainless Steel
6 feet, 1/16" OD, 0.020" ID
cat.# 20524

OFID Procedure:

Rtx®-1
60m, 0.25mm ID, 1.0µm
cat.# 10156

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Have Questions? Call Restek's technical service staff at:
800-356-1688, ext. 4

**A rugged Silcosteel® MXT®-1 column can be used as an alternative to a fused silica column in applications where column breakage is a concern.*

ASTM D5599-94 Oxygenate Calibration Mixtures

ASTM Oxygenate Calibration Kit: cat.# 36223 and cat.# 36223-500 (w/data pack)
Includes 1ml each of the 9 mixes below, plus the ASTM Surrogate Base Gas, cat.# 36222.

Compound	Mix #1	Mix #2	Mix #3	Mix #4	Mix #5	Mix #6	Mix #7	Mix #8	Mix #9
tert-amyl methyl ether	—	13.5	—	4.50	1.00	9.00	—	18.0	—
butanol	2.00	1.00	0.80	0.50	0.10	—	—	—	—
sec-butanol	—	0.10	1.00	0.70	0.50	—	—	2.50	—
t-butanol	0.10	1.00	2.00	—	0.50	—	—	0.30	—
diisopropyl ether	0.50	1.00	0.30	2.00	—	0.10	—	—	—
1,2-dimethoxyethane (IS)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
ethanol	—	5.00	7.50	10.0	12.0	—	—	1.00	—
ethyl-tert-butyl ether	—	—	9.00	4.50	1.00	—	18.0	—	13.5
isobutanol	1.00	2.00	—	—	0.50	0.30	—	0.10	—
isopropanol	0.10	—	0.50	—	2.00	—	0.30	1.00	—
methanol	0.10	—	1.00	2.50	5.00	0.50	—	—	—
methyl-tert-butyl ether	17.0	—	—	—	1.00	15.0	6.00	—	11.5
t-pentanol	2.00	1.00	0.70	—	0.50	—	—	0.10	—
propanol	2.00	0.40	1.00	—	0.70	—	—	0.20	—
Total Oxygenates	29.80%	30.00%	28.80%	29.70%	29.80%	29.90%	29.30%	28.20%	30.00%
Surrogate Base Gasoline	70.20%	70.00%	71.20%	70.30%	70.20%	70.10%	70.70%	71.80%	70.00%
Quantity	***Cat.#***	***Cat.#***	***Cat.#***	***Cat.#***	***Cat.#***	***Cat.#***	***Cat.#***	***Cat.#***	***Cat.#***
each	36213	36214	36215	36216	36217	36218	36219	36220	36221
each w/data pk.	36213-500	36214-500	36215-500	36216-500	36217-500	36218-500	36219-500	36220-500	36221-500
5pk.	36213-510	36214-510	36215-510	36216-510	36217-510	36218-510	36219-510	36220-510	36221-510
5pk. w/data pk.	36213-520	36214-520	36215-520	36216-520	36217-520	36218-520	36219-520	36220-520	36221-520
10pk. w/data pk.	36313	36314	36315	36316	36317	36318	36319	36320	36321

Mixtures 1-9 prepared on a WT/WT% basis, 1ml ampul.

Restek also offers a complete set of standards for five-point calibration to meet ASTM and EPA calibration requirements (see our new product guide).

[1] Peaff, George, *C&EN,* September 26, 1994, pp 8-13.

[2] 40 CFR Part 30, Federal Register, 59(32): 7716-7878, Feb. 16, 1994.

[3] ASTM Test Method D5599-94, *Determination of Oxygenates in Gasoline by Gas Chromatography and Selective Flame Ionization Detection.*

[4] ASTM Test Method D4815-93, *Standard Test Method for Determination of MTBE, ETBE, TAME, DIPE, tertiary-amyl Alcohol and C1 to C4 Alcohols in Gasoline by Gas Chromatography.*

Call (800) 356-1688, ext. 5, to request other Application Notes:

Environmental

Pesticide Analysis (# 59555)
Analysis of Priority Pollutants (# 59556)
SPE Extraction for EPA Method 525.1 (# 59557)
PCB Screening in Less Than 10 Minutes (# 59558)
Optimizing the Analysis of Chlorophenoxy Herbicides (# 59559)
Analysis of Triazine Herbicides (# 59586)
P&T of Volatile Organics Using Narrowbore Cap. Columns (# 59560)
Fused Silica Lined Canisters for TO-14 Ambient Air Sampling (# 59561)
Florisil® Cleanup for Organochlorine Pesticides & PCBs (# 59562)
Extraction of Paraquat & Diquat from Water Using Resprep™-C8-47 Extraction Disks (# 59563)
Extraction of PCBs Using Resprep™-C18-47 SPE Disks and Resprep™-6D (# 59564)
Extraction of PAHs Using Resprep™-C18-47 SPE Disks and Resprep™-6D (# 59565)

Clinical/Forensic

Fire Debris Analysis (# 59574)
Barbiturate Analysis (# 59575)
Opiate Analysis (# 59576)
Updates on USP 467 (# 59577)

Technical Tips

Inlet Sleeve Deactivation (# 59578)
Restek's Thermal Gas Purifier vs. Conventional Traps (# 59588)

Food & Flavor

Analyzing Essential Oils (# 59554)
Analyze Flavor Volatiles in Alcoholic Beverages (# 59579)
Fast Selective Triglyceride Analysis (# 59580)
Analysis of Cholesterol and Other Dietary Solids (# 59581)
Detection of Synthetic and Natural Antioxidants in Food (# 59582)
Analyzing Free Fatty Acids (# 59583)
Analyzing FAMES (# 59584)
Using Computer Modeling to Optimize FAME Analysis (# 59585)

Capillary Columns

The New Rtx®-Wax Column (# 59571)
Aromatics in Reformulated Gasoline by GC/MS (# 59572)

Petrochemical

Analyze Fixed Gases Using the New Rt-Msieve™ 13XPLOT Column (# 59566)
New Cap. Column for Simulated Distillation of Petroleum Fractions (# 59567)
New Rtx®-1 PONA Column for Analysis of Petroleum Products (# 59568)
Analyzing Oxygenates in Gasoline (# 59587)
Restek's Al_2O_3 PLOT Column (# 59569)
Rtx®-1: A New Bonded Packed Column for Simulated Distillation (# 59570)

Micro & Packed

Analyzing Sulfur Comp. @ Trace Levels Using RT-Sulfur P. Cols. (# 59573)

Have Questions? Call Restek's technical service staff at:
800-356-1688, ext. 4

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Fairacres Industrial Centre, Dedworth Road, Windsor, Berkshire, England SL4 4LE
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RESTEK

clinical/forensic

Applications note

Dual-Column Confirmational GC Analysis of Blood Alcohols Using the Rtx®-BAC1 and Rtx®-BAC2 Columns Optimized for the Perkin-Elmer HS 40 Headspace Autosampler

Testing for the presence and subsequent quantitation of ethanol in blood, breath, and urine are the highest volume tests performed in forensic laboratories. In addition to ethanol, the detection of several other significant alcohols and their metabolites is necessary. Gas chromatographic (GC) assays provide the greatest amount of flexibility and specificity in analyzing for these volatile compounds. Direct injection of biological samples into GC columns has been used in the past as the method of sample introduction. This typically leads to column contamination and decreased performance. The incorporation of headspace sampling into the method prevents the buildup of non-volatile contamination at the head of the column and helps to maintain consistent performance and extend column lifetime.

Analysis time and resolution are two critical factors when developing a GC assay for ethanol. Analysis time for each sample should be as short as possible, while still maintaining baseline resolution for all analytes. Isothermal analysis is the method of choice because it eliminates the cool-down period between temperature programmed runs. Overall analysis time can be reduced in isothermal analysis by raising the temperature of the analysis or by increasing carrier gas flow rate. However, in attempting to shorten the analysis time, either by increasing the flow rate or by raising the temperature, many traditional capillary column stationary phases fail to provide adequate resolution of all of the components commonly tested during blood alcohol analysis. Restek has designed two novel capillary column stationary phases to meet all of these requirements.

Our experimentation focused on using these two unique stationary phases in combination with headspace sampling to provide dual-column confirmational analysis of blood alcohol in less than three minutes. The equipment used included a Perkin-Elmer Auto SYS GC with a split injection port and dual-flame ionization detectors, and the Perkin-Elmer HS 40 headspace autosampler. Instrument control and data collection were provided by using Perkin-Elmer Nelson Turbochrom software, interfaced through a Perkin-Elmer Nelson Link 900.

The HS 40 uses a balanced pressure sampling procedure to transport the sample to the GC. This type of sampling works better with columns that require higher head pressure. 0.32mm ID analytical columns were chosen for this application because of their higher operating pressure. Optimal performance of these columns during headspace analysis depends on GC/headspace system set up. Band broadening can occur if there is excess dead volume in the sample flow path between the sample valve and the head of the column. Low volume inlet liners or interfaces in the injection port should be used to reduce the amount of excess volume at the exit end of the transfer line. A 2mm ID sleeve was used in this analysis to reduce dead volume and maintain narrow peak widths. High carrier gas flow rates through the transfer line also can be used to maintain narrow sample bandwidths and speed up sample transfer to the head of the column. A flow of 40mL-per-minute was used to optimize the analysis on the HS 40.

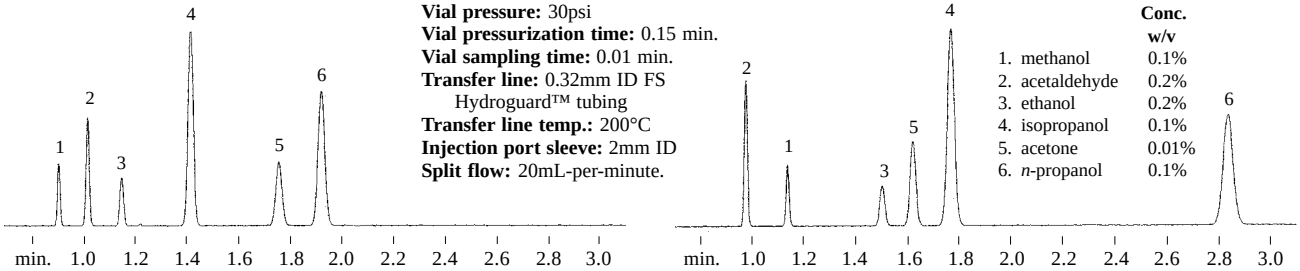
Figure 1

Achieve baseline resolution of all blood alcohol components in less than 3 minutes using the Rtx®-BAC1 and Rtx®-BAC2 columns and a Perkin-Elmer HS 40 headspace autosampler.

30m, 0.32mm ID, 1.8µm Rtx®-BAC1 (cat.# 18003)

30m, 0.32mm ID, 1.2µm Rtx®-BAC2 (cat.# 18002)

Dual column analysis using a two-hole ferrule.
1.0mL headspace sample of a blood alcohol mix
Oven temp.: 40°C isothermal
Inj. temp.: 200°C
Carrier gas: He
Sample equilibration temp.: 70°C
Sample equilibration time: 15 min.
Vial pressure: 30psi
Vial pressurization time: 0.15 min.
Vial sampling time: 0.01 min.
Transfer line: 0.32mm ID FS Hydroguard™ tubing
Transfer line temp.: 200°C
Injection port sleeve: 2mm ID
Split flow: 20mL-per-minute.



	Conc. w/v
1. methanol	0.1%
2. acetaldehyde	0.2%
3. ethanol	0.2%
4. isopropanol	0.1%
5. acetone	0.01%
6. *n*-propanol	0.1%

Simulated blood alcohol samples were prepared and analyzed using a modification of a procedure published by Christmore et al.[1] *n*-Propanol was used as the internal standard and was prepared at a concentration of 0.03g/dL in 1.0M ammonium sulfate as a diluent. Five milliliters of diluent was added to 1mL of sample in a 20mL headspace vial.

Performance Results

The PE Auto SYS GC and HS 40 headspace autosampler, combined with Restek's Rtx®-BAC1 and Rtx®-BAC2 columns, provided excellent accuracy and precision in the analysis of blood alcohol. Calibration curves were constructed using concentrations ranging from 0.01% to 0.5% ethanol. Correlation coefficients above 0.999 were easily obtained for all compounds. Response factor repeatability was less than ±1% standard deviation while analyzing six samples at a concentration of 0.2% ethanol. Based on our experimentation, a system detection limit of 0.001% ethanol should be achievable while maintaining a minimum signal-to-noise ratio of 10.

Conclusion

Successful dual-column confirmational blood alcohol analysis can be achieved in less than 3 minutes by combining Restek's 0.32mm ID Rtx®-BAC1 and Rtx®-BAC2 columns with the Perkin-Elmer Auto SYS and HS 40 headspace autosampler.

References

1. D. S. Christmore, R. C. Kelly, and L. A. Doshier, Journal of Forensic Science, Volume 29, No. 4, October 1984, pp.1038-1044.

Product Listing

Rtx®-BAC1 GC Columns

Length	ID	df (µm)	cat.#
30m	0.53mm	3.0	18001
30m	0.32mm	1.8	18003

Rtx®-BAC2 GC Columns

Length	ID	df (µm)	cat.#
30m	0.53mm	2.0	18000
30m	0.32mm	1.2	18002

Hydroguard™ Fused Silica Transfer Lines

Length	ID	OD	cat.#
5m	0.18mm	0.34mm	10078
5m	0.25mm	0.37mm	10079
5m	0.32mm	0.45mm	10080
5m	0.53mm	0.69mm	10081

Splitless Sleeves for Perkin-Elmer GCs

Description	each	5-pk.	25-pk.
2mm ID	20730	20731	20732
Auto SYS 2mm ID w/wool	20829	20830	20831
Auto SYS XL 2mm ID	21717	21718	——

Headspace Autosampler Vials & Seals

Description	100-pk.	1000-pk.
10mL clear vial, rounded bottom	21164	21165
silver seal w/PTFE/Silicone septa	21763	21764

Dual-Column Analysis Replacement Ferrules

Two-hole ferrule size	Fits column ID	Graphite	Vespel®/ Graphite
0.4mm (1/16")	0.25mm	20235 (5-pk.)	20241 (5-pk.)
0.5mm (1/16")	0.32mm	20235 (5-pk.)	20242 (5-pk.)
0.8mm (1/8")	0.53mm	20245 (5-pk.)	20246 (5-pk.)

RESTEK
HPLC

Applicationsnote

Pinnacle™ DB HPLC Columns as Replacements for Hypersil® BDS

Why choose Pinnacle™ DB columns?

- Performance—equivalent replacement for Hypersil® BDS columns.
- Superior base deactivation and low total metals content-ideal for basic compounds.
- Excellent lot-to-lot and column-to-column reproducibility.
- In stock, ready to ship - 90% of columns ship same day/next day of order.
- Wide selection of stock column dimensions, including semi-prep and prep scale.
- ISO 9001:2000 registered facility.
- Satisfaction guaranteed.

When we set out to create a new base-deactivated silica, we had five specific goals:

- A material that would match or exceed the performance of any base-deactivated "Type B" silica.
- A material that would provide chromatographic separations equivalent to Hypersil® BDS material.
- A material with low metals content, for sharp, symmetric peaks for basic analytes.
- A rugged material compatible with the pressures and liquid environment of typical HPLC applications.
- An effective and efficient manufacturing process that gives consistent, reproducible quality.

We have attained each of these goals.

Pinnacle™ DB silica will meet or exceed the performance of any Type B base-deactivated silica in its class. In Figure 1, peak symmetry for the basic compound pyridine is significantly better, and analysis time is notably shorter on the Pinnacle™ DB C18 column than on a typical Type B C18 column. Expect similar results in your own comparisons.

We have matched the desirable physical characteristics of Hypersil® BDS material. Figures 2 and 3 show the excellent agreement between particle size and pore size distributions for Pinnacle™ DB and Hypersil® BDS materials.

Figure 1: Pyridine/phenol test mix.

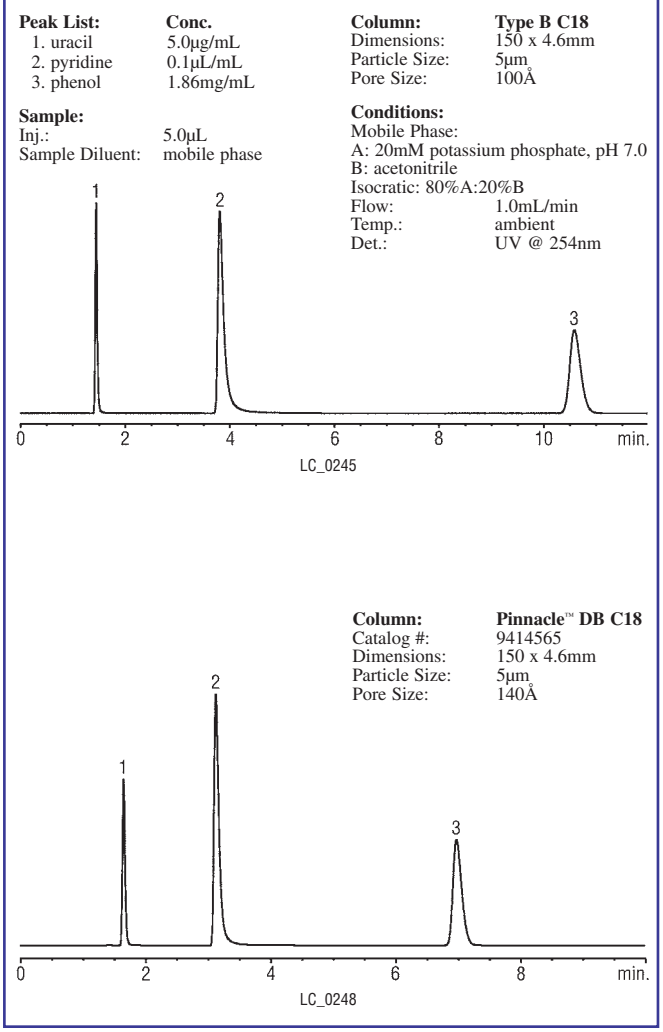
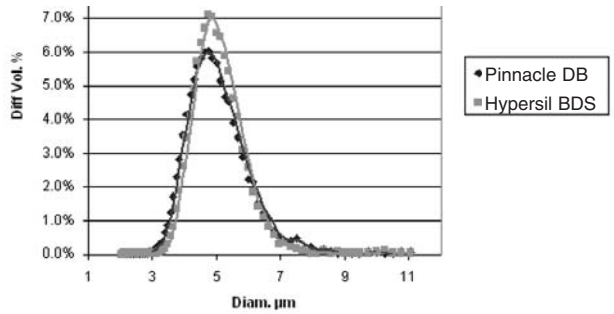
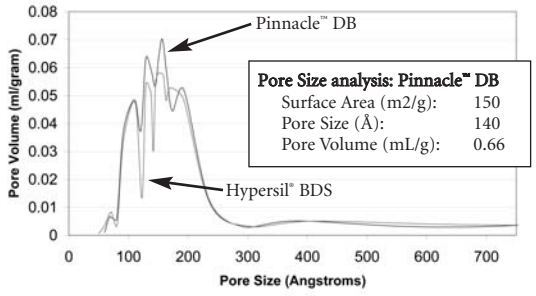


Figure 2: Equivalent particle size distribution for Pinnacle™ DB silica and Hypersil® BDS silica.



Particle Size analysis	Pinnacle DB	Hypersil BDS
Geometric Mean (µm):	4.81±1.20	4.89±1.70
Arithmetic Mean (µm):	4.89±0.94	4.95±0.82
Median (µm):	4.69	4.81
Mode:	4.69	4.79
Max Diff. (%):	6.04	7.11

Figure 3: Pinnacle™ DB silica and Hypersil® BDS silica have remarkably equivalent pore volume distribution.

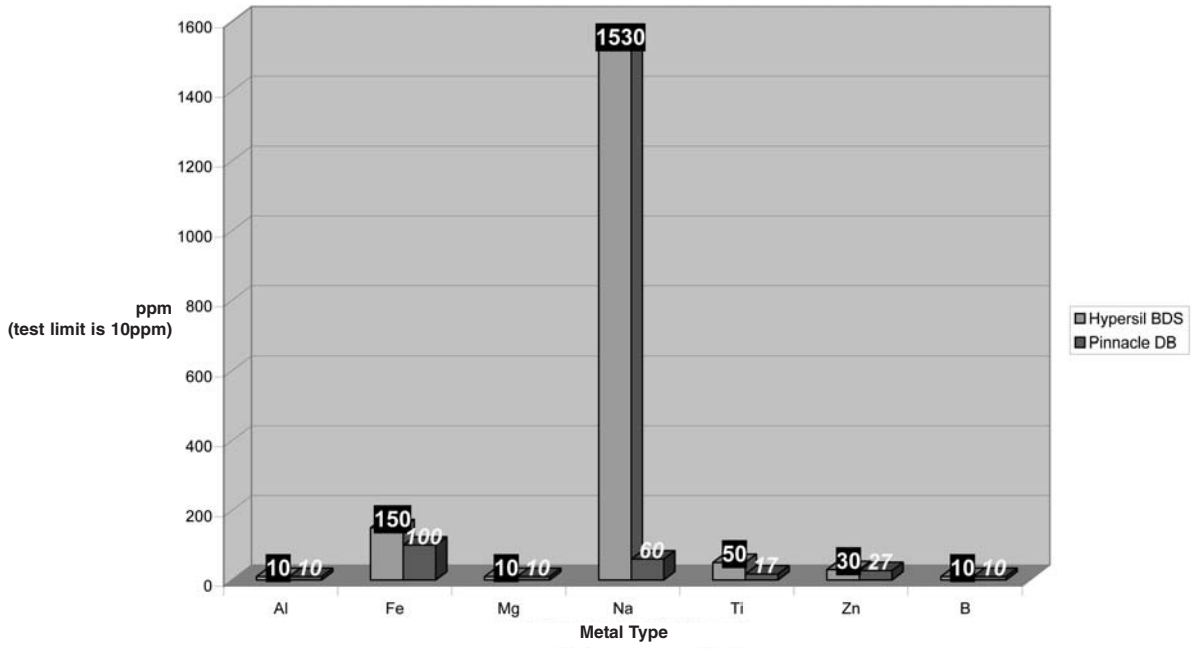


For certain physical parameters we intentionally deviated from Hypersil® BDS material - total metals content, for example. Fewer metal ions on the surface of a silica particle make for sharper and more symmetric peaks for basic analytes. Our new manufacturing process strips metals from the silica surface, and smoothes the surface as well. Figure 4 compares the content of various metals in Pinnacle™ DB and Hypersil® BDS materials. For every metal measured, Pinnacle™ DB silica and Hypersil® BDS silica have equivalent content, or the content in Pinnacle™ DB is lower.

Of course, what really matters is how separations on Pinnacle™ DB columns and Hypersil® BDS columns compare. Figures 5–11 pair chromatograms for various analyte mixes from C18 bonded phase versions of the two silicas; each pair was obtained using the same instrument and mobile phase. Note the similarity in retention, asymmetry, and efficiency in each set of chromatograms.

Behavior of each of these analytes will vary, based on differences for factors such as carbon load, ligand density, degree of base deactivation, endcapping, and metals content. Columns that are similar for these characteristics should provide similar chromatography. Figures 5–11 show a Pinnacle™ DB C18 column and a Hypersil® BDS column perform nearly identically.

Figure 4: Consistently low metals content in Pinnacle™ DB silica ensures sharper, more symmetric peaks for basic analytes.



How important is metals content?

Silica manufacturers and most chromatographers well know that metals, especially metal ions, on the surface of a silica particle negatively affect peak symmetry and otherwise interfere with chromatography, particularly for basic analytes. Consequently, silica manufacturers use various treatments to eliminate surface metals or prevent contact between surface metals and sample components.

Silicas that have a high metal content – especially sodium – are structurally inferior to silicas containing small amounts of metals. To strengthen silica particles containing high concentrations of sodium and other metals, some manufacturers use an annealing process to embed the sodium into the framework of the particles. Although annealing imparts strength to the particles, it destroys some of their useable surface area. Worse, as the particles age or crack, shielded metals that were embedded in the silica through the annealing procedure, and thereby escaped surface deactivation treatments, are re-exposed to wreak havoc with a separation. The analyst observes loss of base deactivation and further erosion of the particles' stability in highly aqueous mobile phases.

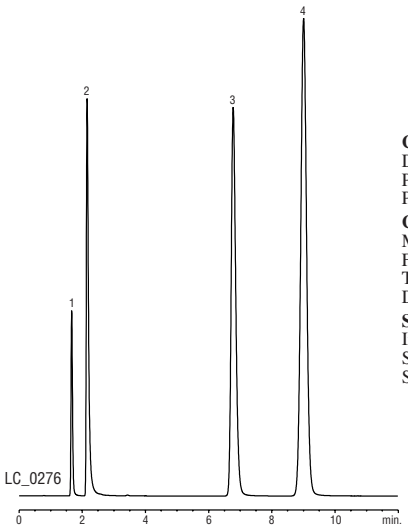
Pinnacle™ DB silica has a particularly low total sodium value; it does not need annealing to impart strength, as strength comes from Restek's use of self-assembled materials technology. By removing excess metals and other impurities, we make a more rugged silica. This means a potentially longer lifetime for a column made from this material, and more consistent peak shapes for bases as the column ages. Lowering total metals content during the creation of the product increases the usable lifetime of the product.

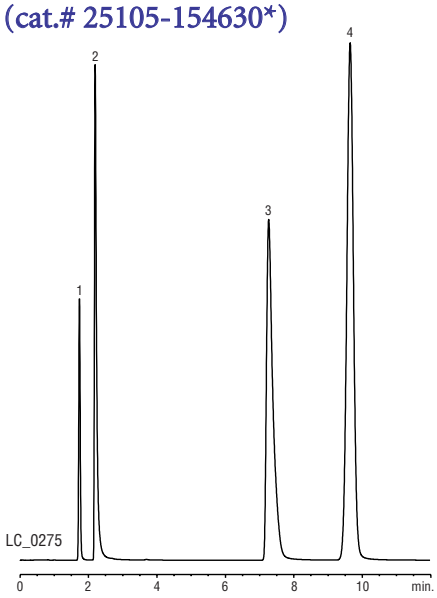
Figure 5: Test mix on Pinnacle™ DB and Hypersil® BDS C18 columns.

Pinnacle™ DB C18 Column (cat.# 9414565)

Nearly identical retention, peak symmetry, and efficiency!

Hypersil® BDS C18 Column (cat.# 25105-154630*)





Column:
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å/130Å

Conditions:
Mobile Phase: water:methanol (40:60 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

Sample:
Inj.: 5µL
Sample Diluent: methanol:water (50:50 v/v)
Sample Temp.: ambient

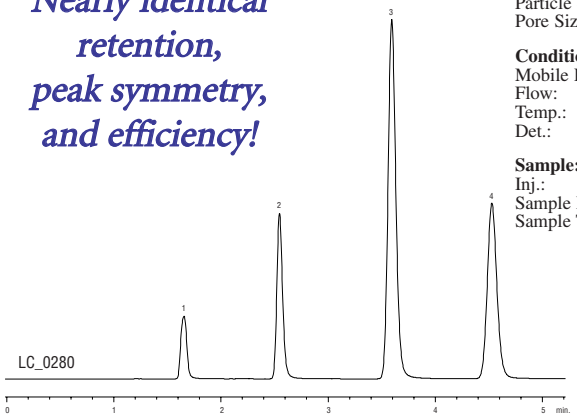
Peak List:	Conc.	Retention Times Pinnacle DB	Hypersil BDS
1. uracil	1µg/mL	1.666	1.737
2. pyridine	0.64mg/mL	2.155	2.196
3. N,N-dimethylaniline	0.45mg/mL	6.778	7.267
4. benzophenone	0.59mg/mL	9.006	9.646

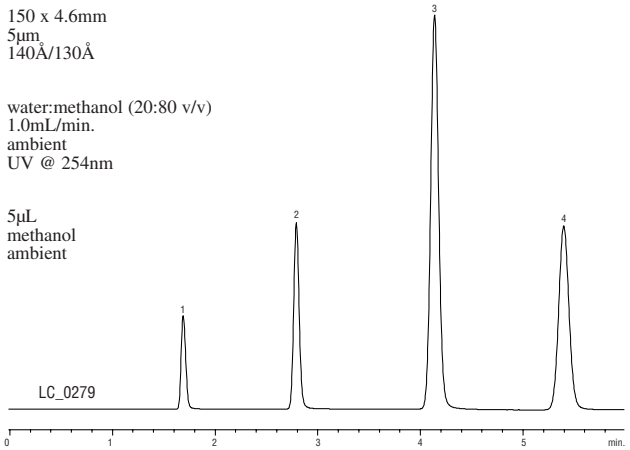
Figure 6: Reversed phase test mix on Pinnacle™ DB and Hypersil® BDS C18 columns.

Pinnacle™ DB C18 Column (cat.# 9414565)

Nearly identical retention, peak symmetry, and efficiency!

Hypersil® BDS C18 Column (cat.# 25105-154630*)





Column:
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å/130Å

Conditions:
Mobile Phase: water:methanol (20:80 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

Sample:
Inj.: 5µL
Sample Diluent: methanol
Sample Temp.: ambient

Peak List:	Conc.	Retention Times Pinnacle DB	Hypersil BDS
1. uracil	20µg/mL	1.655	1.689
2. benzene	3mg/mL	2.546	2.791
3. naphthalene	0.5mg/mL	3.594	4.138
4. biphenyl	60µg/mL	4.529	5.396

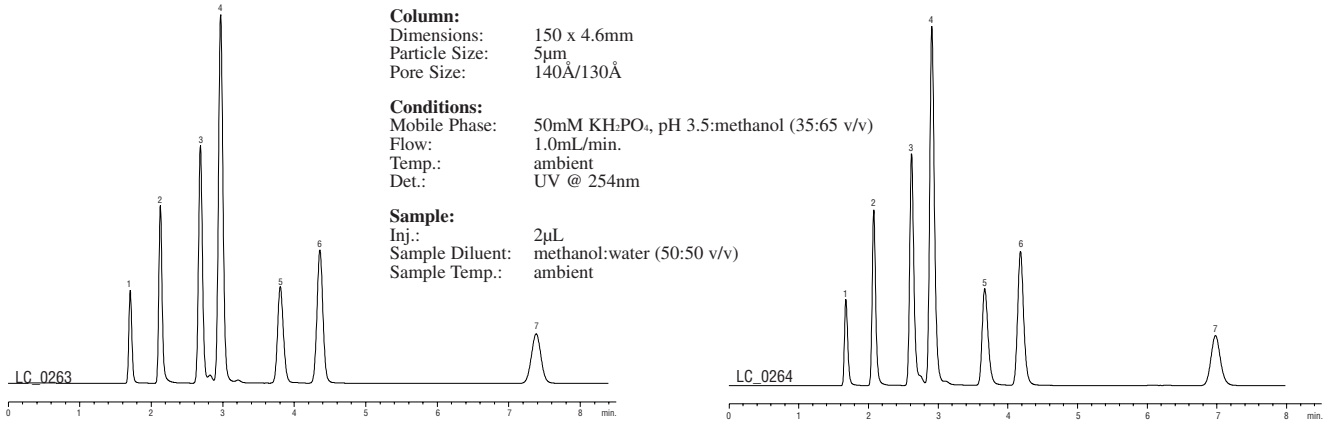
**Hypersil® catalog number; column not available from Restek.*

Figure 7: Base/neutral test mix on Pinnacle™ DB and Hypersil® BDS C18 columns.

Pinnacle™ DB C18 Column (cat.# 9414565)

Nearly identical retention, peak symmetry, and efficiency!

Hypersil® BDS C18 Column (cat.# 25105-154630*)



Column:
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å/130Å

Conditions:
Mobile Phase: 50mM KH_2PO_4, pH 3.5:methanol (35:65 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

Sample:
Inj.: 2µL
Sample Diluent: methanol:water (50:50 v/v)
Sample Temp.: ambient

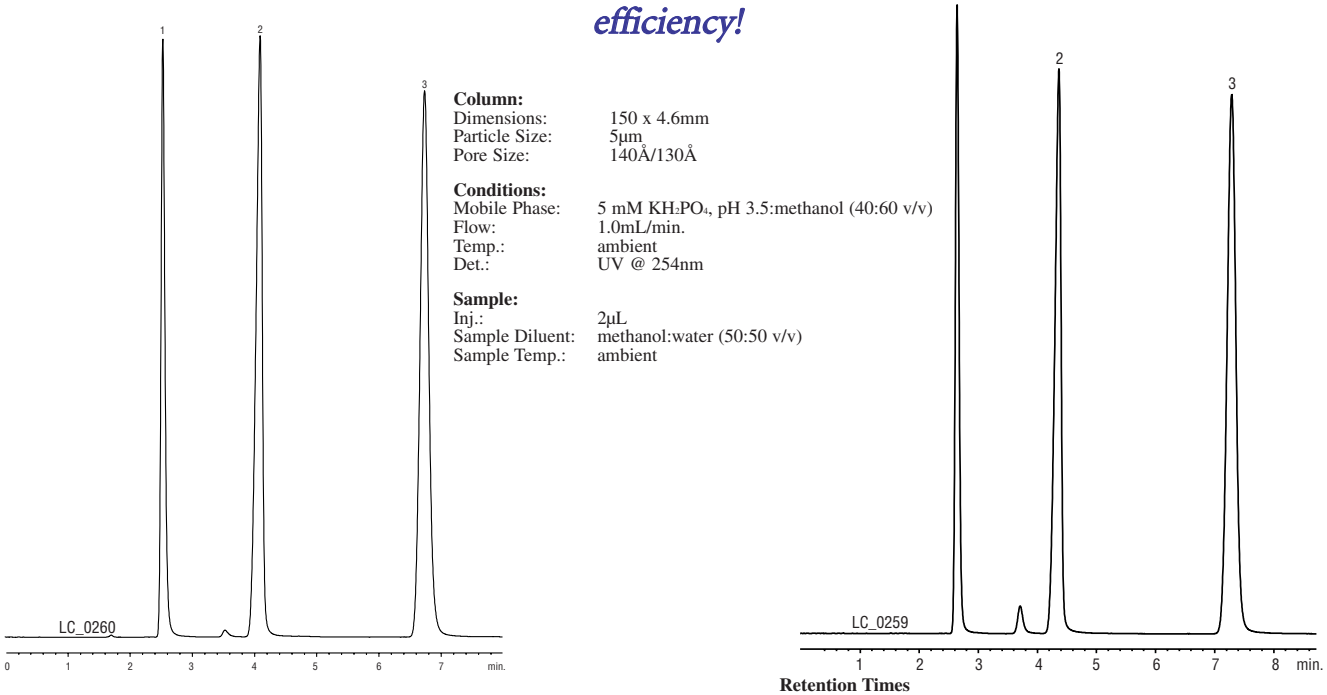
Peak List:	Conc.	Retention Times Pinnacle DB	Hypersil BDS
1. uracil	50µg/mL	1.676	1.705
2. aniline	0.75mg/mL	2.077	2.127
3. 2-nitroaniline	0.35mg/mL	2.618	2.687
4. 2,4-nitroaniline	0.35mg/mL	2.909	2.970
5. 3,5-xylenol	2.5mg/mL	3.668	3.804
6. anisole	2.5mg/mL	4.181	4.359
7. toluene	3.5mg/mL	6.979	7.383

Figure 8: Acid base test mix on Pinnacle™ DB and Hypersil® BDS C18 columns.

Pinnacle™ DB C18 Column (cat.# 9414565)

Nearly identical retention, peak symmetry, and efficiency!

Hypersil® BDS C18 Column (cat.# 25105-154630*)



Column:
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å/130Å

Conditions:
Mobile Phase: 5 mM KH_2PO_4, pH 3.5:methanol (40:60 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

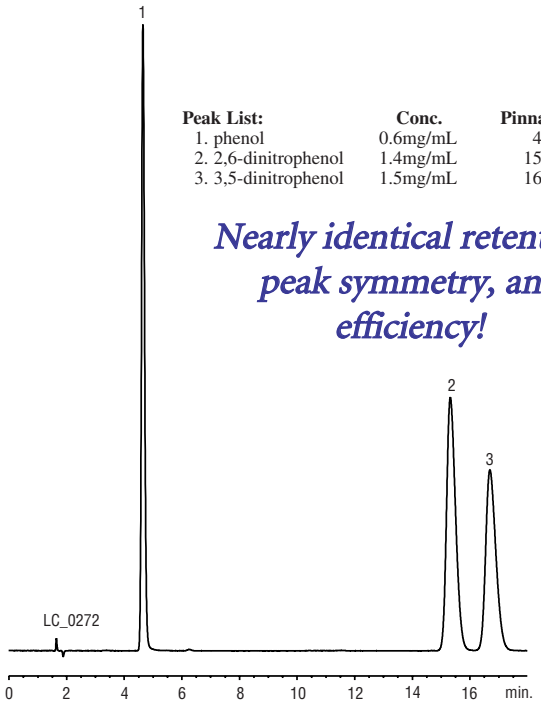
Sample:
Inj.: 2µL
Sample Diluent: methanol:water (50:50 v/v)
Sample Temp.: ambient

Peak List:	Conc.	Retention Times Pinnacle DB	Hypersil BDS
1. caffeine	0.5mg/mL	2.525	2.640
2. acetylsalicylic acid	3.5mg/mL	4.088	4.363
3. benzoic acid	2.8mg/mL	6.733	7.288

**Hypersil® catalog number; column not available from Restek.*

Figure 9: Phenols on Pinnacle™ DB and Hypersil® BDS C18 columns.

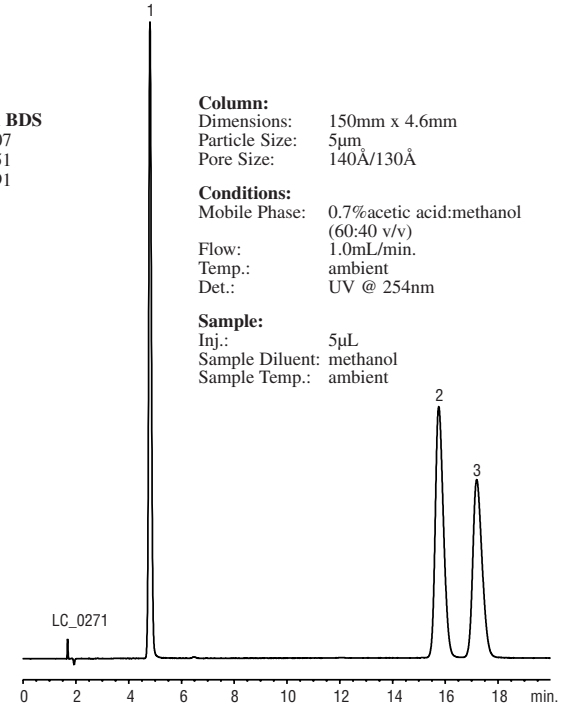
Pinnacle™ DB C18 Column (cat.# 9414565)



Peak List:	Conc.	Retention Times Pinnacle DB	Hypersil BDS
1. phenol	0.6mg/mL	4.647	4.807
2. 2,6-dinitrophenol	1.4mg/mL	15.318	15.751
3. 3,5-dinitrophenol	1.5mg/mL	16.691	17.191

Nearly identical retention, peak symmetry, and efficiency!

Hypersil® BDS C18 Column (cat.# 25105-154630*)



Column:
Dimensions: 150mm x 4.6mm
Particle Size: 5µm
Pore Size: 140Å/130Å

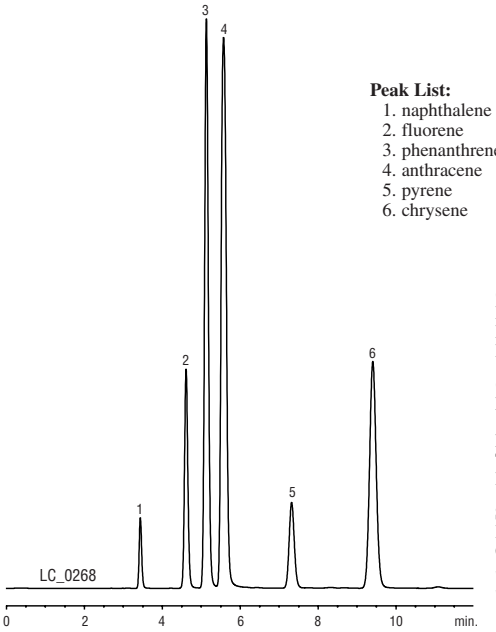
Conditions:
Mobile Phase: 0.7%acetic acid:methanol (60:40 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

Sample:
Inj.: 5µL
Sample Diluent: methanol
Sample Temp.: ambient

Figure 10: PAHs on Pinnacle™ DB and Hypersil® BDS C18 columns.

Pinnacle™ DB C18 Column (cat.# 9414565)

Nearly identical retention, peak symmetry, and efficiency!



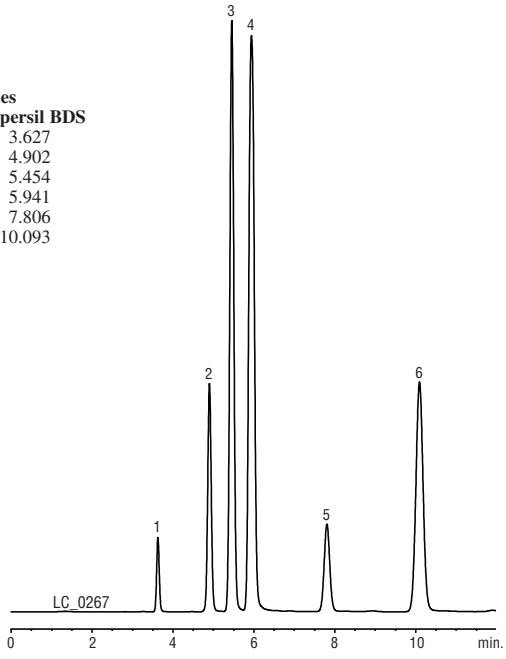
Peak List:	Retention Times Pinnacle DB	Hypersil BDS
1. naphthalene	3.436	3.627
2. fluorene	4.611	4.902
3. phenanthrene	5.132	5.454
4. anthracene	5.574	5.941
5. pyrene	7.320	7.806
6. chrysene	9.404	10.093

Column:
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å/130Å

Conditions:
Mobile Phase: water:acetonitrile (25:75 v/v)
Flow: 1.0mL/min
Temp.: ambient
Det.: UV @ 254nm

Sample:
Inj.: 5µL
Conc.: 100µg/mL each
Sample Diluent: acetonitrile
Sample Temp.: ambient

Hypersil® BDS C18 Column (cat.# 25105-154630*)



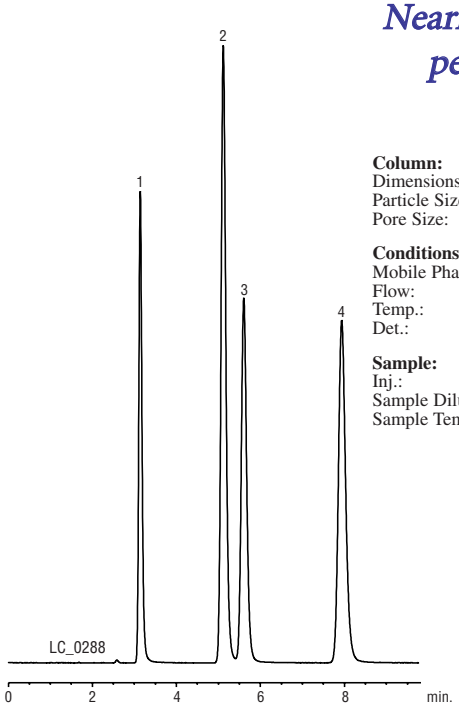
**Hypersil® catalog number; column not available from Restek.*

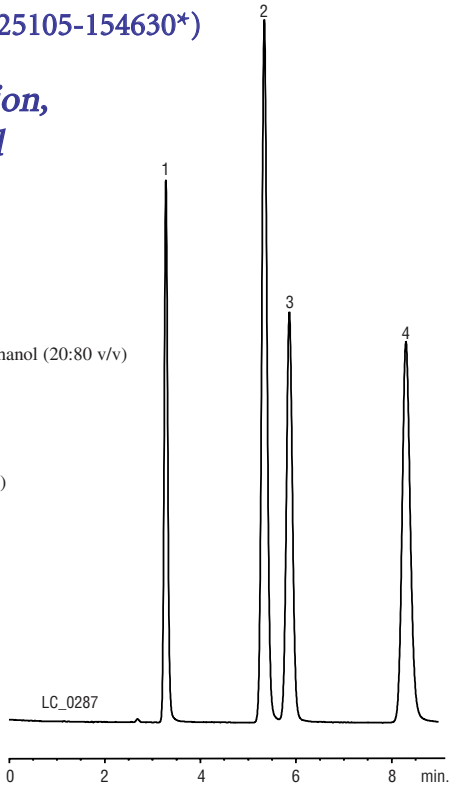
Figure 11: Xanthenes on Pinnacle™ DB and Hypersil® BDS C18 columns.

Pinnacle™ DB C18 Column (cat.# 9414565)

Hypersil® BDS C18 Column (cat.# 25105-154630*)

Nearly identical retention, peak symmetry, and efficiency!





Column:
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å/130Å

Conditions:
Mobile Phase: 20mM KH_2PO_4, pH 3:methanol (20:80 v/v)
Flow: 1.0mL/min
Temp.: ambient
Det.: UV @ 254nm

Sample:
Inj.: 2µL
Sample Diluent: water:methanol (75:25 v/v)
Sample Temp.: ambient

Peak List:	Conc.	Retention Times Pinnacle DB	Hypersil BDS
1. theobromine	200µg/mL	3.141	3.278
2. theophylline	390µg/mL	5.117	5.336
3. β-hydroxytheophylline	390µg/mL	5.605	5.860
4. caffeine	390µg/mL	7.936	8.298

The Pinnacle™ DB line currently is available with three bonded phases: C18, C8, and cyano. Figures 12–14 are representative "real world" chromatograms for each of these phases.

Large, 140Å, pores make Pinnacle™ DB material an excellent choice when you are seeking to transfer an analysis to a shorter column, for faster separations. Figure 15 shows vanilla flavor profiles can be obtained in less than 6 minutes on a 50 x 4.0mm column. These analyses typically are performed on a 150 x 4.6mm column, and analysis times approach 20 minutes.

Satisfaction Guaranteed

Chromatographic and physical data comparisons show Pinnacle™ DB and Hypersil® BDS materials are very closely matched, but we want you to be completely comfortable in evaluating our new columns. We guarantee separations of your samples on Pinnacle™ DB columns will be comparable to separations with Hypersil® BDS columns. If a Pinnacle™ DB column does not meet your satisfaction, simply supply us with copies of chromatograms for your application on your Pinnacle™ DB column and on your Hypersil® BDS column. We will credit your account for the price of the Pinnacle™ DB column—and you can keep the column, free!

If you are looking for a second source for Hypersil® BDS-type columns, or if you simply are looking for a rugged, high quality, base-deactivated material from a supplier who can provide columns quickly, Pinnacle™ DB columns are your answer. Of course, as always, you'll get Plus 1™ service and prompt, expert technical help when you deal with Restek.

Cleaning Industry Application

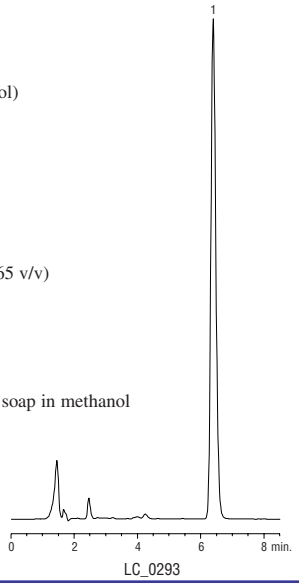
Figure 12: PCMX in hand soap on Pinnacle™ DB C18

Peak List:
1. PCMX (4-chloro-3,5-dimethylphenol)

Column:
Cat. #: 9414565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å

Conditions:
Mobile Phase: water:methanol (35:65 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 280nm

Sample:
Inj.: 10µL
Conc.: 5% solution of hand soap in methanol
Sample Diluent: methanol
Sample Temp.: ambient



Pharmaceutical Applications

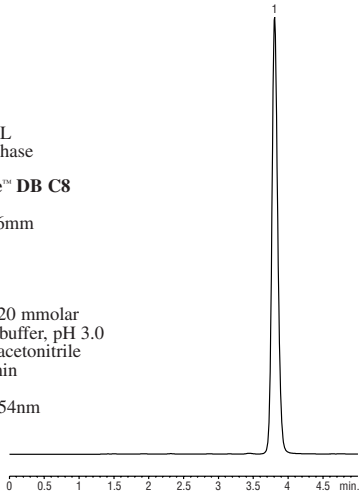
Figure 13: Naproxen on Pinnacle™ DB C8

Peak List:
1. naproxen

Sample:
Inj.: 2µL
Conc.: 550µg/mL
Sample Diluent: mobile phase

Column: **Pinnacle™ DB C8**
Catalog #: 9413565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å

Conditions:
Mobile Phase: A: 90% 20 mmolar KH_2PO_4 buffer, pH 3.0
B: 10% acetonitrile
Flow: 1.0mL/min
Temp.: 27°C
Det.: UV @ 254nm



LC_0240

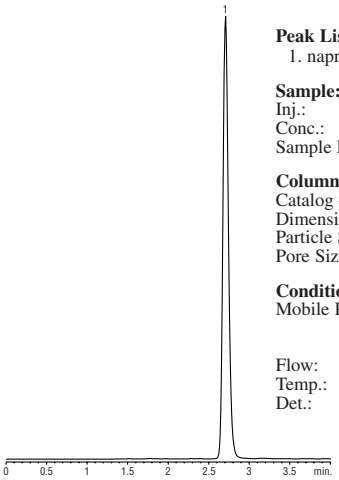
Figure 12: Naproxen on Pinnacle™ DB Cyano

Peak List:
1. naproxen

Sample:
Inj.: 2µL
Conc.: 550µg/mL
Sample Diluent: mobile phase

Column: **Pinnacle™ DB Cyano**
Catalog #: 9416565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å

Conditions:
Mobile Phase: A: 90% 20 mmolar KH_2PO_4 buffer, pH 3.0
B: 10% acetonitrile
Flow: 1.0mL/min
Temp.: 27°C
Det.: UV @ 254nm



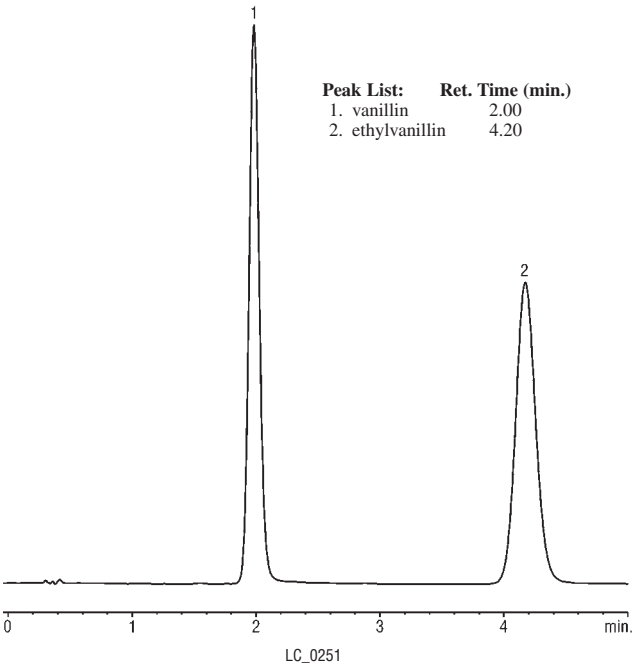
LC_0239

Food, Flavor, and Fragrance Application

Figure 15: Vanilla flavor profiles on Pinnacle™ DB C18: Fast LC

Vanillin and Ethylvanillin on Pinnacle™ DB C18

Peak List: **Ret. Time (min.)**
1. vanillin 2.00
2. ethylvanillin 4.20

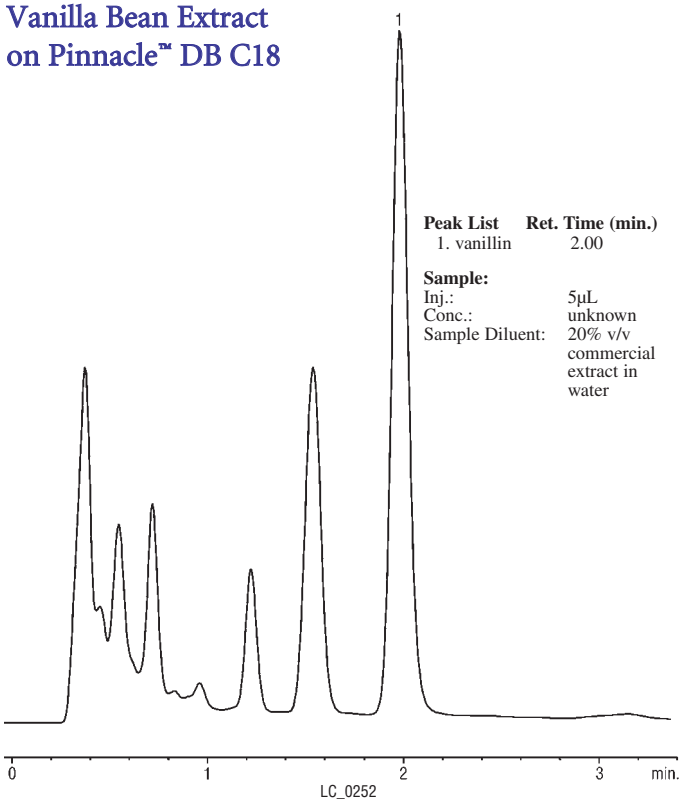


LC_0251

Vanilla Bean Extract on Pinnacle™ DB C18

Peak List **Ret. Time (min.)**
1. vanillin 2.00

Sample:
Inj.: 5µL
Conc.: unknown
Sample Diluent: 20% v/v commercial extract in water



LC_0252

Column: Pinnacle™ DB C18
Catalog #: 9414555
Dimensions: 50 x 4.6mm
Particle Size: 5µm
Pore Size: 140Å

Conditions:
Mobile Phase: A: 0.2% v/v conc. phosphoric acid in water
B: methanol
Isocratic: 80% A: 20% B
Flow: 2mL/min
Temp.: 27°C
Det.: UV @ 254nm

Table I: Cross-reference of catalog numbers.

	Pinnacle™ DB Restek	Hypersil® BDS Thermo Hypersil-Keystone	Phenomenex
250mm length			
1.0mm	9414571	28105-251030	
2.1mm	9414572	28105-252130	
3.2mm	9414573	28105-253030	
4.6mm	9414575	28105-254630	00G-4022-E0
200mm length			
1.0mm	9414521	28105-201030	
2.1mm	9414522	28105-202130	
3.2mm	9414523	28105-203030	
4.6mm	9414525	28105-204630	
150mm length			
1.0mm	9414561	28105-151030	
2.1mm	9414562	28105-152130	00F-4022-B0
3.2mm	9414563	28105-153030	00F-4022-R0
4.6mm	9414565	28105-154630	00F-4022-E0
100mm length			
1.0mm	9414511	28105-101030	
2.1mm	9414512	28105-102130	00D-4022-B0
3.2mm	9414513	28105-103030	
4.6mm	9414515	28105-104630	00D-4022-E0
50mm length			
1.0mm	9414551	28105-051030	
2.1mm	9414552	28105-052130	00B-4022-B0
3.2mm	9414553	28105-053030	00B-4022-R0
4.6mm	9414555	28105-054630	00B-4022-E0
30mm length			
1.0mm	9414531	28105-031030	
2.1mm	9414532	28105-032130	
3.2mm	9414533	28105-033030	
4.6mm	9414535	28105-034630	00A-4022-E0

Product Listing

Pinnacle™ DB C18 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9414531	9414532	9414533	9414535
50mm	9414551	9414552	9414553	9414555
100mm	9414511	9414512	9414513	9414515
150mm	9414561	9414562	9414563	9414565
200mm	9414521	9414522	9414523	9414525
250mm	9414571	9414572	9414573	9414575

Pinnacle™ DB C8 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9413531	9413532	9413533	9413535
50mm	9413551	9413552	9413553	9413555
100mm	9413511	9413512	9413513	9413515
150mm	9413561	9413562	9413563	9413565
200mm	9413521	9413522	9413523	9413525
250mm	9413571	9413572	9413573	9413575

Pinnacle™ DB Cyano 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9416531	9416532	9416533	9416535
50mm	9416551	9416552	9416553	9416555
100mm	9416511	9416512	9416513	9416515
150mm	9416561	9416562	9416563	9416565
200mm	9416521	9416522	9416523	9416525
250mm	9416571	9416572	9416573	9416575

Pinnacle™ DB Silica 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9410531	9410532	9410533	9410535
50mm	9410551	9410552	9410553	9410555
100mm	9410511	9410512	9410513	9410515
150mm	9410561	9410562	9410563	9410565
200mm	9410521	9410522	9410523	9410525
250mm	9410571	9410572	9410573	9410575

Purge and Trap Procedures

Table 4 summarizes the purge and trap conditions used for recovering oxygenates from gasoline. Samples were heated using the Infra-Sparge™ sample heater on an O.I. 4560 concentrator. The minimum purge temperature effective for detecting TBA at a concentration of 25ppb in 10mL of water was 40°C. Purge flow rate was carefully adjusted to 38mL/min.; lower flows dramatically affect recovery of the brominated compounds, higher flows contribute to analyte breakthrough and excessive water retention on the trap.

Analytical Procedures

An Agilent 5890 Series II GC coupled with an Agilent 5971A GC/MS detector fitted with a K&M electron multiplier was used for the analysis. Helium carrier gas was adjusted to 1.3mL/min. constant flow. The oven temperature program was optimized as follows: 35°C (hold 7 min.) to 90°C @ 4°C/min. (hold 0 min.), to 220°C @ 45°C/min. (hold 1 min.). Analysis time was 25 minutes; cycle time was 30 minutes. The MS was set for full scan from 35amu to 260amu and was initially tuned with FC-43 or PFTBA calibration gas, followed by BFB. A 30m x 0.25mm x 1.4µm Rtx®-VMS column (cat.# 19915) was used for the separations.

Standards

The five points of the calibration were 5, 10, 20, 40, and 80 ppb, with internal standards (IS) and surrogate standards (SS) added to each calibration standard at 20ppb. Intermediate standards were made separately for each calibration point, to maintain equal amounts of methanol added to the 10mL volume of water. Samples were spiked and were transferred to the concentrator by hand. Calibration was performed using the analyte list in Table 3, but all compounds shown in Figure 2 were added in order to check for critical coelutions between oxygenates and Method 8260B target compounds. Calibration verification standards (CVS) were added at 10ppb; recoveries were within 20% of expected values. Two blanks were analyzed, followed by a 5ppb QC standard to verify recoveries at the low point of the curve.[4] Reference materials used are listed in Table 5.

Gasoline-Spiked Samples

Analysis of the 5ppb QC standard was followed by analysis of a 1ppm non-oxygenated gasoline standard, then by analysis of the non-oxygenated standard with 5ppb of each target compound added. % recoveries for the 5ppb oxygenate standards were calculated from this high concentration gasoline matrix. The final standard analyzed was 1ppm (unweathered) unleaded gasoline. All samples were spiked with the appropriate IS and SS. The calibration curve passed EPA 8260 criteria for response factors and relative standard deviations. Only two compounds in the test set showed poor response: acetone and TBA.

Table 4:

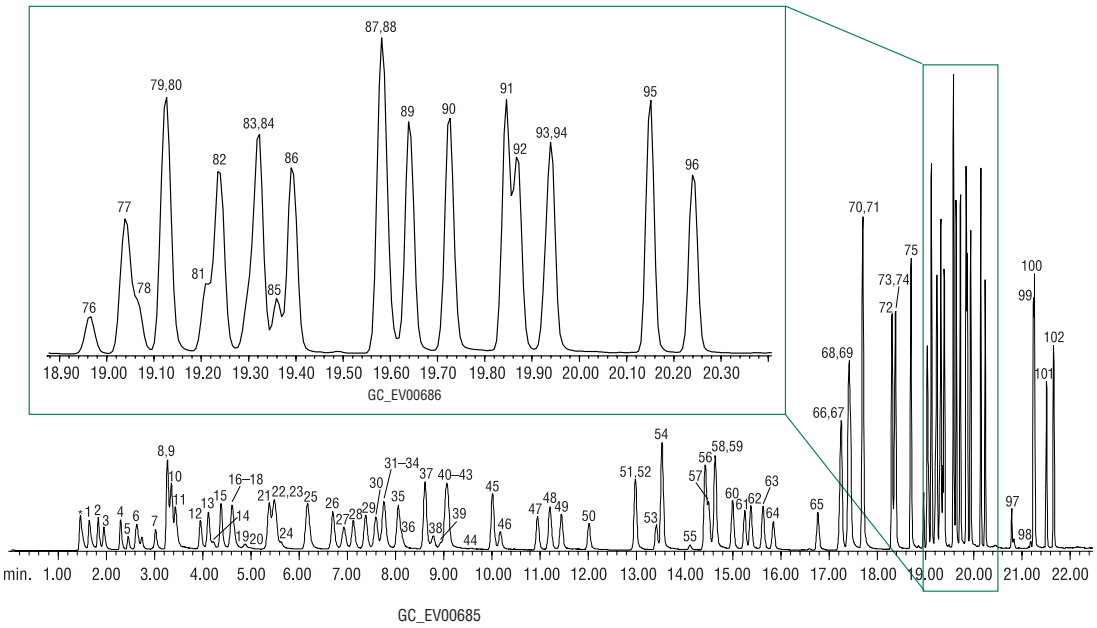
Purge and trap conditions for recovering oxygenates from gasoline (O.I. 4560 concentrator).

Trap:	#10 (Tenax®/silica gel/carbon molecular sieve)
Purge Time:	11 min.
Purge Flow Rate:	38mL/min.
Desorb Flow Rate:	32mL/min.
Desorb Time:	1.0 min.
Bake Time:	10 min.
Sample Size:	10mL
Water Management:	110°C purge, 0°C desorb, 240°C bake
Split Ratio:	1:25
Temperatures:	
Sample:	40°C
Trap:	20°C purge, 190°C desorb, 210°C bake
6-Port Valve:	110°C
Transfer Line:	110°C
Sparge Mount:	45°C
Desorb Preheat:	150°C
Valve Manifold:	50°C
Other Conditions:	pre-purge, pre-heat, dry purge OFF

Conditions suggested by O.I. Analytical.

Figure 2:

Detecting oxygenates in gasoline-water samples: 80ppb each analyte.



Rtx®-VMS, 30m, 0.25mm ID, 1.4µm (cat.# 19915)
Sample: Calibration, internal standard, surrogate standard mixes (cat.# 30475B, 30465, 30006, 30240, 30074)

Purge and trap conditions

Trap:	#10 (Tenax®/silica gel/carbon molecular sieve)
Purge Time:	11 min.
Purge Flow Rate:	38mL/min.
Desorb Flow Rate:	32mL/min.
Desorb Time:	1.0 min.
Bake Time:	10 min.
Sample Size:	10mL
Water Management:	110°C purge, 0°C desorb, 240°C bake
Split Ratio:	1:25
Temperatures:	
Sample:	40°C
Trap:	20°C purge, 190°C desorb, 210°C bake
6-Port Valve:	110°C
Transfer Line:	110°C
Sparge Mount:	45°C
Desorb Preheat:	150°C
Valve Manifold:	50°C
Other Conditions:	pre-purge, pre-heat, dry purge OFF

Chromatography

Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.3 mL/min.
Dead time:	1.47 min. @ 35°C
Oven temp.:	35°C (hold 7 min.) to 90°C @ 4°C/min.(no hold) to 220°C @ 45°C/min. (hold 1 min.).
Det.:	Agilent 5971A GC/MS
Transfer line temp.:	280°C
Scan range:	35-260 amu
Tune:	PFTBA/BFB

*carbon dioxide
1. dichlorodifluoromethane
2. chloromethane
3. vinyl chloride
4. bromomethane
5. chloroethane
6. trichlorofluoromethane
7. diethylether
8. 1,1-dichloroethene
9. carbon disulfide
10. Freon® 113
11. iodomethane
12. allyl chloride
13. methylene chloride
14. acetone
15. *trans*-1,2-dichloroethene
16. methyl-d3-*tert*-butyl-ether
17. methyl acetate
18. methyl-*tert*-butyl-ether
19. *tert*-butyl alcohol
20. acetonitrile
21. diisopropyl ether
22. chloroprene
23. 1,1-dichloroethane
24. acrylonitrile
25. ethyl-*tert*-butyl ether
26. *cis*-1,2-dichloroethene
27. 2,2-dichloropropane
28. bromochloromethane
29. chloroform
30. carbon tetrachloride
31. tetrahydrofuran
32. methyl acrylate
33. 1,1,1-trichloroethane
34. dibromofluoromethane
35. 1,1-dichloropropene
36. 2-butanone
37. benzene
38. propionitrile
39. methacrylonitrile
40. 1,2-dichloroethane-d4
41. pentafluorobenzene
42. *tert*-amyl-methyl ether
43. 1,2-dichloroethane
44. isobutyl alcohol
45. trichloroethene
46. 1,4-difluorobenzene
47. dibromomethane
48. 1,2-dichloropropane
49. bromodichloromethane
50. methyl methacrylate
51. *cis*-1,3-dichloropropene
52. 2-chloroethyl vinyl ether
53. toluene-d8
54. toluene
55. 2-nitropropane
56. tetrachloroethene
57. 2-bromo-1-chloropropane
58. 4-methyl-2-pentanone
59. *trans*-1,3-dichloropropene
60. 1,1,2-trichloroethane
61. ethyl methacrylate
62. dibromochloromethane
63. 1,3-dichloropropane
64. 1,2-dibromoethane
65. 2-hexanone
66. chlorobenzene-d5
67. chlorobenzene
68. ethylbenzene
69. 1,1,1,2-tetrachloroethane
70. *m*-xylene
71. *p*-xylene
72. *o*-xylene
73. bromoform
74. stryrene
75. isopropylbenzene
76. 4-bromo-1-fluorobenzene (SS)
77. bromobenzene
78. *cis*-1,4-dichloro-2-butene
79. 1,4-dichlorobutane
80. *n*-propylbenzene
81. 1,1,2,2-tetrachloroethane
82. 2-chlorotoluene
83. 1,2,3-trichloropropane
84. 1,3,5-trimethylbenzene
85. *trans*-1,4-dichloro-2-butene
86. 4-chlorotoluene
87. *tert*-butylbenzene
88. pentachloroethane
89. 1,2,4-trimethylbenzene
90. *sec*-butylbenzene
91. *p*-isopropyltoluene
92. 1,3-dichlorobenzene
93. 1,4-dichlorobenzene-d4
94. 1,4-dichlorobenzene
95. *n*-butylbenzene
96. 1,2-dichlorobenzene
97. 1,2-dibromo-3-chloropropane
98. nitrobenzene
99. hexachlorobutadiene
100. 1,2,4-trichlorobenzene
101. naphthalene
102. 1,2,3-trichlorobenzene

Acknowledgment: purge & trap courtesy of O.I. Analytical.

Not detected: 2-chloroethanol

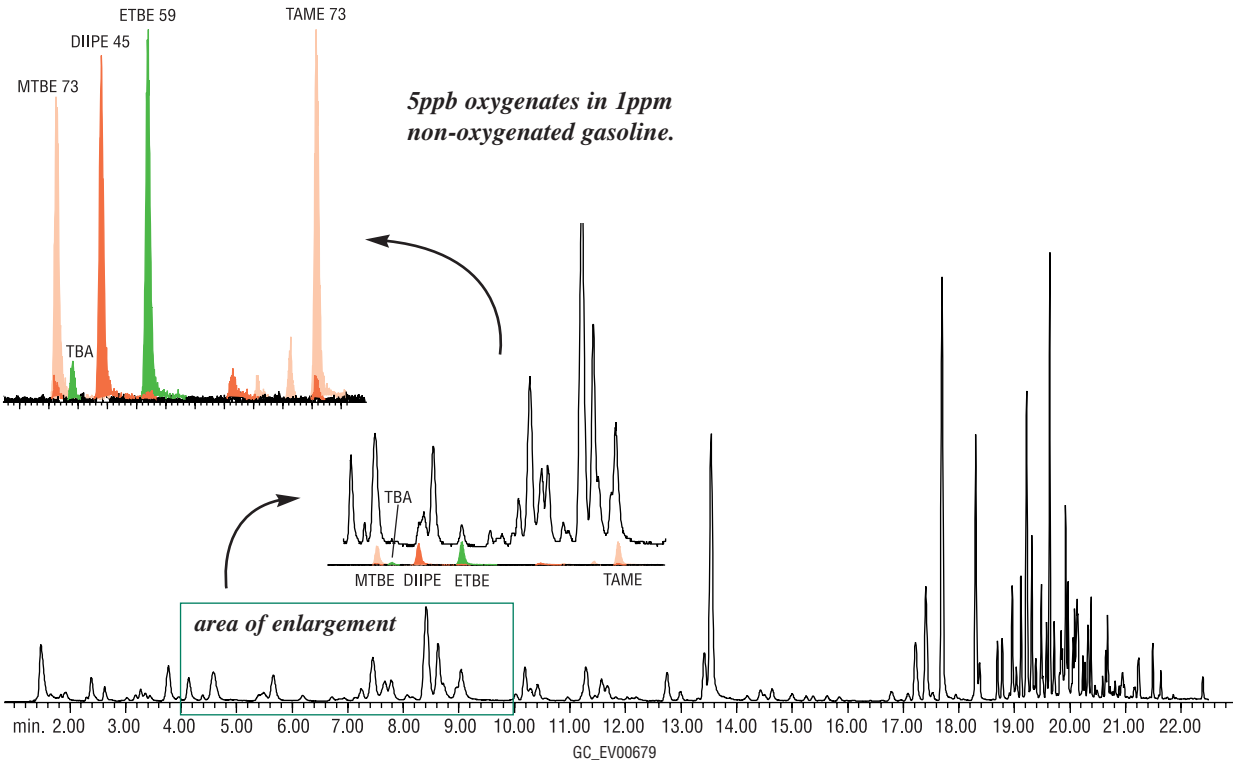
Results

Table 6 summarizes results. All oxygenates were resolved from other target compounds and potentially interfering gasoline components. TBA and MTBE were well resolved using the 35°C starting temperature. Figure 3 represents a TIC of 1ppm non-oxygenated unleaded gasoline spiked with 5ppb of oxygenates. The inset is an extracted ion chromatogram showing oxygenates are recovered without interference from the gasoline matrix.

The internal standard methyl-d3 *tert*-butyl ether was added to compensate for variations in purging efficiency specific to the ethers. After the data were acquired we observed a small ion 73 component as part of the IS spectra (Figure 4). MTBE and the IS share retention time and MTBE produces ion 73. Our initial thought was to discard the data, but we determined the distribution of the relative abundance of ion 73 to ion 76 for the IS was between 0.3% and 0.5%. This would affect calculated concentrations of MTBE by no more than 2%. Analysts in environmental laboratories can decide if this is an acceptable degree of error. We are currently evaluating other potential internal standards that behave in a manner similar to the ethers. One such substitution used in some laboratories is TBA-d9, which accounts for variations common to alcohols, but not to ethers.

Figure 3:

GC/MS with an Rtx-VMS® column allows reliable identification of 5ppb oxygenates added to 1ppm gasoline.



Rtx®-VMS, 30m, 0.25mm ID, 1.4µm (cat.# 19915)
Sample: calibration mixes: cat.# 30475B, 30465, 30006, 30042;
unleaded gasoline (cat.# 30096); non-oxygenated unleaded gasoline (custom)
internal / surrogate standards: cat.# 30240, 30074

Purge and trap conditions

O.I. 4560 Purge and Trap Concentrator

Sample:	10mL
Sample temp.:	40°C
Trap:	#10 (Tenax®/silica gel/carbon molecular sieve)
Trap temp.:	purge: 20°C; desorb: 190°C; bake: 210°C
Purge:	11 min., 38mL/min.
Desorb:	1.0 min., 32mL/min.
Bake:	10 min.

Chromatography

Inj. temp.:	250°C, split 1:25
Carrier gas:	helium, constant flow
Flow rate:	1.3mL/min.
Dead time:	1.47 min. @ 35°C
Oven temp.:	35°C (hold 7 min.) to 90°C @ 4°C/min. (no hold) to 220°C @ 45°C/min. (hold 1 min.)
Det.:	Agilent 5971A GC/MS
Scan range:	35–260 amu
Tune:	PFTBA/BFB

Acknowledgement: purge & trap courtesy of O.I. Analytical.

Table 5:

Calibration mixes and other reference materials for oxygenates analysis.

Reference Material	Restek cat.# / lot#	
Internal Standard & Surrogate Standard Mix	cat.# 30240	lot# A025538
	custom	lot# 03010401s
	cat.# 30074	lot# A022472
Calibration Mix	cat.# 30475A	lot# A020908
	cat.# 30465	lot# A024826
	cat.# 30006	lot# A024175
	cat.# 30042	lot# A024616
QC Second Source Calibration Mix	cat.# 553595	lot# A025888
Unleaded Gasoline (Unweathered)	cat.# 30096	lot# A022384
Non-Oxygenated Unleaded Gasoline (Unweathered)	custom	lot# OFR-TK253

Table 6:

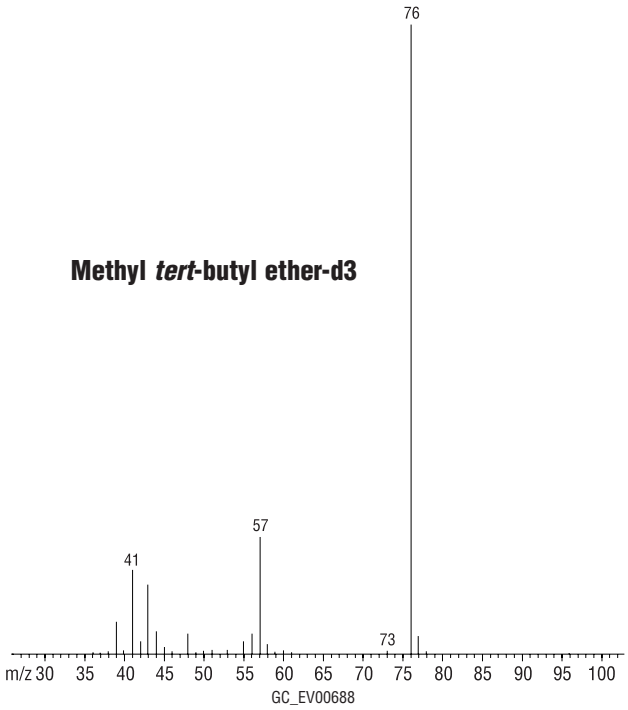
Efficient recovery of ppb oxygenates, using an Rtx®-VMS column.

Compound	5-Point Curve (RF)	5-Point Curve (%RSD)	Method Blank (%Recov.)	5ppb Standard (%Recov.)	1ppm Non-oxygenated Standard (%Recov.)	1ppm Non-oxygenated Standard, 5ppb Spike (%Recov.)	1ppm Gasoline Composite Standard (%Recov.)
methyl-d3-*tert*-butyl ether (IS)							
tert-butyl alcohol (x5)*	0.13	17		93		90	
methyl-*tert*-butyl ether	0.91	7		99		92	6.2ppb
diisopropyl ether	1.08	7		100		94	
ethyl-*tert*-butyl ether	1.21	8		98		90	
tert-amyl-methyl ether	1.15	5		105		98	< D.L.
pentafluorobenzene (IS)							
acetone (x2.5)*	0.05	12		62	< D.L.	N.D.	
dibromofluoromethane (SS)	0.43	3	102	89	106	97	98
1,4-difluorobenzene (IS)							
1,2-dichloroethane-d4 (SS)	0.22	6	103	111	102	100	102
toluene-d8 (SS)	1.02	4	101	108	98	97	98
toluene (CCC)	0.78	3		106	94.4ppb	89.6ppb	81.9ppb
bromofluorobenzene (SS)	0.45	2	91	103	90	90	95
chlorobenzene-d5 (IS)							
chlorobenzene (SPCC)	1.06	6		108			
ethylbenzene (CCC)	1.71	5		110	21.4ppb	24.9ppb	23.9ppb
1,4-dichlorobenzene-d4 (IS)							
naphthalene	2.08	3		93	4.7ppb	8.8ppb	5.0ppb

**Compound added at 5 times or 2.5 times the concentration of other target analytes.*
<D.L. - below detection limit. N.D. - not detected.
Column: Rtx®-VMS, 30m x 0.25mm x 1.4µm (cat.# 19915)
Conditions: given in text

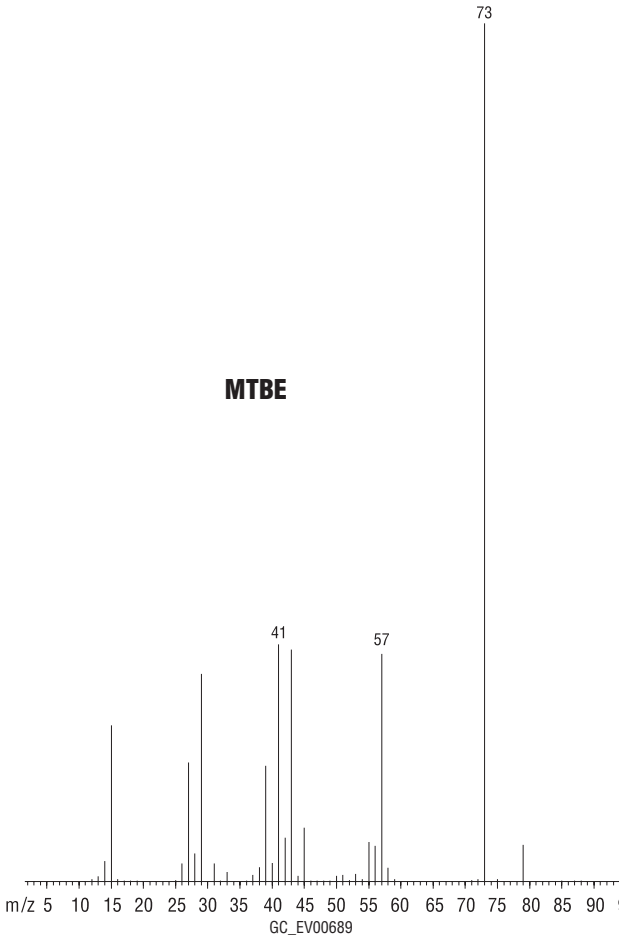
Figure 4:

MTBE and internal standard methyl-d3-*tert*-butyl ether share identification ion 73.



Rtx®-VMS, 30m, 0.25mm ID, 1.4µm (cat.# 19915)

Sample:	custom
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.3mL/min.
Dead time:	1.47 min. @ 35°C
Oven temp.:	35°C (hold 7 min.) to 90°C @ 4°C/min.(no hold) to 220°C @ 45°C/min. (hold 1 min.).
Det:	Agilent 5971A GC/MS
Transfer line temp.:	280°C
Scan range:	35-260 amu
Tune:	PFTBA/BFB
Ionization:	EI



Conclusion

With an expanding target list and difficult sample matrixes, such as petroleum distillates, extreme care must be taken, even with GC/MS, to assure correct identification of oxygenates in the presence of interfering analytes. Under the conditions used here, an Rtx®-VMS column is suitable for analyzing low levels of oxygenates in the presence of other gasoline components. For additional information, see reference 5.

References

1. C. English, C. Cox, F. Dorman, D. Patwardhan, *The Analysis of Gasoline Oxygenates Using a New Capillary Column Stationary Phase,* Pittsburgh Conference 2001, Session 199 (poster). http://www.restekcorp.com/2001/1868P.pdf
2. Happel, A.M., E.H. Beckenbach, R.U. Halden, *An Evaluation of MTBE Impacts to California Groundwater Resources* Lawrence Livermore National Laboratory, UCRL-AR-130897 (1988). http://www-erd.llnl.gov/mtbe/pdf/mtbe.pdf
3. U.S. Environmental Protection Agency, *Volatile Organic Compounds by Gas Chromatography/Mass Spectroscopy (GC/MS): Capillary Column Technique Method 8260.* Revision 0, July 1992, US EPA Office of Solid Waste. Washington, D.C.
4. U.S. Environmental Protection Agency, *Volatile Organic Compounds by Gas Chromatography/Mass Spectroscopy (GC/MS): Capillary Column Technique Method 8260B.* Revision 2, December 1996, US EPA Office of Solid Waste. Washington, D.C.
5. C.M. English, F.L. Dorman, G.B. Stidsen, *The Analysis of Gasoline Oxygenates by EPA Method 8260B* Pittsburgh Conference 2003, Session 590-6P (poster). http://www.restekcorp/pittcon2003.htm#slides

Acknowledgement

We are grateful to O.I. Analytical for supplying the purge and trap unit used in this study, and for their help with establishing analytical conditions.

Product Listing

Rtx®-VMS Columns (fused silica)

ID	df (µm)	temp. limits	30-Meter	60-Meter	75-Meter
0.25mm	1.40	-40 to 240/260°C	19915	19916	
0.32mm	1.80	-40 to 240/260°C	19919	19920	
0.45mm	2.55	-40 to 240/260°C	19908	19909	
0.53mm	3.00	-40 to 240/260°C	19985	19988	19974
ID	**df (µm)**	**temp. limits**	**20-Meter**	**40-Meter**	
0.18mm	1.00	-40 to 240/260°C	49914	49915	

8260A/B Surrogate Mix

4-bromofluorobenzene
dibromofluoromethane
1,2-dichloroethane-d4
toluene-d8

2,500µg/mL each in P&T methanol, 1mL/ampul

Each	5-pk.	10-pk.
30240	30240-510	—
	w/data pack	
30240-500	30240-520	30340

8260 Internal Standard Mix

chlorobenzene-d5
1,4-dichlorobenzene-d4
1,4-difluorobenzene
pentafluorobenzene

2,500µg/mL each in P&T methanol, 1mL/ampul

Each	5-pk.	10-pk.
30074	30074-510	—
	w/data pack	
30074-500	30074-520	30174

8260B MegaMix™ Calibration Mix (76 + 1 components)

Note: This product is provided as a two ampul set:

2,000µg/mL each component in P&T methanol, 1mL/ampul
2,000µg/mL 2-chloroethyl vinyl ether in P&T methanol, 1mL/ampul

Each	5-pk.	10-pk.
30475	30475-510	—
	w/data pack	
30475-500	30475-520	30575

California Oxygenates Mix

diisopropyl ether	*2,000µg/mL*
ethyl-tert-butyl ether	*2,000*
tert-amyl methyl ether	*2,000*
tert-butyl alcohol	*10,000*
methyl tert-butyl ether	*2,000*

In P&T methanol, 1mL/ampul

Each	5-pk.	10-pk.
30465	30465-510	—
	w/data pack	
30465-500	30465-520	30565

VOA Calibration Mix #1 (ketones)

acetone
2-butanone
2-hexanone
4-methyl-2-pentanone

5,000µg/mL each in P&T methanol:water (90:10), 1mL/ampul

Each	5-pk.	10-pk.
30006	30006-510	—
	w/data pack	
30006-500	30006-520	30106

502.2 Calibration Mix #1 (gases)

bromomethane
chloroethane
chloromethane
dichlorodifluoromethane
trichlorofluoromethane
vinyl chloride

2,000µg/mL each in P&T methanol, 1mL/ampul

Each	5-pk.	10-pk.
30042	30042-510	—
	w/data pack	
30042-500	30042-520	30142

Unleaded Gasoline Standard: Unweathered

Prepared from a single source (one refinery) product. Samples of regular and premium grade unleaded gasoline were collected, then blended in equal volumes.

5,000µg/mL in P&T methanol, 1mL/ampul

Each	5-pk.	10-pk.
30096	30096-510	—
	w/data pack	
30096-500	30096-520	30196

8240/8260 Calibration Check Mix

chloroform
1,1-dichloroethene
1,2-dichloropropane
ethylbenzene
toluene
vinyl chloride

2,000µg/mL each in P&T methanol, 1mL/ampul

Each	5-pk.	10-pk.
30427	30427-510	—
	w/data pack	
30427-500	30427-520	30527

Other reference materials prepared on request. Please inquire.

Organochlorine Pesticides by GCxGC-ECD

Introduction

United States Environmental Protection Agency Method (EPA) 8081 uses gas chromatography–electron capture detection (GC-ECD) to quantify organochlorine pesticides (OCPs) in extracts from solid and liquid matrices. The method includes a parallel dual-column option in which the GC is configured with one injection port, directing extracts to two different GC columns that terminate in two ECDs. This configuration helps to confirm quantified values that are obtained when a non-specific detector such as the ECD is used. Conversely, biases that arise due to pesticide coelutions with interferences are illuminated.

A relatively new way to solve separation problems is to use comprehensive two-dimensional GC (GCxGC). GCxGC is a way to increase peak capacity by applying two independent separations to a sample in one analysis with one detector. Typically, GCxGC involves a serial column configuration (differing phases) separated by a thermal modulator. A separation is performed on the first column, and then effluent from the first column is continually (and quickly) focused and "injected" onto the second column. By keeping the second column short, a series of high-speed chromatograms are generated, and the first column separation can be maintained. Separation results can be plotted as a retention plane (column 1 time x column 2 time), also known as a contour plot. By using GCxGC, the chances for coeluting interferences are reduced, and an analogy can be drawn between GCxGC and parallel dual-column analysis.

This application note describes a comparison of OCP results obtained from a parallel dual-column GC-ECD method and a GCxGC-ECD method. Soil and water extracts were quantified using the external standard method.

Standards

The standards were obtained from Restek and contained the following OCPs: aldrin, alpha-chlordane, alpha-hexachlorocyclohexane (HCH), beta-HCH, DDD, DDE, DDT, delta-HCH, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, gamma-chlordane, gamma-HCH, heptachlor, heptachlor epoxide, and methoxychlor. Decachlorobiphenyl (209) and tetrachloro-m-xylene (TCMX) were also in the standards and serve as surrogate compounds for method 8081.

The standards were diluted in hexane to achieve the following concentrations (in pg/µL) for calibration curves.

- HCHs, heptachlors, aldrin, chlordanes, endosulfans (5, 10, 20, 40, 80)
- 209, TCMX, dieldrin, endrins, endosulfan sulfate, DDT compounds (10, 20, 40, 80, 160)
- Methoxychlor (50, 100, 200, 400, 800)

Samples

Soil and water extracts were obtained from Severn Trent Laboratories, STL Burlington, in Colchester, Vermont. Soil was extracted using EPA Method 3550, ultrasonic extraction. Additionally, gel permeation chromatography was applied to the soil extracts using EPA Method 3640. Water was liquid-liquid extracted in a separatory funnel according to EPA Method 3510. Both soil and water extracts were subjected to Florisil column clean-up following EPA Method 3620.

Experimental Conditions

Parallel Dual-Column GC-ECD

Column 1:	30 m x 0.32 mm x 0.50 µm Rtx-CLPesticide (Restek)
Column 2:	30 m x 0.32 mm x 0.42 µm Rtx-CLPesticideII (Restek)
Carrier:	Hydrogen at 2.8 mL/min, constant flow
Injection:	2 µL direct at 200°C
Oven Program:	120°C (1 min), 16°/min to 210°, 13°/min to 245°, 12.5°/min to 300° (4 min)
Total Run Time:	17 min
Detector:	ECD, 300°C, argon/methane makeup gas at 140 mL/min

LECO GCxGC-ECD

Agilent 6890 GC-ECD equipped with a LECO Quad Jet—Dual-Stage Thermal Modulator

Column 1:	9 m x 0.18 mm x 0.20 µm Rtx-5 (Restek)
Column 2:	1 m x 0.18 mm x 0.20 µm Rtx-200 (Restek)
Carrier:	Helium at 2 mL/min, constant flow
Injection:	1 µL split at 250°C, split ratio 50:1
Oven 1 Program:	50°C (0.2 min), 30°/min to 140°, 5°/min to 250°
Oven 2 Program:	50°C offset from oven 1
Modulation:	Temperature offset 30°C from oven 1, time 6 sec.
Total Run Time:	25.2 min
Detector:	ECD, 325°C, N2 makeup gas at 148 mL/min, 50Hz

Data Processing

LECO ChromaTOF® software was used to automatically peak find and quantify organochlorine pesticides analyzed with GCxGC-ECD.

Analysis of Standards with GCxGC-ECD

Figure 1 is a contour plot of an OCP standard analyzed with GCxGC-ECD. Note that the X-axis represents the first dimension retention time, and the Y-axis shows the second dimension retention time (the actual retention times are recorded in Table 1). Peak intensity, as defined by detector response, is represented by a color scheme from blue (zero, or baseline detector response) to red (most intense response). Each "spot" represents a peak (and pesticide). Figure 2 demonstrates the power of GCxGC by showing how beta- and gamma-HCH, which coelute on Rtx-5 (in the first dimension), are separated by Rtx-200 in the second dimension.

Another way to visualize GCxGC data is with a surface plot (illustrated in Figure 3). In this plot, the Z-axis represents peak intensity (as defined by ECD response).

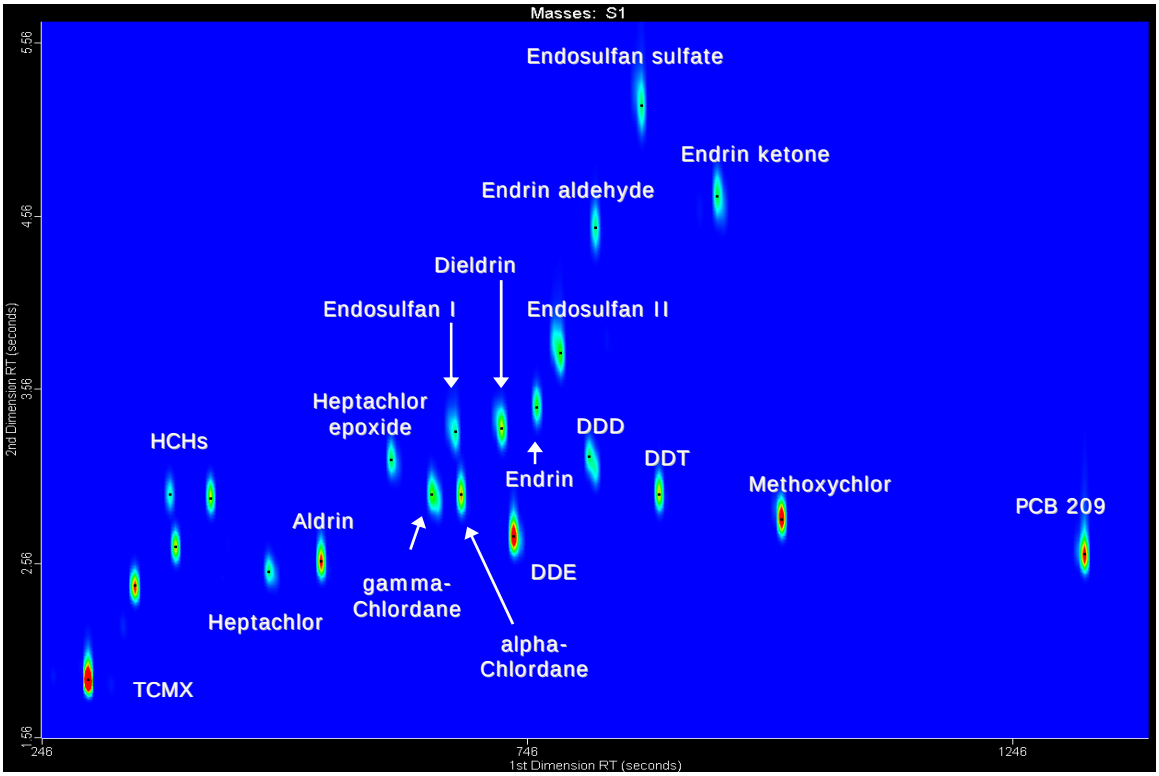


Figure 1. *Contour plot (GCxGC chromatogram) of organochlorine pesticide mix. Note the separation of compounds in two dimensions with the Rtx-5 separation (and retention time) on the X-axis, and the Rtx-200 separation occurring along the Y-axis.*

Table 1. Retention times (RTs) for OCPs via GCxGC-ECD.

Pesticide	RT 1 sec (Rtx-5)	RT 2 sec (Rtx-200)
Tetrachloro-m-xylene	294	1.90
alpha-HCH	342	2.44
beta-HCH	378	2.96
gamma-HCH	384	2.66
delta-HCH	420	2.94
Heptachlor	480	2.52
Aldrin	534	2.58
Heptachlor epoxide	606	3.16
gamma-Chlordane	648	2.96
Endosulfan I	672	3.32
alpha-Chlordane	678	2.96
Dieldrin	720	3.34
4,4'-DDE	732	2.72
Endrin	756	3.46
Endosulfan II	780	3.78
4,4'-DDD	810	3.18
Endrin aldehyde	816	4.50
Endosulfan sulfate	864	5.20
4,4'-DDT	882	2.96
Endrin ketone	942	4.68
Methoxychlor	1008	2.82
Decachlorobiphenyl	1320	2.62

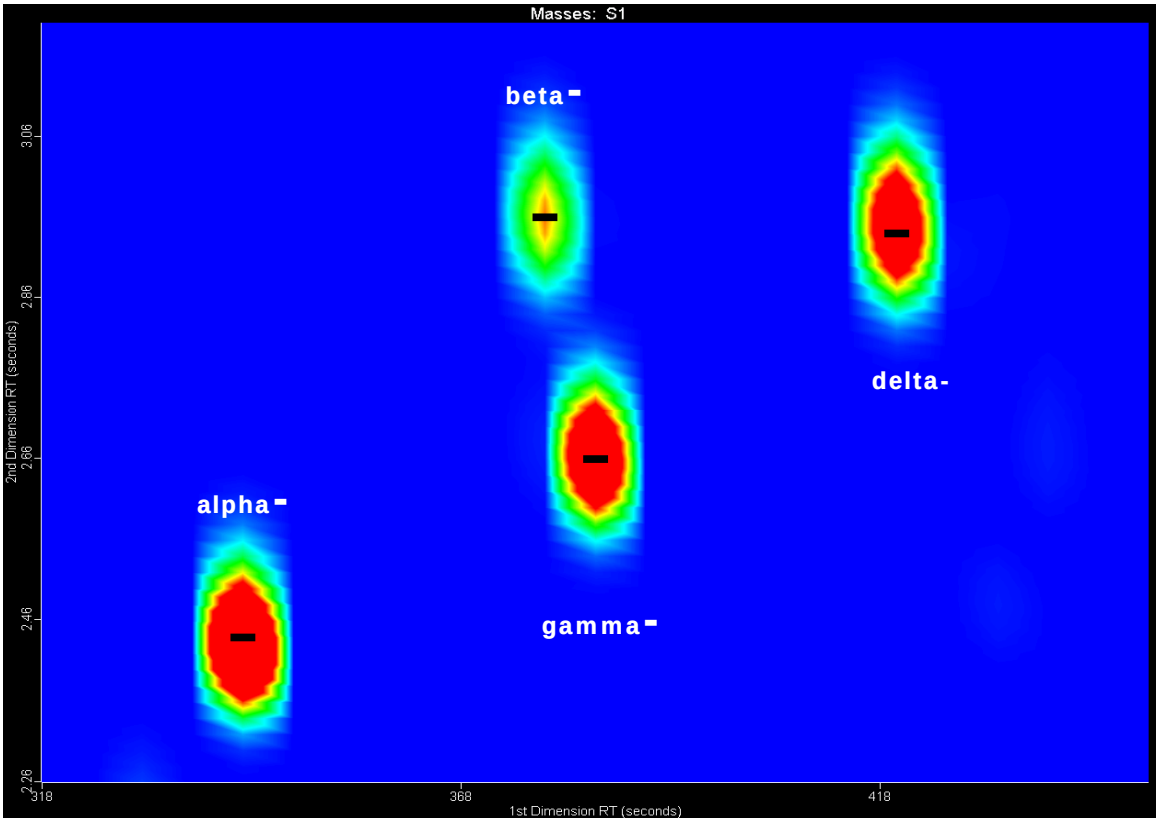


Figure 2. *Separation of HCHs with GCxGC-ECD. Beta- and gamma-HCH coelute in the first dimension on Rtx-5, but are easily separated in the second dimension with Rtx-200.*

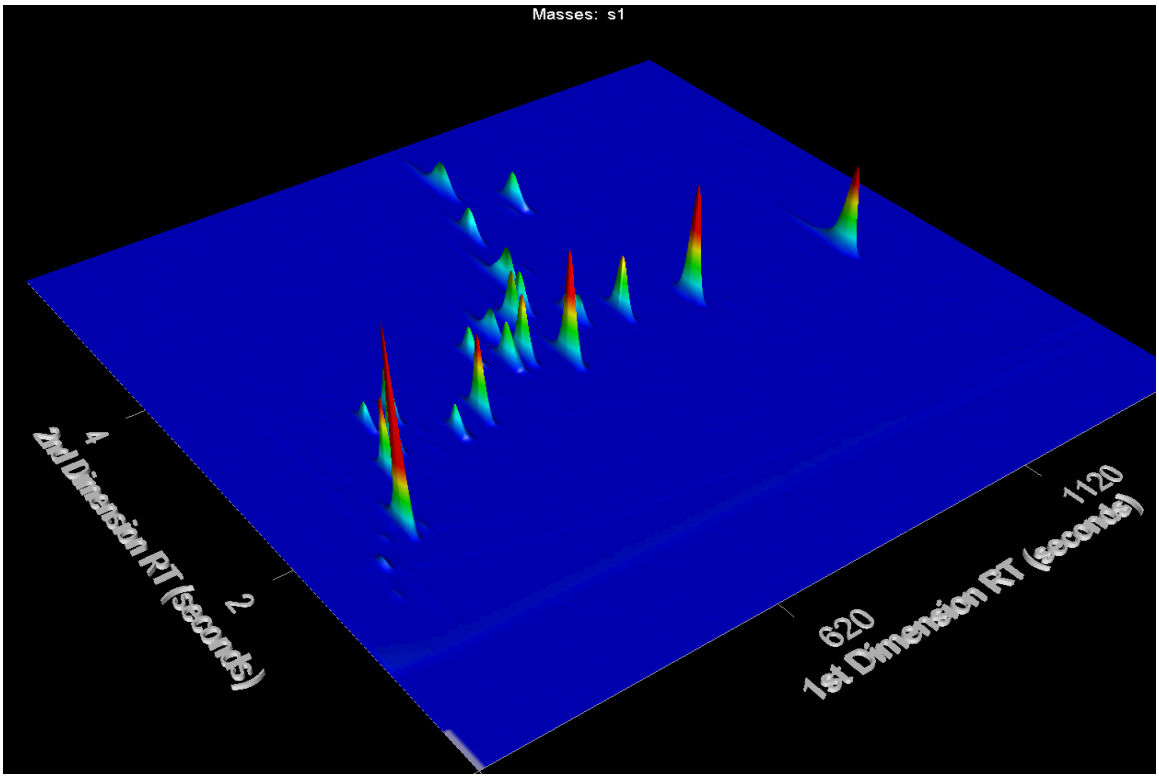


Figure 3. *Surface plot of organochlorine pesticide mix analyzed with GCxGC-ECD. The first dimension retention time is for the Rtx-5 separation, and the second dimension retention time is for the Rtx-200 separation. In addition, there is a Z-axis which represents ECD response for the pesticides.*

Calibration with GCxGC-ECD

Calibration curves were established for GCxGC-ECD by the external standard method using the standard concentrations listed above. Example calibration curves for one of the better ECD responding compounds (gamma-HCH or Lindane), and the worst responding compound (Methoxychlor) are shown in Figures 4 and 5.

Although all of the GCxGC-ECD quantifications for the samples were performed using calibrations established based on concentrations listed in the Standards section, it is possible to go much lower due to the extreme sensitivity afforded by the ECD and the sensitivity enhancement achieved by the focusing effect of GCxGC. Table 2 lists low points and the correlation coefficients when the curve is further extended to even lower OCP concentrations. Again, it is important to point out that since a split injection was used, some of the actual amounts on column (and to the detector) are very low. For example, a 0.5 pg/µL standard represents 10 fg (!) on column.

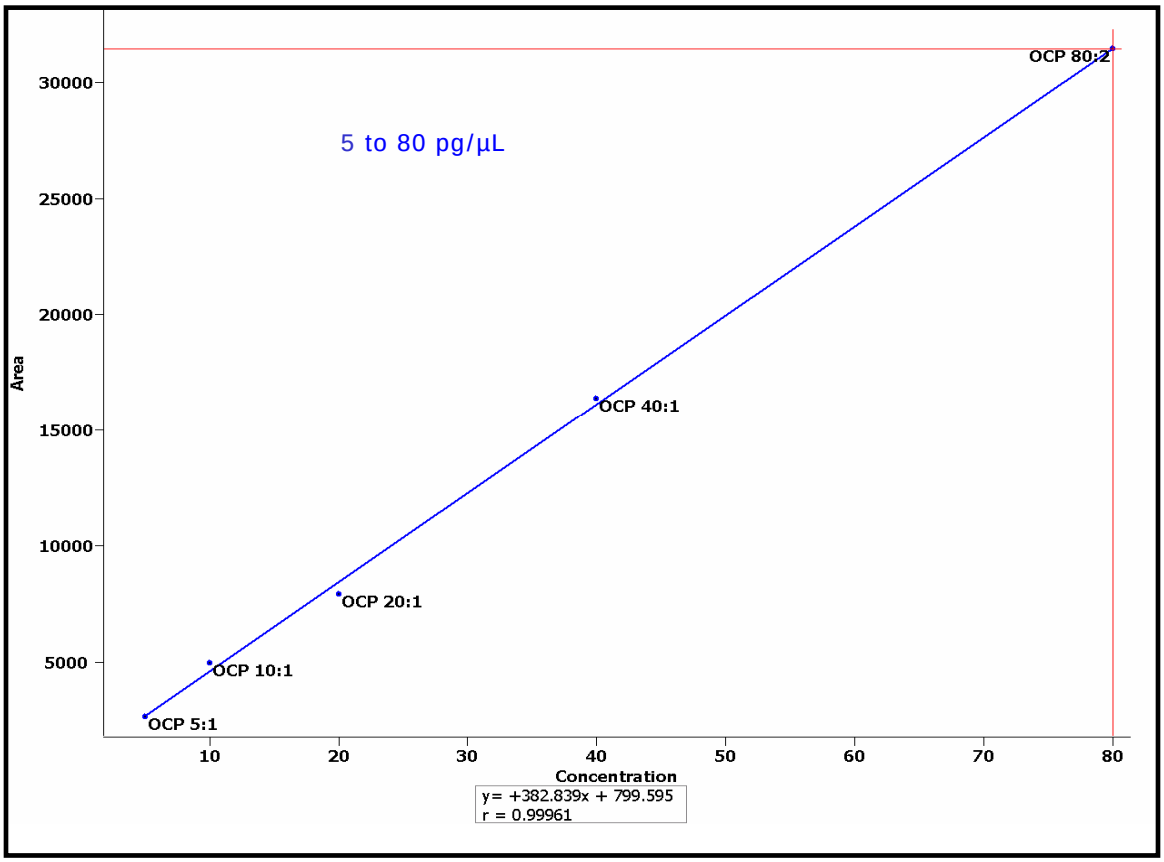


Figure 4. *GCxGC-ECD calibration curve for gamma-HCH (Lindane). Note that the concentrations are in pg/µL (listed as black numbers by the points, e.g. 5:1 = 5 pg/µL), but due to split injection at a ratio of 50:1, the low point represents only 0.1 pg on column.*

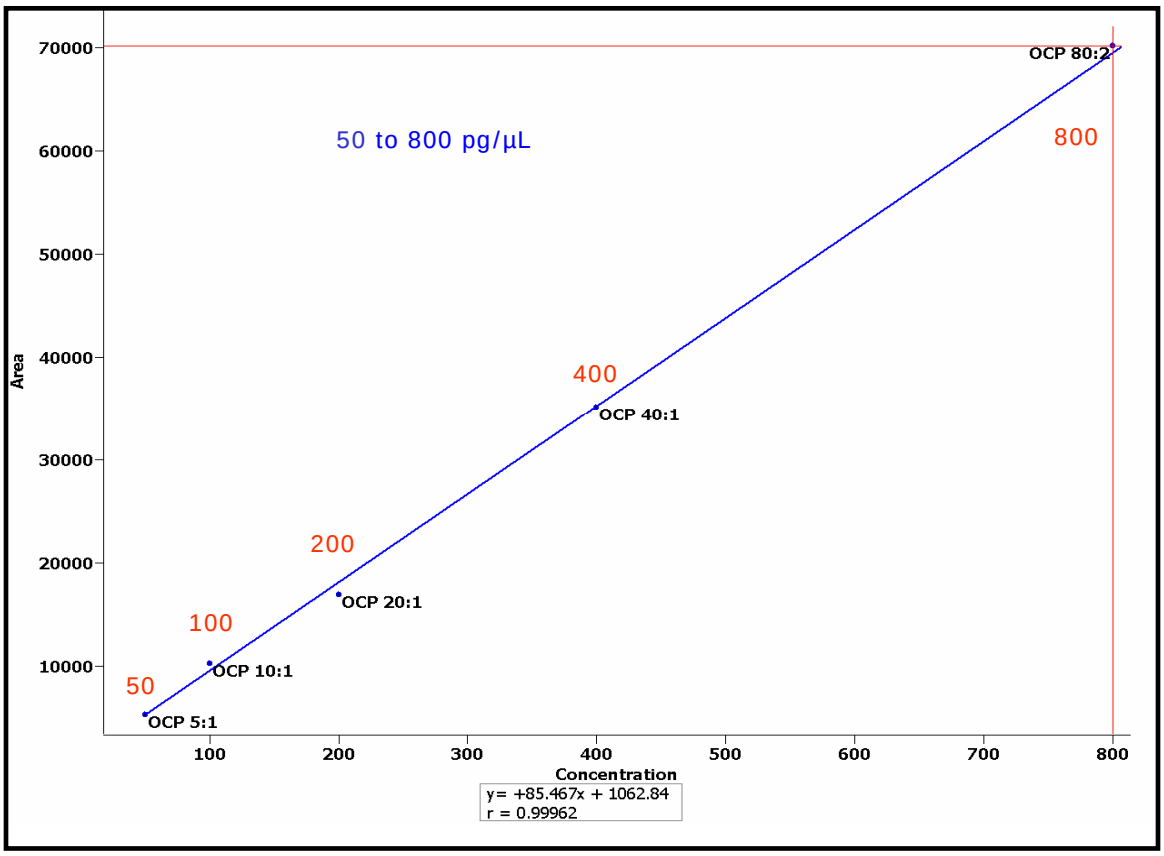


Figure 5. *GCxGC-ECD calibration curve for Methoxychlor from 50 to 800 pg/µL. Note that the concentrations are in pg/µL (listed in red near the points on the curve), but due to split injection at a ratio of 50:1, the low point represents only 1 pg on column.*

Table 2. Calibration curves extended to values lower than those used to quantify samples for OCPs.

Pesticide	Low point (pg / µL)	Correlation Coefficient
Tetrachloro-m-xylene	1	0.9983
alpha-HCH	0.5	0.9996
beta-HCH	0.5	0.9995
gamma-HCH	0.5	0.9995
delta-HCH	0.5	0.9994
Heptachlor	0.5	0.9993
Aldrin	0.5	0.9994
Heptachlor epoxide	0.5	0.9995
gamma-Chlordane	0.5	0.9997
Endosulfan I	0.5	0.9997
alpha-Chlordane	0.5	0.9996

Pesticide	Low point (pg / µL)	Correlation Coefficient
Dieldrin	1	0.9996
4,4'-DDE	1	0.9996
Endrin	1	0.9995
Endosulfan II	1	0.9996
4,4'-DDD	1	0.9996
Endrin aldehyde	1	0.9987
Endosulfan sulfate	1	0.9995
4,4'-DDT	1	0.9996
Endrin ketone	1	0.9994
Methoxychlor	5	0.9997
Decachlorobiphenyl	1	0.9996

Quantitative Comparison of GCxGC-ECD and Parallel Dual-Column GC-ECD

Tables 3 and 4 compare GCxGC-ECD and GC-ECD results for water and soil laboratory control spikes. These samples were uncontaminated with OCPs (and presumably other halogenated components) previous to the spikes so they represent a good foundation for comparing results. As can be seen, the concentration values compare nicely between the two techniques.

Table 3. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/µL) for a water laboratory control spike extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	34.7	41.5
alpha-HCH	ND	ND
beta-HCH	ND	ND
gamma-HCH	57.9	58.9
delta-HCH	ND	ND
Heptachlor	54.5	61.7
Aldrin	49.6	62.8
Heptachlor epoxide	ND	ND
gamma-Chlordane	ND	ND
Endosulfan I	ND	ND
alpha-Chlordane	ND	ND

Pesticide	GCxGC-ECD	GC-ECD
Dieldrin	115	126
4,4'-DDE	ND	ND
Endrin	116	141
Endosulfan II	ND	ND
4,4'-DDD	ND	ND
Endrin aldehyde	ND	ND
Endosulfan sulfate	ND	ND
4,4'-DDT	110	134
Endrin ketone	ND	ND
Methoxychlor	ND	ND
Decachlorobiphenyl	40.0	41.0

ND = not detected.

Table 4. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/µL) for a soil laboratory control spike extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	31.0	35.8
alpha-HCH	21.6	19.3
beta-HCH	22.3	20.3
gamma-HCH	21.7	19.6
delta-HCH	21.8	19.6
Heptachlor	20.8	19.2
Aldrin	19.0	18.9
Heptachlor epoxide	21.7	18.9
gamma-Chlordane	20.3	18.4
Endosulfan I	19.6	18.1
alpha-Chlordane	19.6	18.7

Pesticide	GCxGC-ECD	GC-ECD
Dieldrin	41.9	38.7
4,4'-DDE	40.6	37.1
Endrin	42.3	38.8
Endosulfan II	41.4	38.0
4,4'-DDD	41.4	40.3
Endrin aldehyde	30.3	22.4
Endosulfan sulfate	40.4	43.2
4,4'-DDT	40.0	39.3
Endrin ketone	42.4	43.2
Methoxychlor	195	218
Decachlorobiphenyl	33.7	39.3

ND = not detected.

In Table 5, GCxGC-ECD and GC-ECD results are compared for a "real world" water extract. To start with, looking at the surrogate results (tetrachloro-m-xylene and decachlorobiphenyl) for each method is a good way to gauge the results. As might be expected with water samples, which in general are less complex and less subject to interferences versus soil samples, the numbers are in good agreement.

Table 5. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/µL) for a "real world" water extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	21.4	19.8
alpha-HCH	ND	ND
beta-HCH	10.5	8.91
gamma-HCH	49.9	39.0
delta-HCH	ND	ND
Heptachlor	39.2	31.1
Aldrin	21.7	21.5
Heptachlor epoxide	ND	ND
gamma-Chlordane	35.9	33.4
Endosulfan I	ND	ND
alpha-Chlordane	59.1	61.0

Pesticide	GCxGC-ECD	GC-ECD
Dieldrin	13.9	10.1
4,4'-DDE	35.1	32.6
Endrin	61.0	54.2
Endosulfan II	ND	ND
4,4'-DDD	ND	ND
Endrin aldehyde	ND	ND
Endosulfan sulfate	36.4	31.5
4,4'-DDT	47.9	43.3
Endrin ketone	ND	ND
Methoxychlor	134	114
Decachlorobiphenyl	24.0	20.3

ND = not detected.

For "real world" soil extracts, differences start to show between GCxGC-ECD and parallel dual-column GC-ECD results. In Table 6, highlighted in red are some of the more dramatic concentration differentials seen for a soil extract. Especially note the highly biased value for gamma-HCH with GC-ECD. Based on gas chromatography–time-of-flight mass spectrometry (GC-TOFMS) analysis, this sample was seen to contain polychlorinated biphenyls (PCBs), and that is what is likely causing the high gamma-HCH value for GC-ECD. Interestingly, neither column used in the parallel dual-column work (Rtx-CLPesticides and Rtx-CLPesticidesII) provided an unbiased gamma-HCH concentration.

Table 6. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/µL) for a "real world" soil extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	31.8	25.9
alpha-HCH	ND	15.9
beta-HCH	ND	ND
gamma-HCH	ND	64.9
delta-HCH	ND	9.19
Heptachlor	1.44	ND
Aldrin	ND	ND
Heptachlor epoxide	20.3	10.3
gamma-Chlordane	78.5	57.6
Endosulfan I	ND	ND
alpha-Chlordane	68.3	66.3

Pesticide	GCxGC-ECD	GC-ECD
Dieldrin	10.8	6.92
4,4'-DDE	30.8	20.2
Endrin	0.27	27.8
Endosulfan II	ND	ND
4,4'-DDD	60.4	22.1
Endrin aldehyde	ND	5.18
Endosulfan sulfate	ND	ND
4,4'-DDT	211	145
Endrin ketone	ND	12.7
Methoxychlor	11.8	ND
Decachlorobiphenyl	35.9	34.2

ND = not detected.

In another example (Table 7) where the soil extract had very high concentrations of PCBs, as confirmed by GC-TOFMS, the biases for GC-ECD are even more striking (again highlighted in red).

Table 7. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/µL) for a "real world" soil extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	2.06	1.52
alpha-HCH	ND	4.96
beta-HCH	ND	3.94
gamma-HCH	154	118
delta-HCH	ND	42.1
Heptachlor	28.1	13.1
Aldrin	26.4	70.0
Heptachlor epoxide	ND	42.2
gamma-Chlordane	3.26	ND
Endosulfan I	ND	ND
alpha-Chlordane	ND	99.4

Pesticide	GCxGC-ECD	GC-ECD
Dieldrin	1.45	12.6
4,4'-DDE	124	199
Endrin	ND	216
Endosulfan II	ND	28.4
4,4'-DDD	ND	121
Endrin aldehyde	ND	49.1
Endosulfan sulfate	ND	ND
4,4'-DDT	70.9	480
Endrin ketone	ND	ND
Methoxychlor	60.4	ND
Decachlorobiphenyl	2.80	2.75

ND = not detected. This sample was diluted 30:1 prior to analysis.

The reason for the less biased performance of GCxGC-ECD can be deduced from the contour plot shown in Figure 6. The PCBs tend to elute in a relative straight line along the X-axis since they are not as significantly retained on Rtx-200 as the pesticides marked with yellow ovals in the figure. Moving the pesticides away from this chromatographic line with the second dimension separation leads to less chance of an erroneously high quantitation value for an OCP.

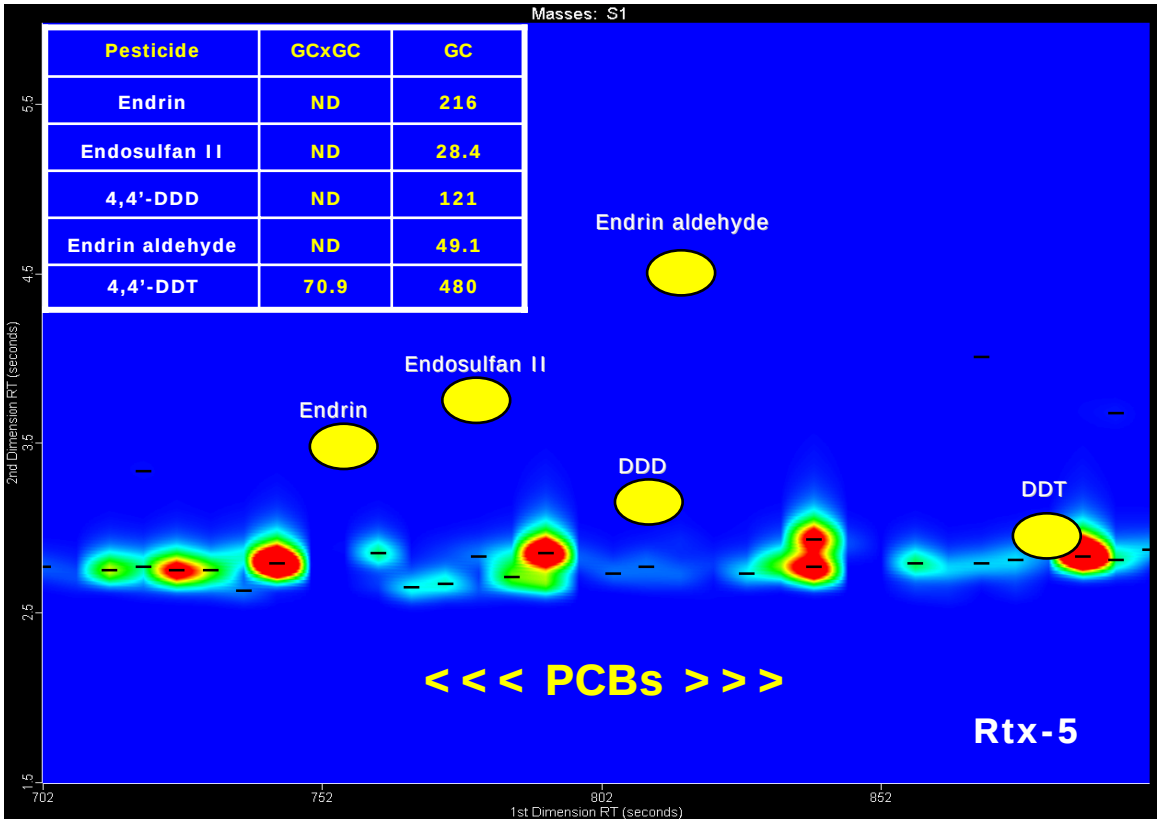


Figure 6. *Zoomed in contour plot of a "real world" soil extract showing PCBs eluting along a relatively straight line in the first dimension. The OCPs (with their chromatographic places marked by yellow ovals), except for DDT, are moved off of the X-axis by the Rtx-200 column, and are less prone to interference (and high quantitative bias), as seen in the inset table results.*

Conclusions

GCxGC is a powerful way to reduce the possibility of quantification bias when using a non-specific detector such as an ECD. As shown in this application note, it may be better than parallel dual-column analysis (while still providing a dual-column approach), for the organochlorine pesticides of EPA Method 8081, especially when the samples contain PCBs.

Calibrations (ECD responses) are linear, even across relatively wide concentration ranges. Due to the focusing effect of GCxGC, where peaks are sharpened close to the detector, sensitivity is improved, which allows detection of low femtogram levels of many OCPs.

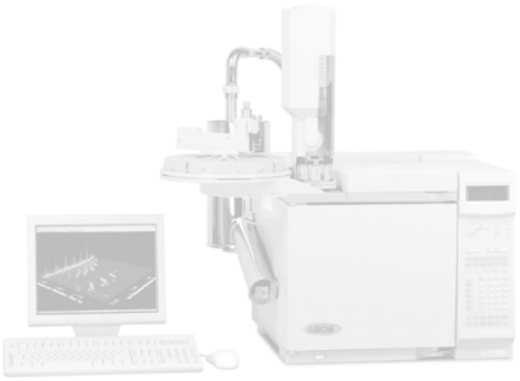
Split injections of dirty samples, possible due to the elevated sensitivity afforded when using GCxGC-ECD, may lead to less downtime due to injector and column maintenance.

Acknowledgment

Jennifer Clements and Bryce Stearns at STL Burlington kindly provided the sample extracts, the GC-ECD results, and also helped with data interpretation.

References

United States Environmental Protection Agency, Test Methods: SW-846 on-line; http://www.epa.gov/epaoswer/hazwaste/test/main.htm



Separation of Polychlorinated Biphenyl Congeners 105, 132, and 153 Using GCxGC-ECD with a Selective Column in the Second Dimension

Introduction

Polychlorinated biphenyl congeners 105, 132, and 153 (with chlorine substitutions 234-34, 234-236, and 245-245, respectively) tend to coelute on 100%-dimethyl-polysiloxane and 95%-dimethyl-5%-diphenyl-polysiloxane gas chromatography (GC) columns. This can thwart attempts to quantify them individually in samples, especially when using the non-specific electron capture detector (ECD).

Comprehensive two-dimensional GC (GCxGC) with a GC phase that is selective for PCBs is one way to solve the 105/132/153 (and other) coelution problems. GCxGC is a way to increase peak capacity by applying two independent separations to a sample in one analysis with one detector. GCxGC involves serially connected columns (differing phases) separated by a thermal modulator. A separation is performed on the first column, and then effluent from the first column is continually (and quickly) focused and "injected" onto the second column. By keeping the second column short, a series of high-speed chromatograms are generated, and the first column separation can be maintained. Separation results can be plotted as a retention plane (column 1 time x column 2 time), also known as a contour plot.

Standards

Aroclor 1254 was obtained from AccuStandard (New Haven, Connecticut, USA).

Experimental Conditions

LECO GCxGC-ECD

Agilent 6890 GC-ECD equipped with a LECO Quad Jet—Dual-Stage Thermal Modulator

Column 1:	50 m x 0.18 mm x 0.18 µm Rtx-1 (Restek)
Column 2:	1.5 m x 0.18 mm x 0.10 µm Rtx-PCB (Restek)
Carrier:	Helium at 1.3 mL/min, constant flow
Injection:	1 µL split at 250°C, split ratio 20:1
Oven 1 Program:	160°C (0.2 min), 2°/min to 280°
Oven 2 Program:	20°C offset from oven 1
Modulation Time:	8 sec.
Detector:	ECD, 325°C, N2 makeup gas at 148.7 mL/min, 50 Hz

Results and Discussion

Figure 1 shows the separation of PCBs 105, 132, and 153 in the second dimension by using GCxGC-ECD with the Rtx-PCB column. Note that these PCBs almost line up in the first dimension (a coelution), which represents their retention times on Rtx-1, 100%-dimethyl-polysiloxane. The Rtx-PCB column is particularly retentive for those PCBs that have lower degrees of ortho-chlorine substitution.

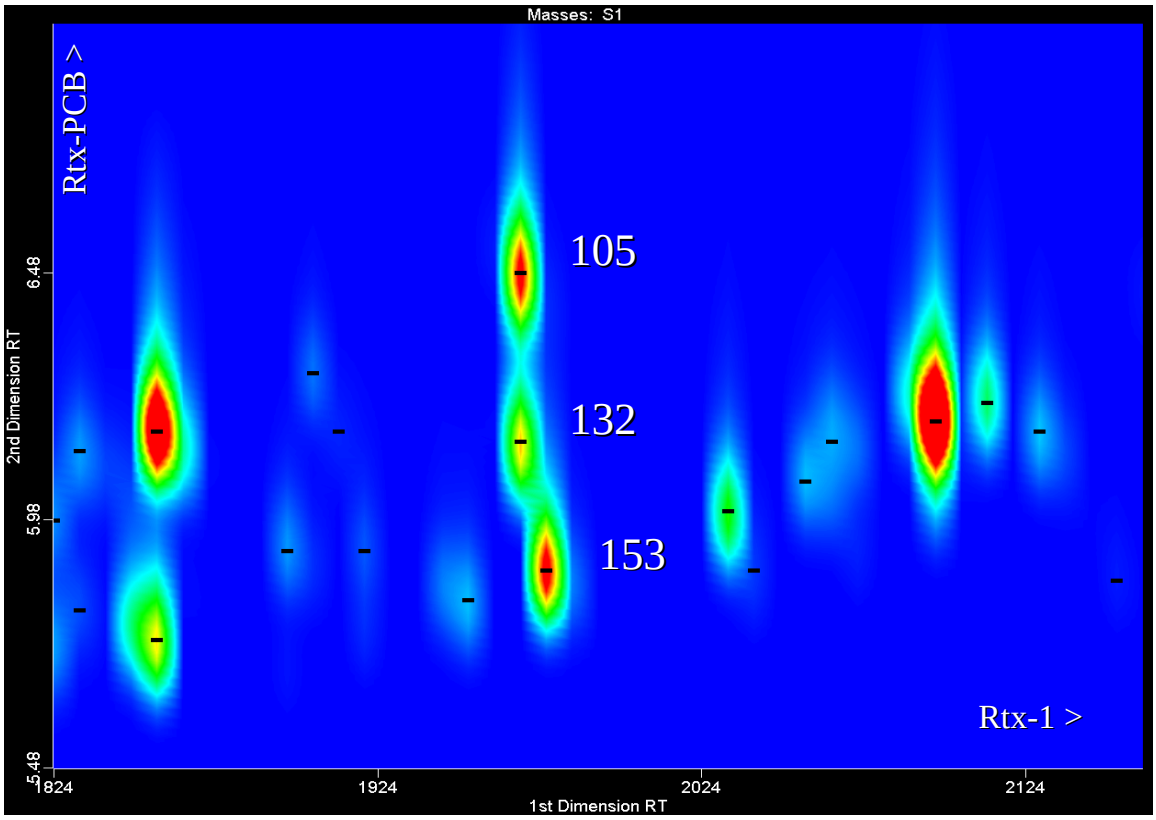


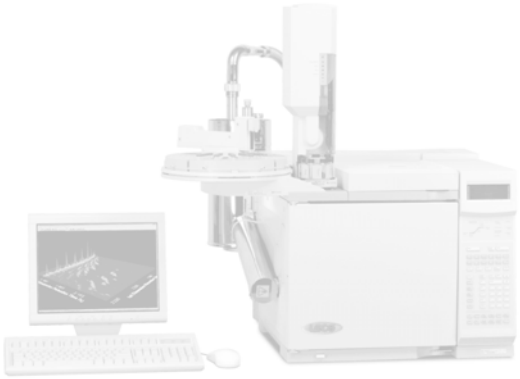
Figure 1. *Contour plot showing GCxGC-ECD separation of PCBs 105, 132, and 153 in the second dimension using the selective Rtx-PCB column. The sample is Aroclor 1254.*

Conclusions

GCxGC-ECD with a selective column in the second dimension offers a way to substantially improve separations for complex halogenated mixtures such as PCBs.

Acknowledgment

Frank Dorman at Restek Corporation provided the GC columns used for this work.



Ion Ratio as Quality Assurance for Pesticide Analysis by Gas Chromatography—Time-of-Flight Mass Spectrometry (GC-TOFMS)

Introduction

Ion Ratio is a ChromaTOF® software feature that was originally developed for clients who prior to their acquisition of a LECO Pegasus Gas Chromatograph (GC)—Time-of-Flight Mass Spectrometer (TOFMS), were measuring polychlorinated biphenyls (PCBs) by GC-Selected Ion Recording (SIR)—high-resolution mass spectrometry (HRMS). With SIR, where a full mass spectrum is not available, to ensure that it was a PCB they were determining and not an interference, they compared the ratios of two molecular ions for each congener (Figure 1). In nature, ^{35}Cl and ^{37}Cl exist in a 76% and 24% proportion, respectively. Exact ion ratios for different chlorination levels can be calculated, and if SIR determined ratios were outside a percentage range for the expected ratios (either theoretical or from analyzed standards), then the particular compound was not a PCB. Conversely, if the measured and expected ratios matched within a certain error (and the GC retention time was correct), then the compound was a PCB. This method is also used in chlorinated dioxin and furan analysis with SIR HRMS. Even though these clients now had the qualitative power of a full mass spectrum from a time-of-flight mass spectrometer, they desired Ion Ratio to supplement PCB verification.

Ion Ratio can be used for pesticide analysis as a Quality Assurance (QA) tool to supplement the full mass spectrum that is always obtained when doing TOFMS. Most importantly, Ion Ratio may illuminate quantification mass bias from interferences in the case where summed ions are used for quantification. This application note demonstrates Ion Ratio for a group of pesticides analyzed in a spiked spinach extract.

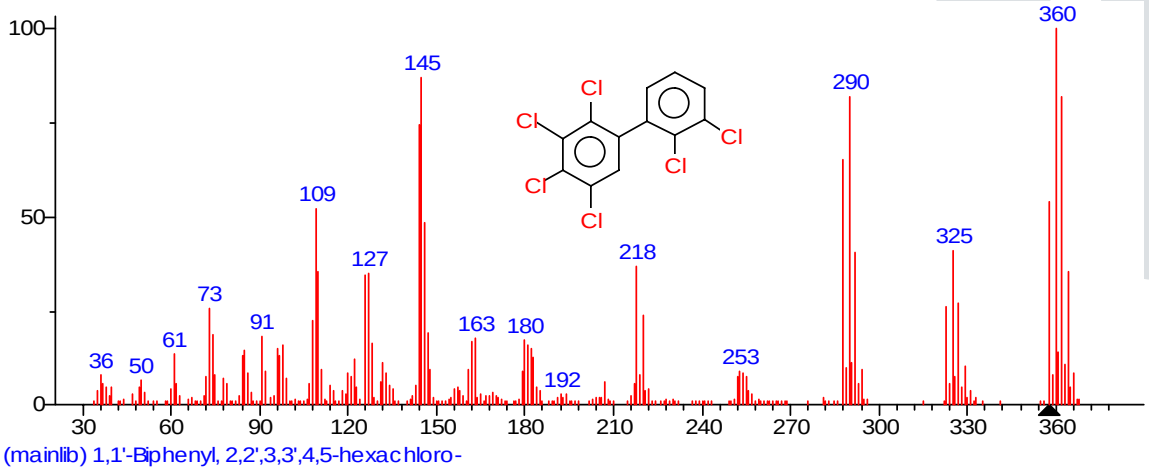


Figure 1. *Mass spectrum of hexachlorobiphenyl. The 360 and 362 ions would be used for selected ion recording—high-resolution mass spectrometry. Their proportion, as calculated from ^{35}Cl and ^{37}Cl abundances in nature, is approximately 1.25 (360/362).*

Experimental Conditions

Gas Chromatography: Agilent 6890 GC

Column:	20 m x 0.18 mm x 0.14 µm CLPII (Restek)
Carrier:	Helium at 1 mL/min, constant flow
Injection:	1 µL splitless at 250°C, 60 sec. valve
Oven Program:	40°C (1 min), 40°/min to 120°, 20°/min to 320°
Total Run Time:	13 min

Mass Spectrometry: LECO Pegasus III TOFMS

Ionization:	Electron ionization at 70eV
Source Temperature:	225°C
Stored Mass Range:	45 to 550 u
Acquisition rate:	20 spectra/sec.

Data Processing

LECO ChromaTOF software with automatic Peak Find and Deconvolution

Extraction and Analysis of Spinach

The Florida-Modified—California Department of Food and Agriculture multiresidue method was used to prepare an extract from frozen spinach purchased at a local grocery store. The spinach extract was spiked with pesticides prior to analysis by GC-TOFMS.

Calibration and Ion Ratio for Pesticide Analysis

With the full mass acquisition that is always available in TOFMS, the choice for identifying a compound in a sample as a pesticide, and then quantifying that pesticide is usually a three step process.

1. Does the compound meet the pesticide Reference Spectrum match factor?
2. Does the compound fall within a certain retention time window?
3. Does the compound meet the S/N or area threshold set by the user?

However, there is a possibility that a mass spectrum of an identified pesticide can meet the Reference Spectrum criterion, but still show bias (due to an interfering compound) on a mass selected for quantification. In this case, Ion Ratio can be an excellent QA feature for flagging the quantification bias.

Ion Ratio is set up in a Calibration Table (Figure 2). After analysis of pesticide standards, Ion Masses are entered in the Calibration Table and their ratios are calculated from a higher level standard. The user can define an Ion Ratio Tolerance (%) that will govern whether the analyzed ratio of a pesticide in a sample will be flagged as "Passed" or "Failed" (Figure 3). An Ion Ratio Result marked "Passed" supplements the Reference Spectrum match, which is the first step in identifying a peak in the proper retention time window as a particular pesticide. "Passed" also assures quantitative accuracy by indicating that no bias exists on masses chosen for quantification.

Calibration Table - "PDP Calib Ion Ratio Ltd"

Analyte	Name	Absolute R.T. (s)	Ion Ratio Masses	Expected Ion Ratio	Ion Ratio Tolerance (%)
1*	Trifluralin	426.394	264/306	1.22	30.0
2	Pentachloronitrobenzene	465.594		Not Defined	30.0
3	Chlorothalonil	503.394	264/266	0.770	30.0
4	DCPA	534.794	299/301	0.800	30.0
5	Thiabendazole	570.01	174/201	1.23	30.0
6	4,4'-DDE	576.85	316/318	0.786	30.0
7	Azinphos methyl	703.144	132/160	1.04	30.0

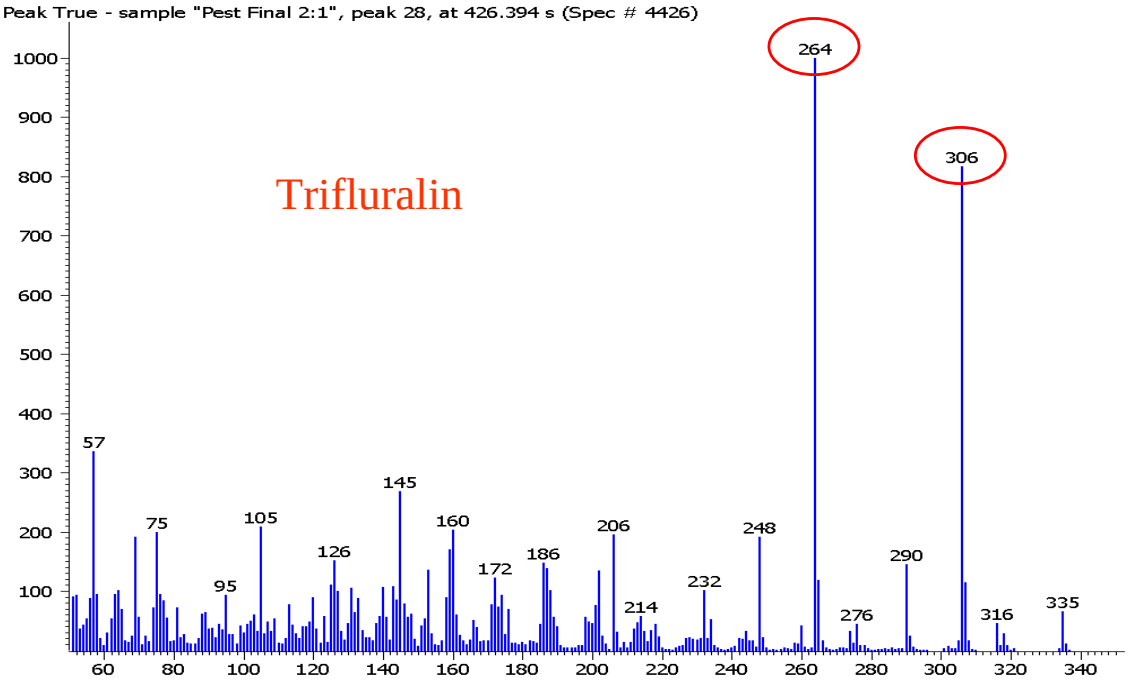


Figure 2. *Ion Ratio for a group of pesticide standards in a Calibration Table. The Trifluralin mass spectrum demonstrates how two substantial ions (264 and 306) have been chosen as the Ion Masses with an Expected Ion Ratio of 1.22.*

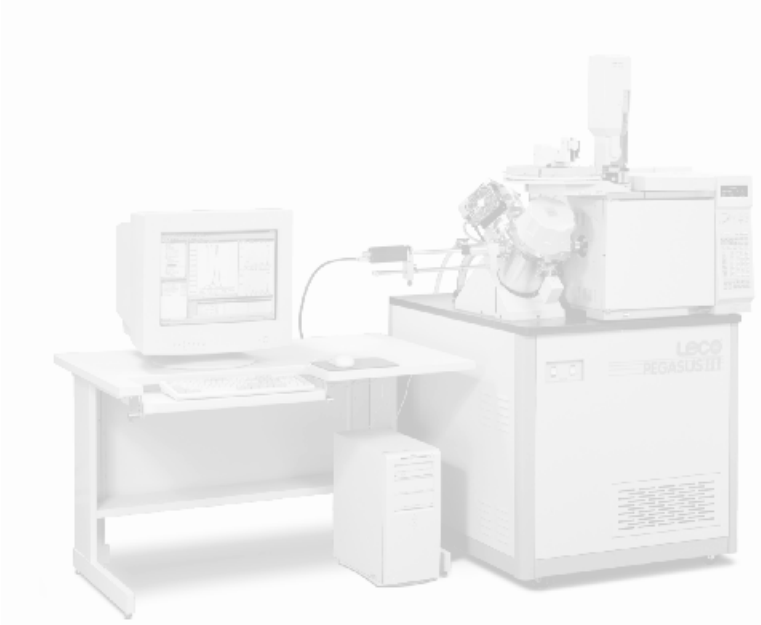
Peak Table - "Spinach 0.5:1"

Peak #	Quantification	R.T. (s)	Ion Ratio Masses	Calculated Ion Ratio	Ion Ratio Result	Quant Masses	Concentration
65*	Trifluralin	426.237	264/306	1.1973	Passed	264+306	0.55
78	Pentachloronitrobenzene	465.487	Not Defined	0.0000	Not Checked	237+249+295	2.00
91	Chlorothalonil	503.287	264/266	0.76961	Passed	264+266+268	0.41
105	DCPA	534.637	299/301	0.77972	Passed	299+301+332	0.53
119	Thiabendazole	571.537	174/201	1.2431	Passed	174+201	1.08
120	4,4'-DDE	576.587	316/318	0.78852	Passed	246+248+318	0.56
142	Azinphos methyl	703.087	132/160	1.0914	Passed	77+132+160	0.83

Figure 3. *Peak Table for a spinach extract showing results of a check of Ion Ratios for the spiked pesticides. Note that the Quant Masses and the Ion Masses overlap in most cases. If Ion Ratio Result were "Failed" instead of "Passed", this would indicate a possible bias in quantification. Note that the Expected Ion Ratio could also be displayed in the Peak Table.*

Conclusions

Ion Ratio can be used to supplement the confidence of a full mass spectrum match for a pesticide in food analysis, while indicating to the analyst that no quantification mass bias exists. It will also highlight those unusual cases where a good Reference Spectrum match was achieved, but the Ion Ratio was out of tolerance, which indicates a bias on the quantification mass or masses that could lead to the reporting of an erroneously high concentration for a pesticide.



Quantification of Dioxin-Like Polychlorinated Biphenyls Using GCxGC-ECD with a Selective Column in the Second Dimension

Introduction

Certain polychlorinated biphenyls (PCBs), those with no or only one ortho-chlorine substitution, exhibit dioxin-like toxicity due to their ability to take planar shapes in space. When determining PCBs in a variety of environmental samples, many analytical schemes focus on those congeners, and it is important that any quantitative values are free from bias. This is sometimes a challenge due to the fact that there are 209 possible PCB congeners, and also because dioxin-like PCBs are almost always at much lower concentrations in samples than other PCBs. For these reasons, the chromatographic separation of PCBs must be highly efficient.

Comprehensive two-dimensional GC (GCxGC) is one way to determine dioxin-like PCBs with a higher degree of selectivity. GCxGC increases peak capacity by applying two independent separations to a sample in one analysis with one detector. This application note will demonstrate the possibility of using GCxGC-ECD to quantify dioxin-like PCBs in environmental samples with an example of their quantification in an Aroclor 1254 dilution.

Standards

PCB standard mixes that included the dioxin-like PCBs shown in Table 1 and Aroclor 1254 were obtained from AccuStandard (New Haven, Connecticut, USA). An organochlorine pesticide standard mix was obtained from Restek Corporation (Bellefonte, Pennsylvania, USA).

Experimental Conditions

LECO GCxGC-ECD

Agilent 6890 GC-ECD equipped with a LECO Quad Jet—Dual-Stage Thermal Modulator

Column 1:	50 m x 0.18 mm x 0.18 µm Rtx-1 (Restek)
Column 2:	1.5 m x 0.18 mm x 0.10 µm Rtx-PCB (Restek)
Carrier:	Helium at 1.3 mL/min, constant flow
Injection:	1 µL split at 250°C, split ratio 20:1
Oven 1 Program:	160°C (0.2 min), 2°/min to 280°
Oven 2 Program:	20°C offset from oven 1
Modulation Time:	8 sec.
Detector:	ECD, 325°C, N_2 makeup gas at 148.7 mL/min, 50 Hz

Table 1. World Health Organization non- and mono-ortho substituted polychlorinated biphenyls.

PCB #	Cl #	Cl Position
77	4	34-34
81	4	345-4
105	5	234-34
114	5	2345-4
118	5	245-34
123	5	345-24
126	5	345-34

PCB #	Cl #	Cl Position
156	6	2345-34
157	6	234-345
167	6	245-345
169	6	345-345
189	7	2345-345

Results and Discussion

The second dimension column chosen for this work is highly selective for those PCBs that can form planar configurations, which makes it an ideal column for use with GCxGC to determine the dioxin-like PCBs. Table 2 shows how the PCBs with fewer ortho-chlorines (which allows the planar shape to occur), generally elute later in the second dimension than those with more ortho-chlorines. It is important to note that even though the retention time differences shown in this table seem rather small, the distances are in most cases adequate for quantification due to the very narrow peaks produced using GCxGC (in this work they are approximately 200 ms at half-height). In addition, the first dimension separation afforded by the Rtx-1 cannot be ignored for reducing the potential for interferences while trying to determine dioxin-like PCBs.

Table 2. Second dimension retention times in seconds (RT 2) for dioxin-like PCBs (on left) compared to those with greater degrees of ortho-chlorine substitution (on right).

PCB #	Cl Position	RT 2
77	34-34	6.42
81	345-4	6.28
105	234-34	6.50
114	2345-4	6.28
118	245-34	5.94
123	345-24	6.04
126	345-34	6.78
156	2345-34	6.72
157	234-345	6.72
167	245-345	6.12
169	345-345	7.08
189	2345-345	6.92

PCB #	Cl Position	RT 2
49	24-25	5.06
52	25-25	5.00
87	234-25	5.82
95	236-25	5.80
99	245-24	5.56
101	245-25	5.50
110	236-34	5.90
132	234-236	6.14
138	234-245	6.18
149	236-245	6.12
153	245-245	5.88
180	2345-245	6.30

To test the accuracy of GCxGC-ECD, dioxin-like PCBs in an Aroclor 1254 dilution were quantified against GCxGC-ECD calibration curves prepared using hexachlorobenzene as an internal standard. The values produced were compared against those from a detailed study conducted by Frame et. al, where numerous GC column sets and mass spectrometry were used to define Aroclor PCB concentrations (Table 3). The numbers are in good agreement, except for PCB 77, where a large concentration of PCB 110, a pentachlorobiphenyl, may have thwarted the quantification effort for PCB 77. This is interesting from the standpoint that these congeners are resolved chromatographically in the second dimension using Rtx-PCB when they exist in approximately equal concentrations in a sample (Figure 1A). Figure 1B shows the same chromatographic area as Figure 1A, but for Aroclor 1254. PCB 110, which is at 325 pg/µL as calculated from Frame et. al, is the dominant PCB, and PCB 77 cannot be detected.

Table 3. Dioxin-like PCB concentrations in Aroclor 1254 (pg/µL) as calculated from the study of Frame et. al, and with GCxGC-ECD.

PCB #	Frame et. al	GCxGC-ECD
77	1.05	**
81	ND	ND
105	105	101
114	6.30	7.41
118	257	201
123	5.25	5.41
126	ND	ND
156	28.7	32.6
157	6.65	6.96
167	9.45	11.1
169	ND	ND
189	0.35	1.44

*ND = not detected. **possible coelution from PCB 110.*

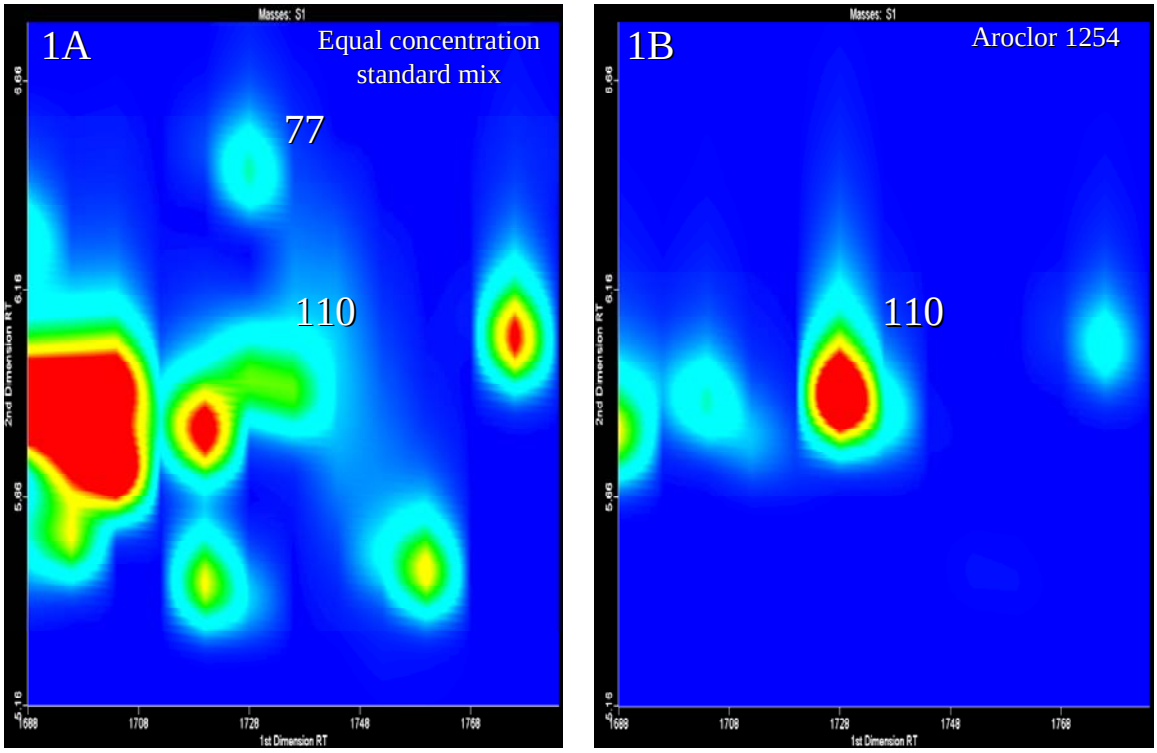


Figure 1. *Contour plots of (1A) standard mix containing equal concentrations of PCBs 110 and the dioxin-like PCB 77 and (1B) Aroclor 1254. As calculated from the study of Frame et. al, PCB 110 is at 325 pg/µL in the Aroclor 1254 dilution, while PCB 77 only has a concentration of 1.05 pg/µL.*

The selectivity of GCxGC-ECD for dioxin-like PCBs, even when they are in the presence of bulk PCBs in an Aroclor, has been adequately demonstrated by the data in Table 3, except for PCB 77. In this case, a better chromatographic separation may be necessary to determine this PCB in an unbiased fashion should PCB 110 be elevated in the same sample. To further demonstrate the selectivity of GCxGC-ECD for dioxin-like PCBs, the Aroclor 1254 dilution was made more complex by spiking it with an organochlorine pesticide (OCP) mix that contained aldrin, chlordanes, DDD, DDE, DDT, dieldrin, endosulfans, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide, hexachlorocyclohexanes, and methoxychlor at 100 pg/µL for each pesticide. In environmental samples it is not unusual to find OCPs with PCBs, which complicates the determination of both species. Table 4 demonstrates that the OCPs did not interfere with the determination of dioxin-like PCBs in an Aroclor mix, highlighting the selectivity of the GCxGC-ECD technique.

Table 4. Dioxin-like PCB concentrations in Aroclor 1254 (pg/µL) as calculated from the study of Frame et. al, and with GCxGC-ECD. The Aroclor was spiked with an OCP standard to make the determination more challenging and demonstrate the selectivity of GCxGC.

PCB #	Frame et. al	GCxGC-ECD
77	0.95	**
81	ND	ND
105	94.2	96.4
114	5.67	6.54
118	232	193
123	4.73	4.90
126	ND	ND
156	25.8	33.1
157	5.99	6.74
167	8.51	10.5
169	ND	ND
189	0.32	0.85

*ND = not detected. **possible coelution from PCB 110.*

Conclusions

GCxGC-ECD with a selective column in the second dimension offers a way to substantially improve separations for the important group of dioxin-like PCBs, even in the presence of PCBs that are normally at higher concentrations in environmental samples. Due to the increase in peak capacity afforded with GCxGC, organochlorine pesticides did not interfere with dioxin-like PCB determinations either.

Reference

G.M. Frame, J.W. Cochran and S.S. Bowadt, **Complete PCB Congener Distributions for 17 Aroclor Mixtures Determined by 3 HRGC Systems Optimized for Comprehensive, Quantitative, Congener-Specific Analysis,** *J. High Resol. Chromatogr.,* 19 (1996) 657-668.

Acknowledgment

Frank Dorman at Restek Corporation provided the GC columns used for this work.

GC-TOFMS and GCxGC-TOFMS of Organophosphate Pesticides in Ash Leaves

Introduction

Organophosphate pesticides such as Chlorpyrifos, Diazinon, and Malathion are sometimes used on ornamental plants to control nuisance insects. Monitoring these plants for the insecticides can be a challenge because the residual levels are often small and the matrix (the plant material) is chemically complex. This note describes the analysis of an ash leaves extract for Chlorpyrifos, Diazinon, and Malathion by Gas Chromatography—Time-of-Flight Mass Spectrometry (GC-TOFMS) and comprehensive two-dimensional GC-TOFMS (GCxGC-TOFMS). Advantages offered by automated peak find and spectral deconvolution routines are discussed, and the benefit of enhanced peak capacity for complex samples is shown.

Sample Extraction

An ash leaves extract was prepared by the United States Department of Agriculture in Gulfport, Mississippi.

Experimental Conditions

GC-TOFMS

GC: Agilent 6890 Gas Chromatograph

Column:	20 m x 0.25 mm x 0.71 µm Rtx-TNT (Restek)
Carrier:	Helium at 2 mL/min, constant flow
Injection:	1 µL splitless at 250°C, valve time 60 sec.
Oven Program:	80°C (1 min), 20°/min to 340°

MS: LECO Pegasus® TOFMS

Ionization:	Electron ionization at 70 eV
Source Temperature:	225°C
Stored Mass Range:	45 to 550 u
Acquisition Rate:	20 spectra/sec.

GCxGC-TOFMS

GCxGC: Agilent 6890 Gas Chromatograph equipped with a LECO GCxGC Thermal Modulator and Secondary Oven

Column 1:	10 m x 0.18 mm x 0.20 µm Rtx-5 (Restek)
Column 2:	2 m x 0.10 mm x 0.10 µm Rtx-PCB (Restek)
Carrier:	Helium at 0.7 mL/min, constant flow
Injection:	1 µL splitless at 275°C, valve time 60 sec.
Oven 1 Program:	80°C (1 min), 10°/min to 300°
Oven 2 Program:	35°C offset from oven 1
Modulation Time:	3 sec.

MS: LECO Pegasus TOFMS

Ionization:	Electron ionization at 70 eV
Source Temperature:	225°C
Stored Mass Range:	45 to 550 u
Acquisition Rate:	100 spectra/sec.

Data Processing

LECO ChromaTOF® software with automated peak find and spectral deconvolution.

Results and Discussion

Figure 1 simultaneously illustrates the complexity of the ash leaves extract and the power of automated peak find and spectral deconvolution afforded by the fast acquisition capability and spectral reproducibility of TOFMS. The matrix peaks, especially the off-scale m/z 124, hide the orange peak representing Diazinon (approximately 50 pg), and a caliper spectrum at the Diazinon peak apex is representative of the background from the ash leaves. Note that the deconvoluted mass spectrum matches nicely with a reference spectrum for Diazinon.

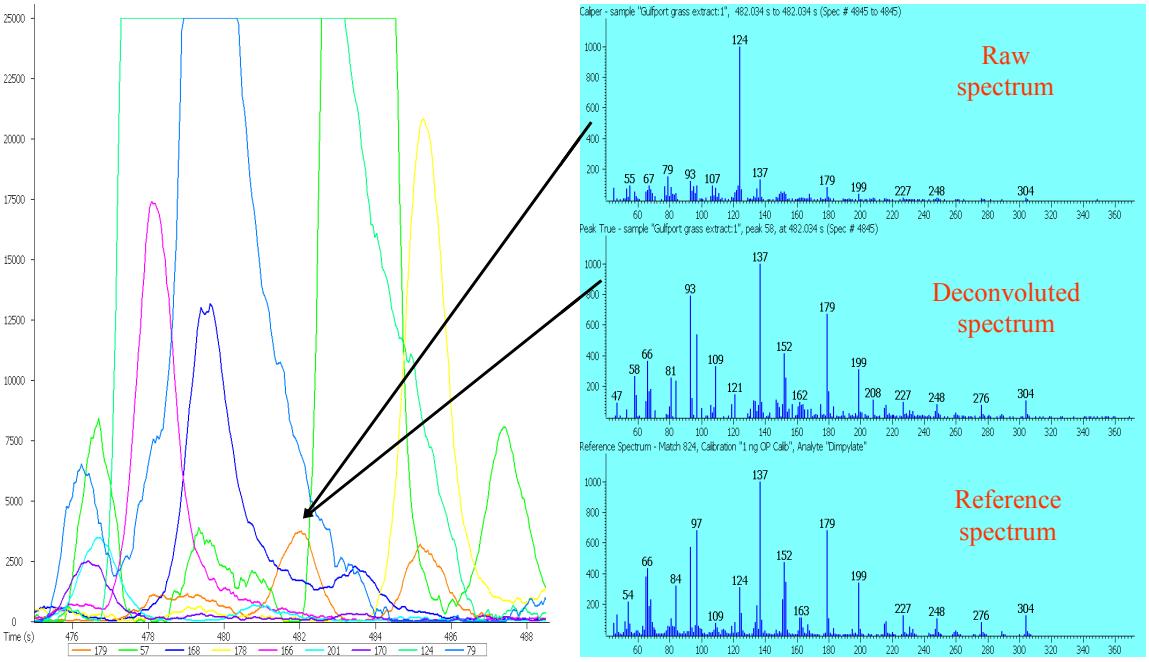


Figure 1. *Chromatogram of 50 pg Diazinon (orange peak) in an ash leaves extract plotted with ions representing the matrix. The raw spectrum taken at the peak apex for Diazinon is representative of the matrix interferences. The deconvoluted TOF mass spectrum matches well with the reference spectrum.*

A similar chromatographic situation exists for Malathion in the complex ash leaves extract as shown in Figure 2. Again, the raw spectrum taken at the peak apex for Malathion (approximately 125 pg) is mainly showing ions for the matrix interferences, particularly 167, the turquoise off-scale peak in Figure 2. The deconvoluted spectrum, while not perfect (it still contains some 167 ion), shows a similarity of 806 (out of 999) against the first-hit library spectrum for Malathion.

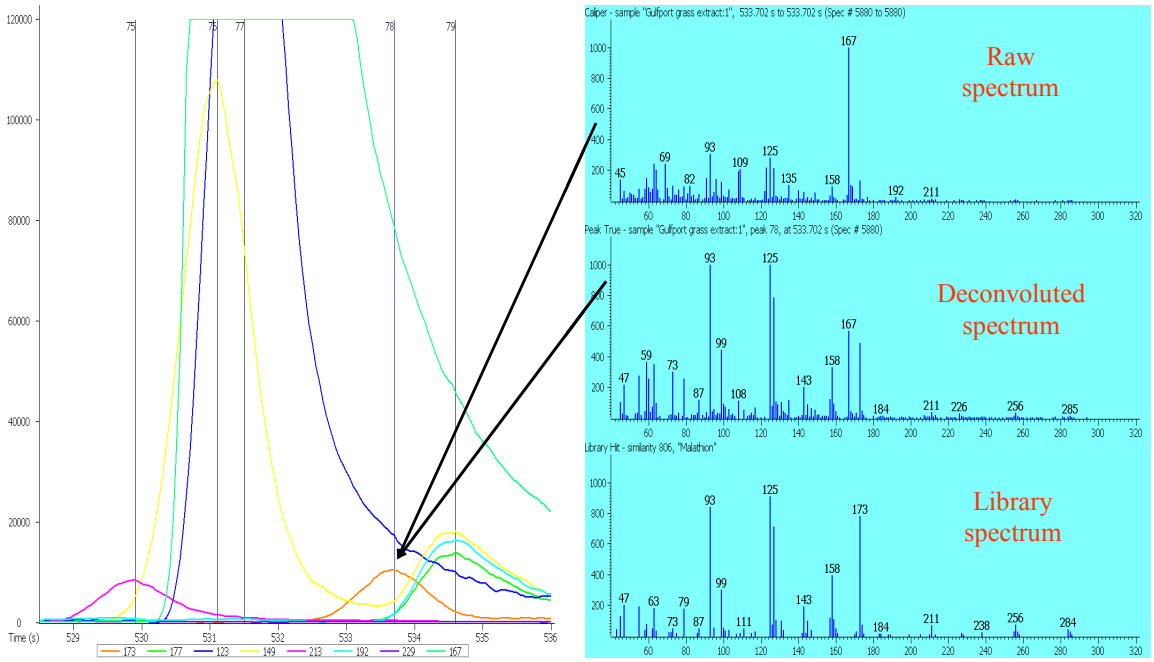


Figure 2. *Chromatogram of 125 pg Malathion (orange peak) in an ash leaves extract plotted with ions representing the matrix. The raw spectrum taken at the peak apex for Malathion hardly hints that Malathion may be present. The deconvoluted TOF mass spectrum, even though it contains some residual 167 ion from the huge matrix interference, matches well with a library spectrum.*

Another way to tackle complex samples is to increase the chromatographic separating power by applying GCxGC. The benefit of this approach is easily visualized when viewing the contour plot, or GCxGC chromatogram, of Figure 3. Notice how separations are now occurring in two dimensions, one along the X-axis (Rtx-5) and the other along the Y-axis (Rtx-PCB). The potential to move pesticides away from matrix interferences is substantially increased. In fact, in this example, both Malathion and Chlorpyrifos would have coeluted with high-concentration matrix interferences in a one-dimensional analysis with Rtx-5. But the Rtx-PCB has located the pesticides away from these interferences in the second dimension (Figure 4), resulting in the high quality, library-searchable spectra seen in Figure 5. The similarities for both pesticides are greater than 900 (out of 999).

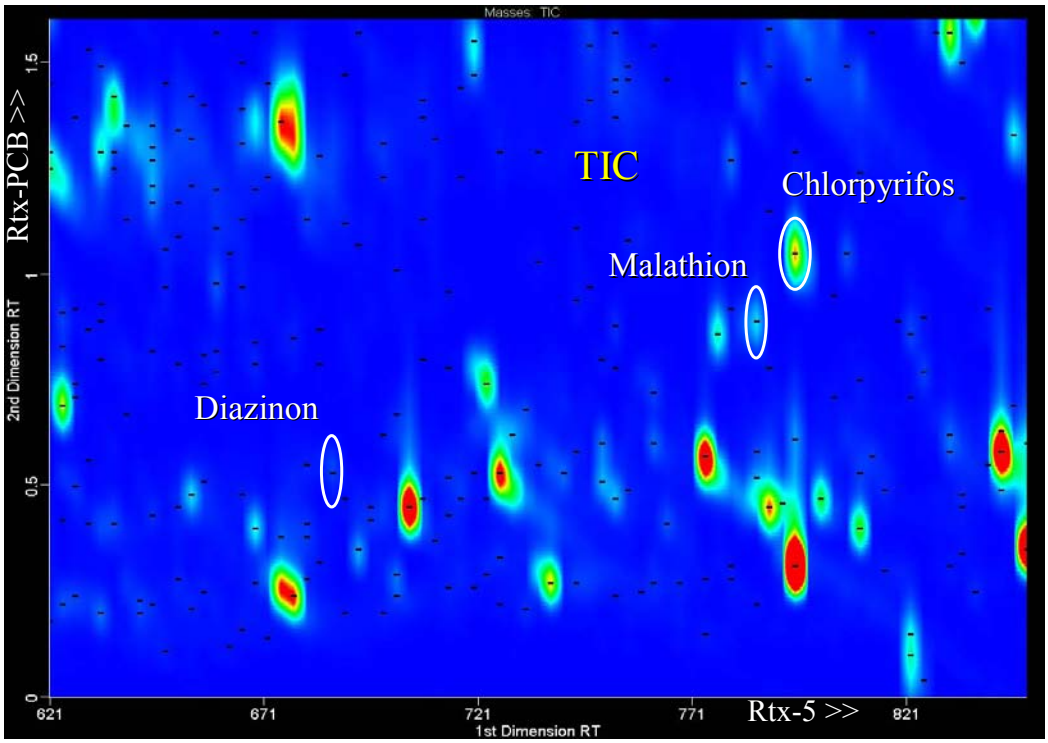


Figure 3. *Contour plot of organophosphate pesticides in an ash leaves extract. Note how separations are occurring in two dimensions, and where Malathion and Chlorpyrifos have been separated in the second dimension from substantial matrix interferences (at a first dimension retention time of approximately 800 seconds).*

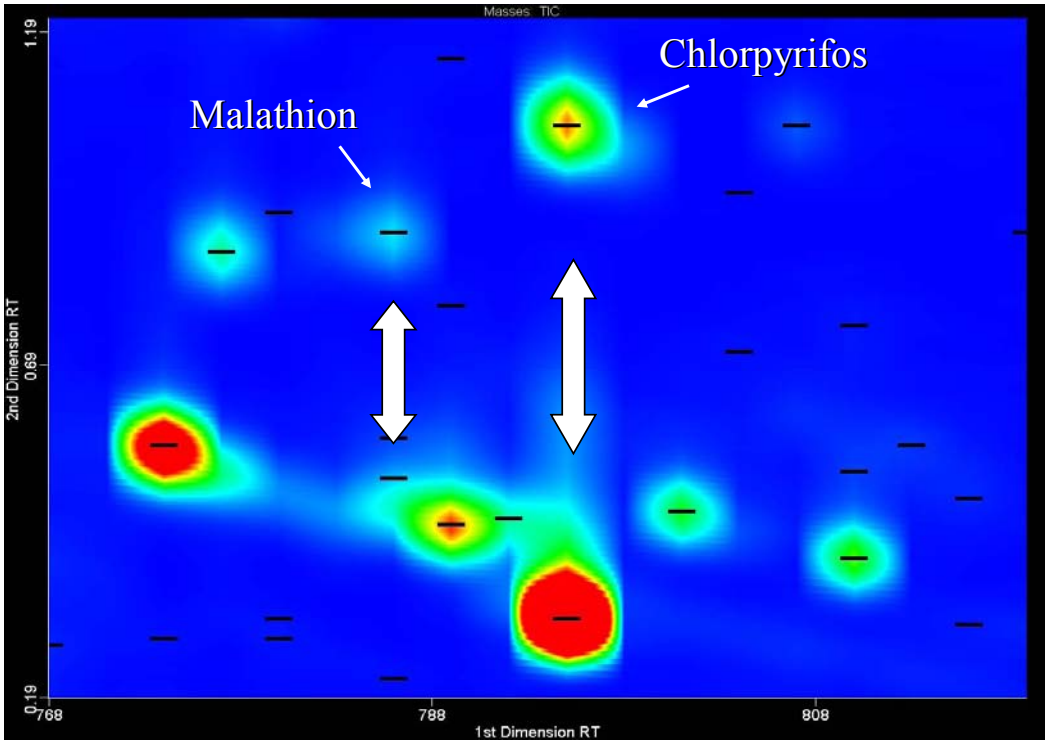


Figure 4. *Zoom of contour plot from Figure 3. Malathion and Chlorpyrifos are separated in the second dimension from substantial matrix interferences as noted by the white arrows.*

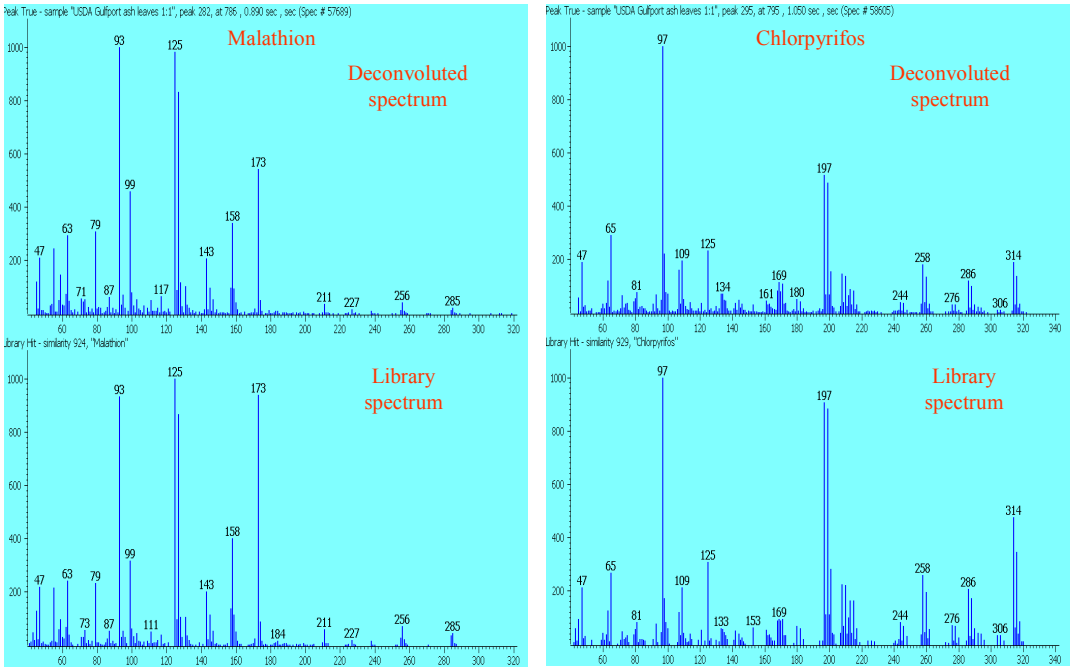


Figure 5. *TOF mass spectra from the GCxGC analysis of Malathion and Chlorpyrifos in an ash leaves extract. The similarities versus library spectra are greater than 900 (out of 999).*

One observation for the spectrum of Malathion produced with GCxGC of ash leaves was the absence of the 167 ion (from matrix) that was present in the deconvoluted spectrum for the one-dimensional analysis. Figure 6 helps illuminate the reason behind this, which simply put, is the increased separating power available with GCxGC. Close inspection of the GCxGC portion of this figure (or of the contour plots in Figures 3 and 4) will reveal that the GCxGC peaks, due to thermal focusing close to the detector, are only about 100-200 ms wide at their base. Because the peaks are so narrow, TOFMS, which can acquire data at up to 500 spectra/second, is the only mass spectrometer appropriate for GCxGC.

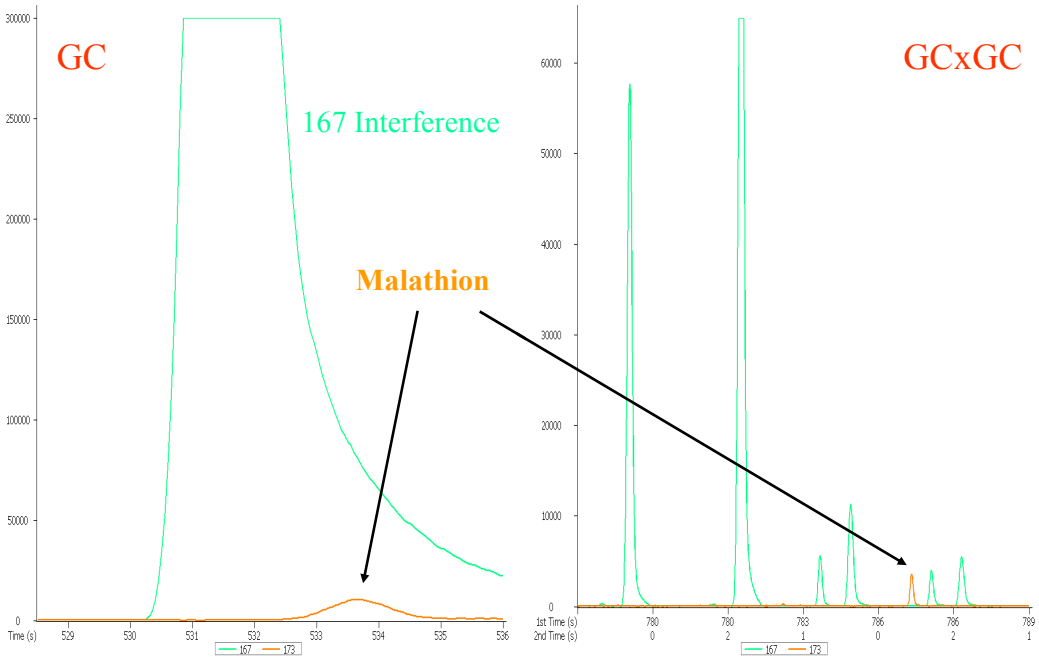


Figure 6. *Linear chromatograms for one-dimensional (GC) and two-dimensional (GCxGC) analyses of Malathion in ash leaves. Malathion coelutes with a large matrix peak in GC, but is easily separated from this same compound using GCxGC.*

The separation of Diazinon from its interference in the one-dimensional analysis was also better when employing GCxGC (Figure 7).

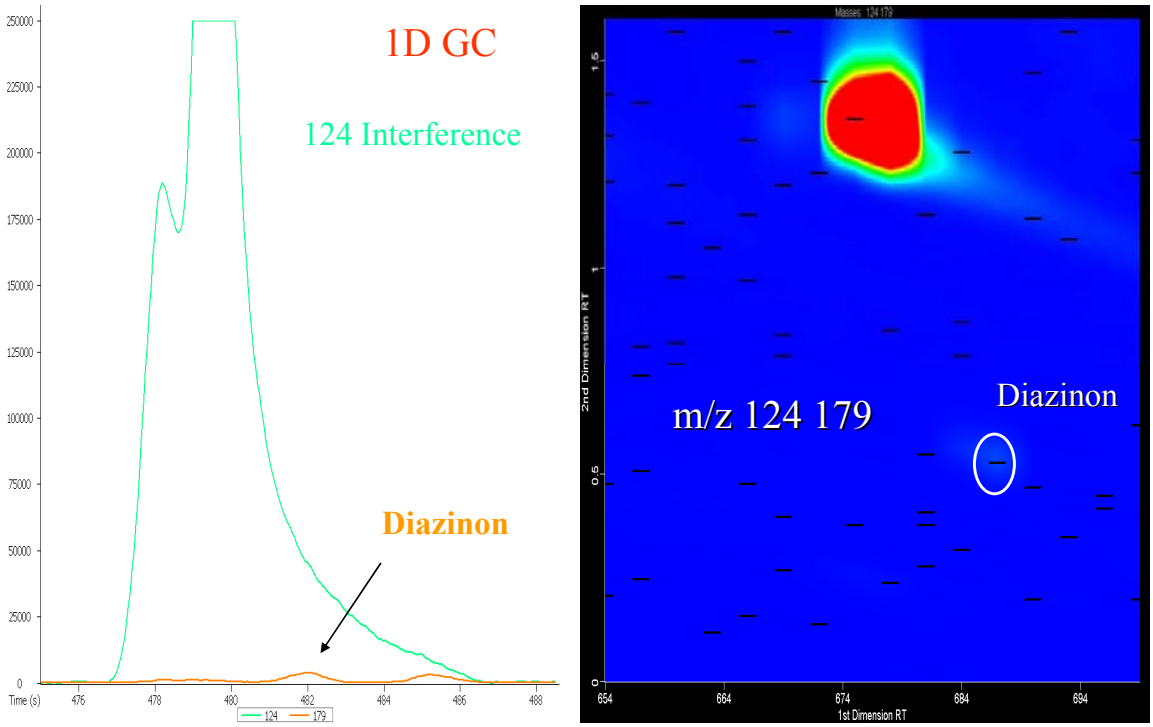


Figure 7. *One-dimensional GC (1D GC) and GCxGC (contour plot on right) analyses for Diazinon in ash leaves. The matrix interference that contains substantial 124 ion and is coeluting with Diazinon in 1D GC is easily resolved from Diazinon using GCxGC. The peak containing the 124 ion is the large red spot in the contour plot.*

Conclusions

GC-TOFMS and GCxGC-TOFMS are both powerful techniques for determining pesticides in matrix due to automated peak find and spectral deconvolution, and because of the peak capacity increase afforded with GCxGC. GCxGC-TOFMS has the potential to produce better spectra for organophosphate pesticides in the most complex matrices. TOFMS is the only mass spectrometer that has the acquisition speed to support GCxGC, and a full mass spectrum is always obtained.

Acknowledgment

John Gallagher at the United States Department of Agriculture in Gulfport, Mississippi provided the ash leaves extract. The GC columns were from Frank Dorman at Restek Corporation.

The Use of Resample, a New ChromaTOF® Feature, to Improve Data Processing for GCxGC-TOFMS

Introduction

Comprehensive two-dimensional Gas Chromatography (GCxGC) peaks generally range from 100 to 200 ms wide. A Time-of-Flight Mass Spectrometer (TOFMS) is the only mass spectrometer capable of proper GCxGC peak definition because it can acquire data at up to 500 spectra/second. Also with TOFMS, a full mass spectrum from 5 to 1000 u can always be acquired if necessary. With fast acquisition rates, full mass range acquisition, and the longer GC runs sometimes seen in GCxGC, file sizes can be rather large (up to 500 MB or more), and automated and flexible data handling is necessary. ChromaTOF®, LECO's fully automated instrument control and data processing software for the Pegasus® 4D, contains many features necessary to handle GCxGC data. Some of these features (all automated) include baseline, peak find, spectral deconvolution, Combine of peak slices, library search, calculate area/height, quantify, Compare, and reporting of data. Recently, a new feature called Resample was added to ChromaTOF that provides additional flexibility for GCxGC data processing.

Resample is a way to create a new data file that is reduced in size from an Acquired Sample File. Often this can lead to faster and more efficient data processing. This note will demonstrate the use of Resample for GCxGC-TOFMS data.

Samples

A petroleum standard containing paraffins, iso-paraffins, aromatics, naphthenes, and olefins (PIANO mix) was used to demonstrate Resample with GCxGC-TOFMS, in addition to another standard containing polybrominated diphenyl ethers (PBDEs).

Experimental Conditions for Petroleum

GCxGC-TOFMS

GCxGC: Agilent 6890 Gas Chromatograph equipped with a LECO GCxGC thermal modulator and secondary oven

Column 1:	100 m x 0.25 mm x 0.50 µm Rtx-1 PONA (Restek)
Column 2:	2 m x 0.25 mm x 0.10 µm Rtx-50 (Restek)
Carrier:	Helium at 2.3 mL/min, constant flow
Injection:	0.05 µL split at 250°C, split ratio 250:1
Oven 1 Program:	40°C (0.2 min), 2°/min to 200°
Oven 2 Program:	5°C offset from oven 1
Modulation time:	2 seconds

MS: LECO Pegasus TOFMS

Ionization:	Electron ionization at 70 eV
Source Temperature:	200°C
Stored Mass Range:	35 to 450 u
Acquisition Rate:	100 spectra/second

Experimental Conditions for PBDE Standard

GCxGC-TOFMS

GCxGC: Agilent 6890 Gas Chromatograph equipped with a LECO GCxGC thermal modulator and secondary oven

Column 1:	9.5 m x 0.18 mm x 0.20 µm Rtx-5 (Restek)
Column 2:	1 m x 0.25 mm x 0.25 µm Rtx-Dioxin 2 (Restek)
Carrier:	Helium at 1.5 mL/min, constant flow
Injection:	1 µL direct injection at 250°C, 4 mm Uniliner (Restek)
Oven 1 Program:	120°C (1 min), 10°/min to 340° (2 min)
Oven 2 Program:	20°C offset from oven 1
Modulation Time:	3 seconds

MS: LECO Pegasus TOFMS

Ionization:	Electron ionization at 70 eV
Source Temperature:	225°C
Stored Mass Range:	200 to 1000 u
Acquisition Rate:	50 spectra/second

Data Processing

LECO ChromaTOF software.

Results and Discussion

To reduce processing time for GCxGC-TOFMS data, it is often desirable to process only part of the chromatogram. Figure 1 is a contour plot, or GCxGC chromatogram, of a PIANO mix that is marked with yellow lines to illustrate an example of limited data processing. The only area of interest in this example is the first dimension retention time region, approximately 1850 to 3550 seconds, which contains the C_3-C_6 benzenes. ChromaTOF already contains segmented processing capability that allows peak finding for only this region (Figure 2), which can reduce the overall processing time.

Resample is a recently added ChromaTOF feature that allows segmented processing by creating a new file for the desired retention time region, with the added benefits of choosing a sample reduction rate (acquisition rate), and/or restricting the mass range for the new file. The sample reduction rate allows a user to create a file of, for example, 50 spectra/second acquisition rate from one of 100 spectra/second, which cuts the file size in half and can speed processing. The nice thing about this part of the feature is that the "extra" spectra are not thrown away, but are instead summed to produce a signal-to-noise enhancement. Mass range restriction, in addition to decreasing file size which leads to processing time improvement, may have the added benefit of improving spectral deconvolution when lower (or higher) m/z ions are discarded that are only from matrix interferences.

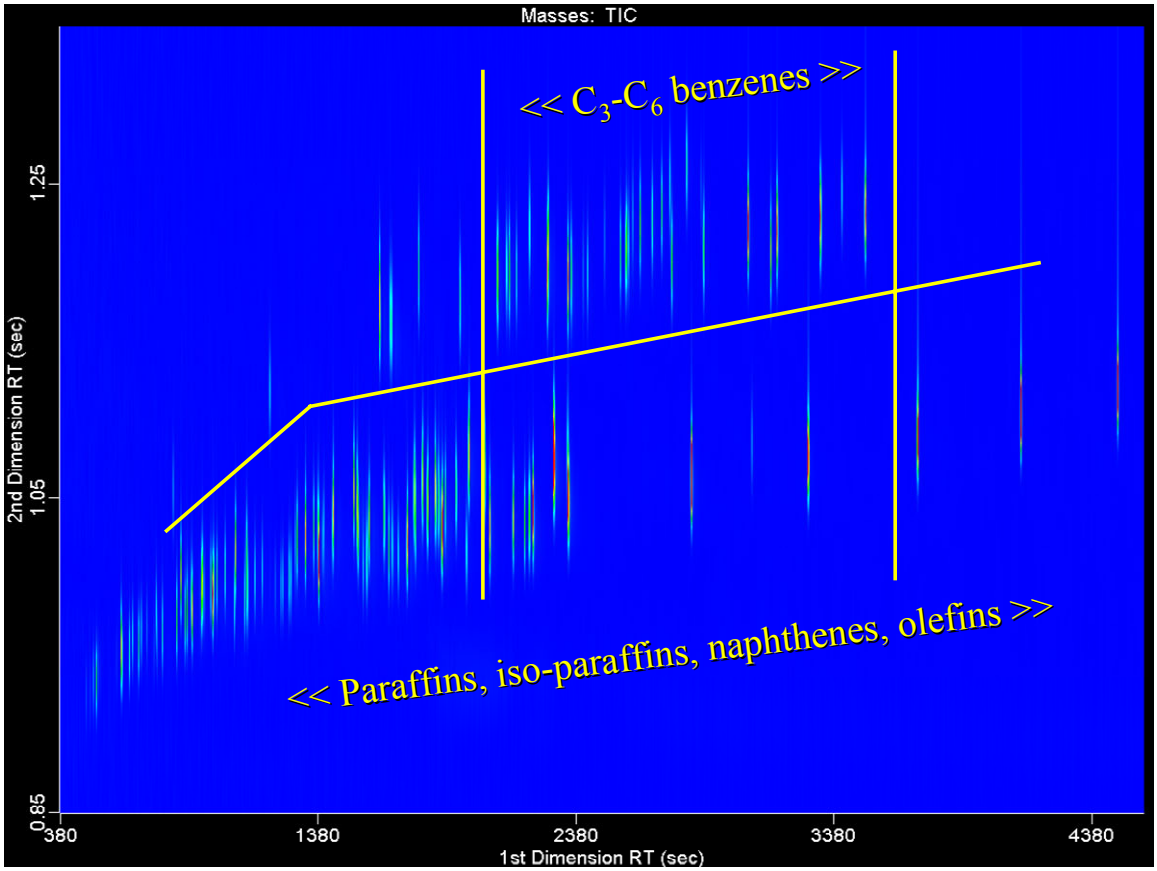


Figure 1. *Contour plot of PIANO mix analyzed with GCxGC-TOFMS. The vertical yellow lines denote the first dimension retention area for processing the C_3-C_6 benzenes. The horizontal yellow line is used only to mark the second dimension separation of aliphatics and aromatics.*

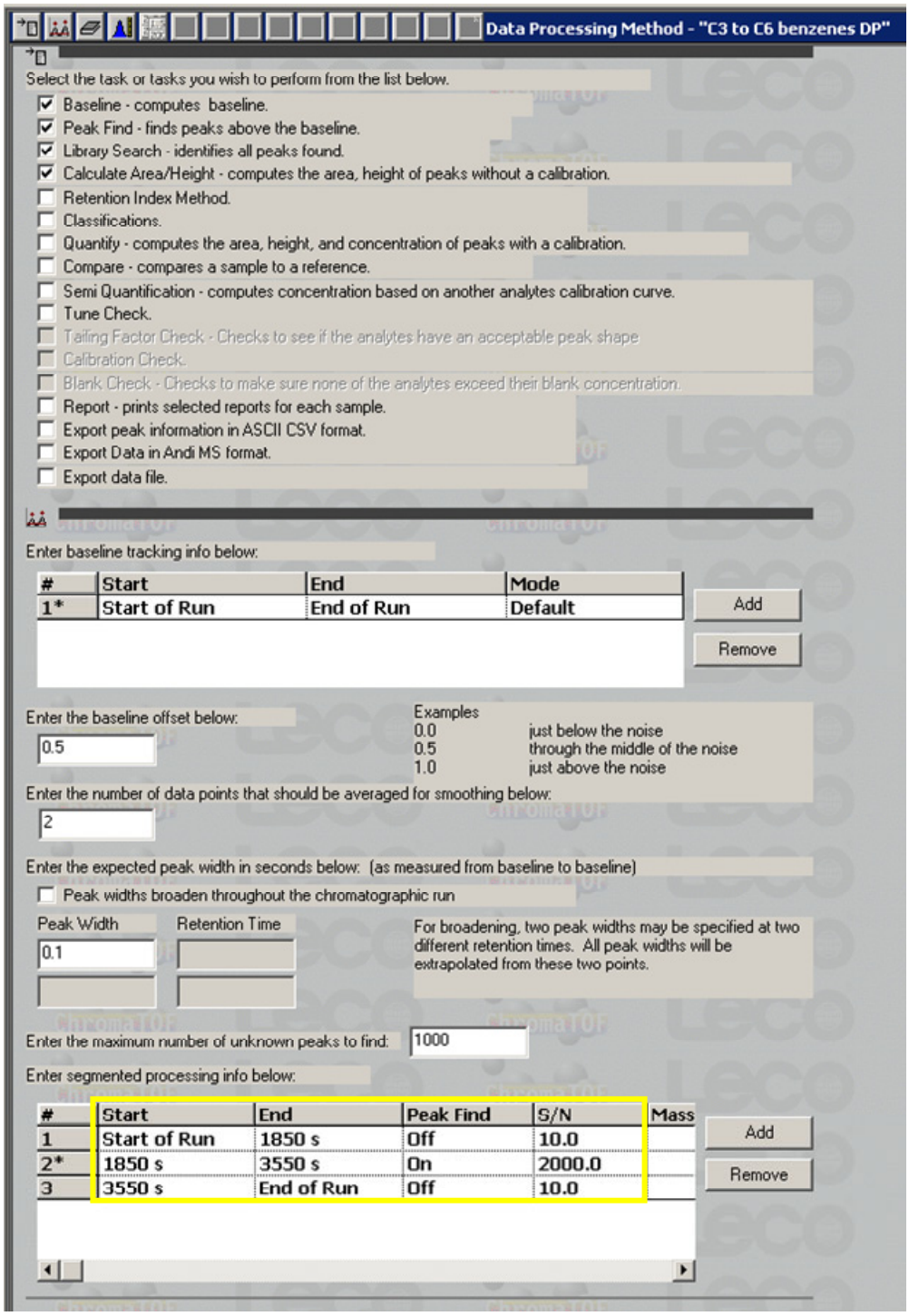


Figure 2. *Segmented processing capability of ChromaTOF where peak finding can be restricted across a narrow retention time range.*

Figure 3, a meld of screen captures from ChromaTOF, shows the Resample dialog boxes with new entries for the C_3-C_6 benzene retention time region of the PIANO mix. And Figure 4 shows the process of Resample occurring, where the new file (peg file extension) is created. After creating the new file in the directory of the user's choice, it can be returned to the Acquired Sample Files database by using Import for peg files.

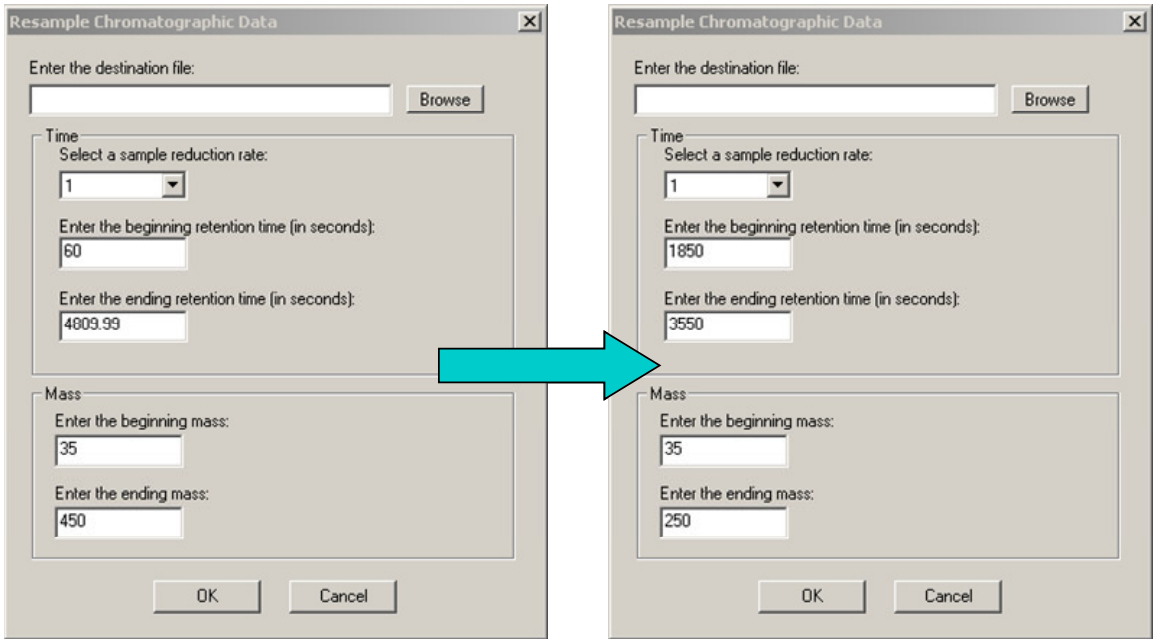


Figure 3. *Example of Resample of a PIANO mix Acquired Sample File where a new file will be created for the retention time range of 1850 to 3500 seconds and the mass range of 35 to 250.*

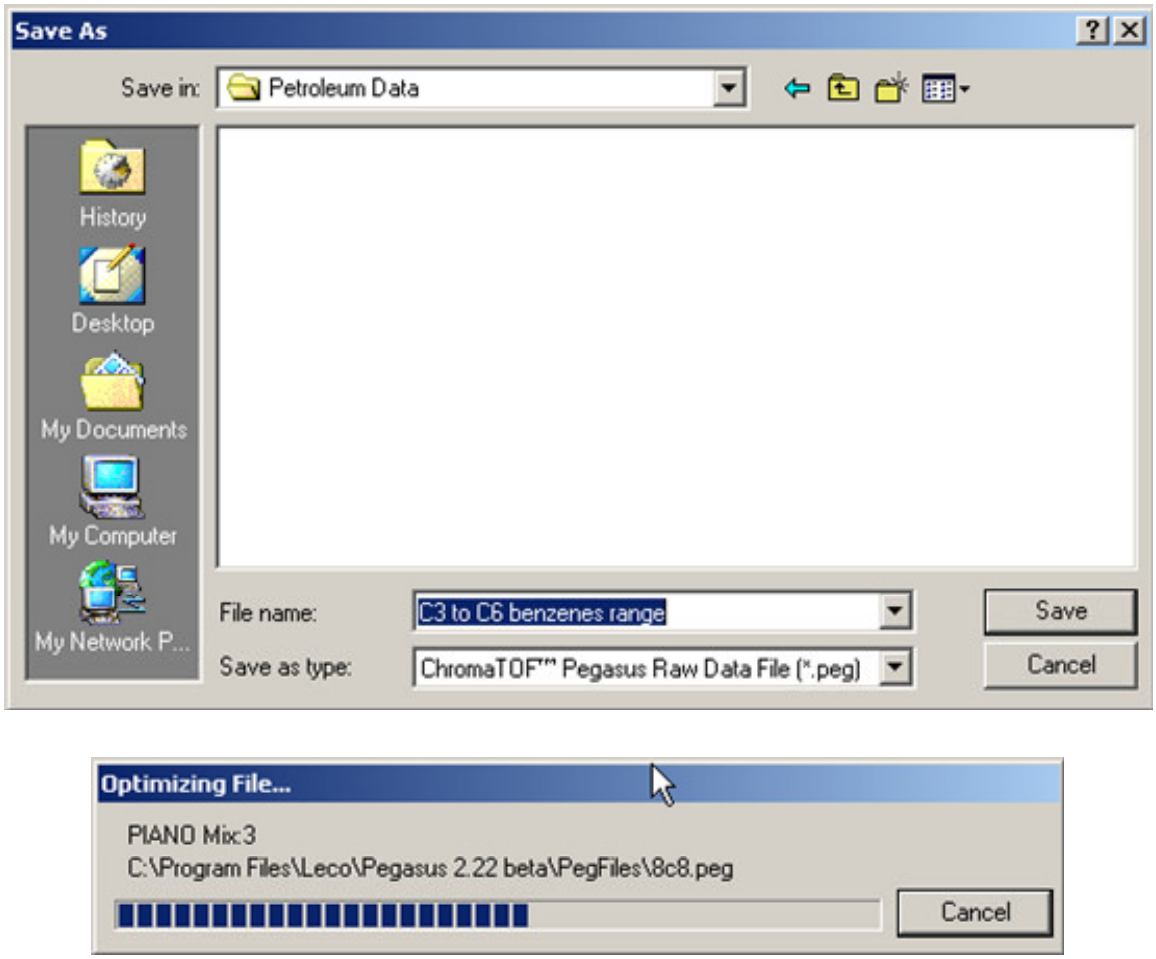


Figure 4. *File being created from Resample parameters seen in Figure 3.*

Table 1 contains the information used to construct the new file from Resample and illustrates the resulting file size reduction. It is important to note that the original file is completely intact and could even be used again to construct a new file with different Resample parameters.

Table 1. Resample parameters and reduced file size.

Resample Parameter	Old File	New File
Sample reduction rate	1	1
Beginning retention time (sec.)	60	1850
Ending retention time (sec.)	4810	3550
Beginning mass	35	35
Ending mass	450	250
File size (Mb)	377.9	70.4

For the previous example of Resample, it was not necessary to use the sample reduction rate parameter, as the peak widths generated for GCxGC of the PIANO mix mandated the 100 spectra/second acquisition rate. During a GCxGC-TOFMS analysis for PBDEs though, decabromodiphenyl ether (BDE 209), which is notoriously tough to chromatograph, showed very broad peaks (slices) that were over-sampled at the 50 spectra/second rate that was appropriate for other PBDEs (Figure 5).

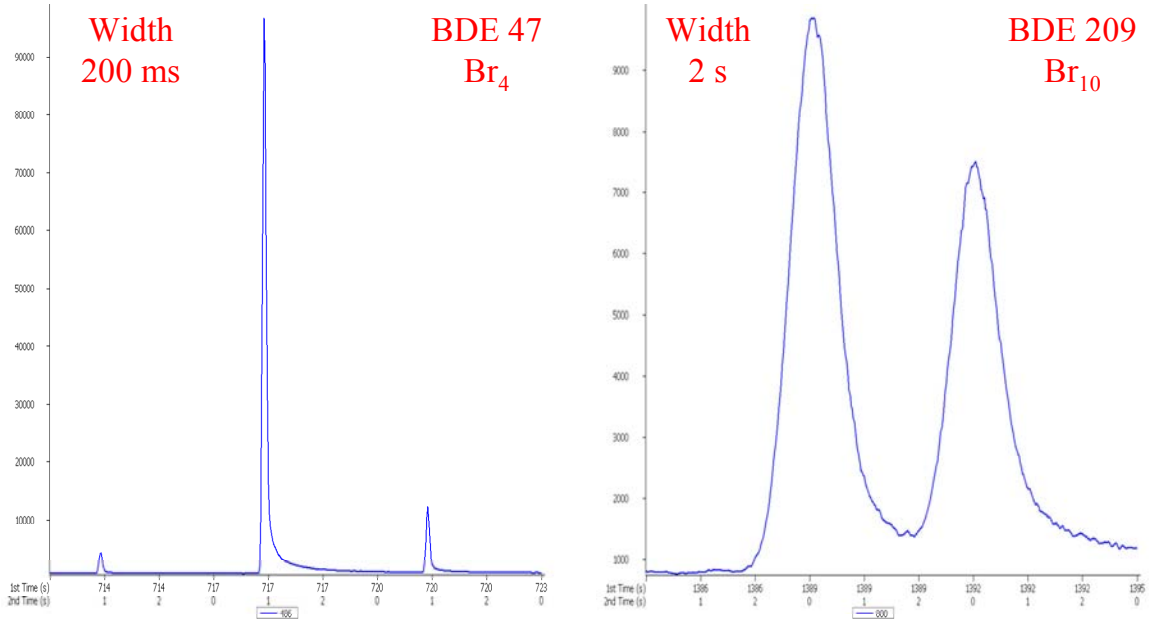


Figure 5. *Linear chromatograms showing modulated peaks for BDEs 47 (tetrabromo) and 209 (decabromo). 50 spectra/second is an appropriate acquisition rate for the 200 ms wide peaks of BDE 47, but the rate is too fast for the broad peaks of BDE 209 (which results in a sensitivity decrease).*

Using Resample with sample reduction rates of 2, 5, and 10 (a dialog box demonstrating this, with retention time and mass range reductions, is shown in Figure 6) better peak responses are seen for BDE 209 (Figure 7). As mentioned earlier, this enhanced peak response is due to spectral summing.

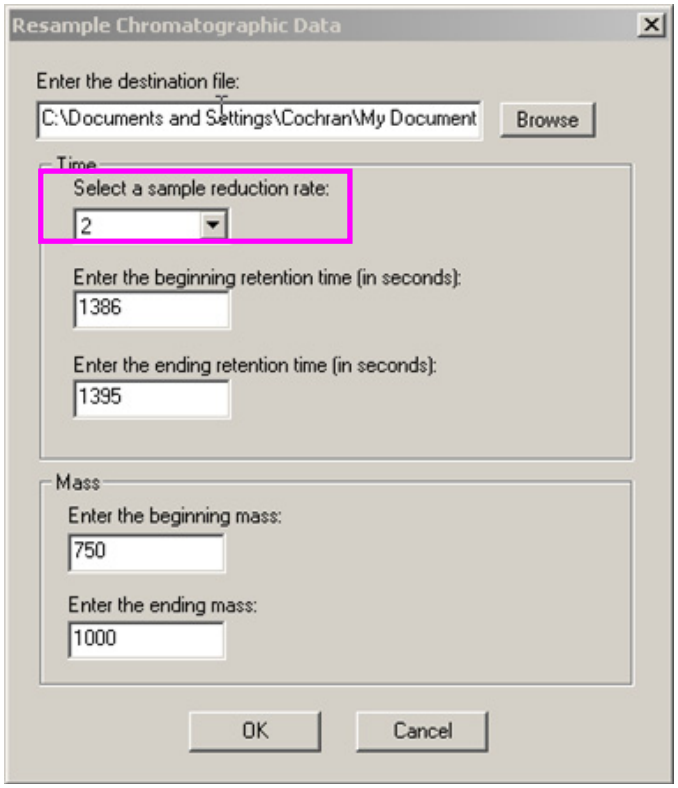


Figure 6. *Dialog box showing Resample for BDE 209. Because the data acquisition rate was too fast for the broad peaks, a sample reduction rate of 2 was used for the retention time range for where BDE 209 eluted.*

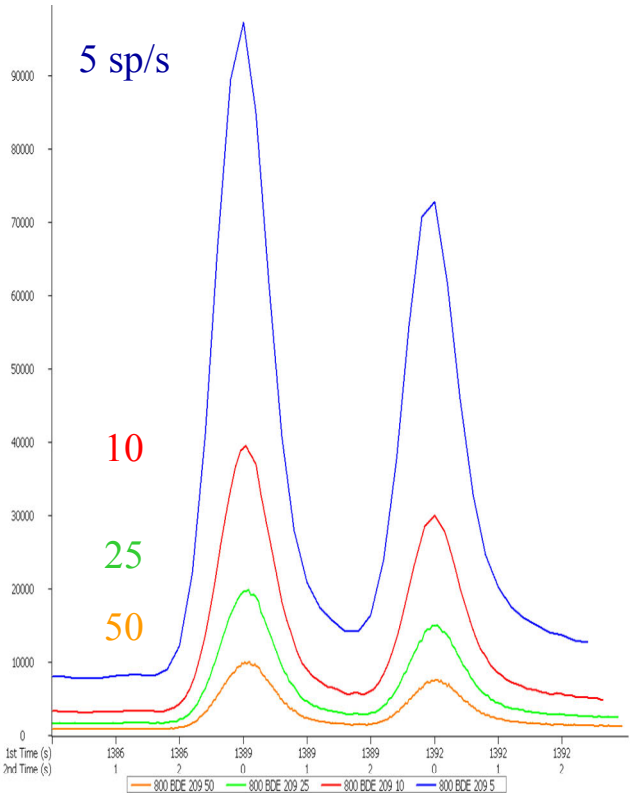


Figure 7. *Linear chromatograms for modulated BDE 209 at original acquisition rate of 50 spectra/second (orange) and when sample reduction rates (of 2, 5, and 10) were used in the ChromaTOF Resample feature to produce new files at 25 (green), 10 (red), and 5 (blue) spectra/second. The enhanced peak responses are due to spectral summing.*

Conclusions

Resample is a new ChromaTOF feature that adds flexibility to GCxGC-TOFMS data processing. New files can be created that reflect sample rate reductions, restricted retention time regions, and narrower mass ranges. The resulting smaller file sizes speed up automated processing routines. In addition, spectral deconvolution may be better due to elimination of low or high m/z matrix ions. When Resample is used to match peak widths properly to acquisition rates through the sample rate reduction, a sensitivity enhancement can occur. No matter how Resample is used, the original data file is still intact.

Acknowledgment

Victoria Jacobs at AccuStandard, Inc. provided the PIANO mix and Frank Dorman at Restek Corporation supplied the GC columns.

Characterization of Ballpoint Pen Inks by Solid-Phase Microextraction Gas Chromatography—Time-of-Flight Mass Spectrometry

Introduction

Time-of-Flight Mass Spectrometry (TOFMS) offers benefits for Gas Chromatography (GC) not available with other mass spectrometers (e.g. quadrupoles and magnetic sectors) because of its fast acquisition rates (up to 500 spectra/second) and the spectral continuity associated with an almost instantaneous mass analysis. Two of these benefits are automated Peak Find and Spectral Deconvolution, algorithms that can be built into the data processing software.

Characterization of ink samples with GC-MS is important for several reasons, including formulation determination for competitive motives, quality assurance and control, counterfeit cases, and cases where age of a document is in question. In this contribution, Solid-Phase Microextraction (SPME) GC-TOFMS, with automated peak find and spectral deconvolution, is used to characterize ballpoint pen inks. In addition, an automated Compare algorithm is employed to identify components associated with aged ballpoint inks in a complex background matrix of volatile compounds generated from the heating of paper to sample the aged ink by SPME.

Experiments

Two experiments were conducted for this work. The first involved characterization of black (8 samples) and blue (5 samples) ballpoint inks for volatile organic compounds (VOCs) with SPME GC-TOFMS. The second was determining VOCs remaining in ballpoint ink from aged scribbles on paper.

Experimental Conditions

Solid-Phase Microextraction

A divinylbenzene/Carboxen/PDMS (50/30 µm) fiber from Supelco was used. 5 mm punched paper holes with ink scribbles were placed into a 4 mL vial that was capped with a PTFE-silicone septum for SPME sampling. Five holes were used for the characterization experiment and eight were used for the ink aging work. The vial was sampled by headspace SPME while either at 22°C (characterization) or 70°C (aging). For the aging work, the vial was equilibrated at 70°C for 10 minutes prior to SPME. A 10 minute SPME sampling time was used to collect ink volatiles. The fiber was desorbed into a 0.75 mm injection sleeve (Supelco) in a split/splitless injector at 270°C either at a 20:1 split ratio (characterization) or splitless with a valve time of 60 seconds (aging).

GC-TOFMS

Column:	20 m x 0.25 mm x 0.71 µm Rtx-TNT (Restek)
Carrier:	Helium at 2 mL/min, constant flow
Injection:	See above.
Oven Program:	40°C (1 min), 40°/min to 340°
Total Run Time:	9 min

MS: LECO Pegasus TOFMS

Ionization:	Electron ionization at 70 eV
Source Temperature:	200°C
Stored Mass Range:	35 to 450 u
Acquisition Rate:	10 spectra/second

Data Processing

LECO ChromaTOF® software with automated peak find and spectral deconvolution.

Results and Discussion

The automated Peak Find algorithm that is integral to ChromaTOF software was used to locate all ballpoint ink VOCs that eluted in a retention range approximately between acetone (not included in tables) and 2-phenoxyethanol (included in tables). Separate reference tables were prepared of major volatiles in black and blue inks. Tables 1 and 2 contain the results for major components of black and blue ballpoint pen inks. All of the values in the table are relative to mixed ink samples and do not necessarily indicate the major volatile component of each ink. Interestingly, all of the ballpoint pen inks, except for black pens 4 and 5, are distinctly different as to their volatile constituents. This was quickly and easily determined through fast GC-TOFMS analysis and the fully automated data processing available with ChromaTOF.

Table 1. Relative component amounts for eight black ballpoint pen inks analyzed using SPME GC-TOFMS.

Compound Name	1	2	3	4	5	6	7	8
Ethylene glycol						930		
Propylene glycol		100	13			310		
Hexylene glycol			630					
Phenol	8	100	82	2	4		52	140
Aniline		150	85			120		
Ethoxy ethoxyethanol	350	3						
2-Ethylhexanol	17	97	170	140	120	35		
Benzyl alcohol		91	120	170	150			
Phenylethanol	170	15	53	85	62			
2-Phenoxyethanol	33	46	41	38	22			75
Phenoxypropanol			210					95

Table 2. Relative component amounts for five blue ballpoint pen inks analyzed using SPME GC-TOFMS.

Compound Name	1	2	3	4	5
Propylene glycol		38			160
Hexylene glycol		81			
Phenol	48			30	
Aniline		29	130		
Methoxy methylethoxy propanol 1	550	15	62		
Ethoxy ethoxyethanol	520				
Methoxy methylethoxy propanol 2	530				
Dimethoxy propane	620				
Benzyl alcohol	780				
Phenylethanol	900				
2-Aminotoluene			130		
2-Phenoxyethanol	71	16	18	38	
Diethylaniline				100	

An example of the automated peak find capability of ChromaTOF for a complex ink sample is seen in Figure 1. Each vertical line with a number represents a found peak, and even where there are significant coelutions (some of the peak apexes are only ms apart), peaks were easily located.

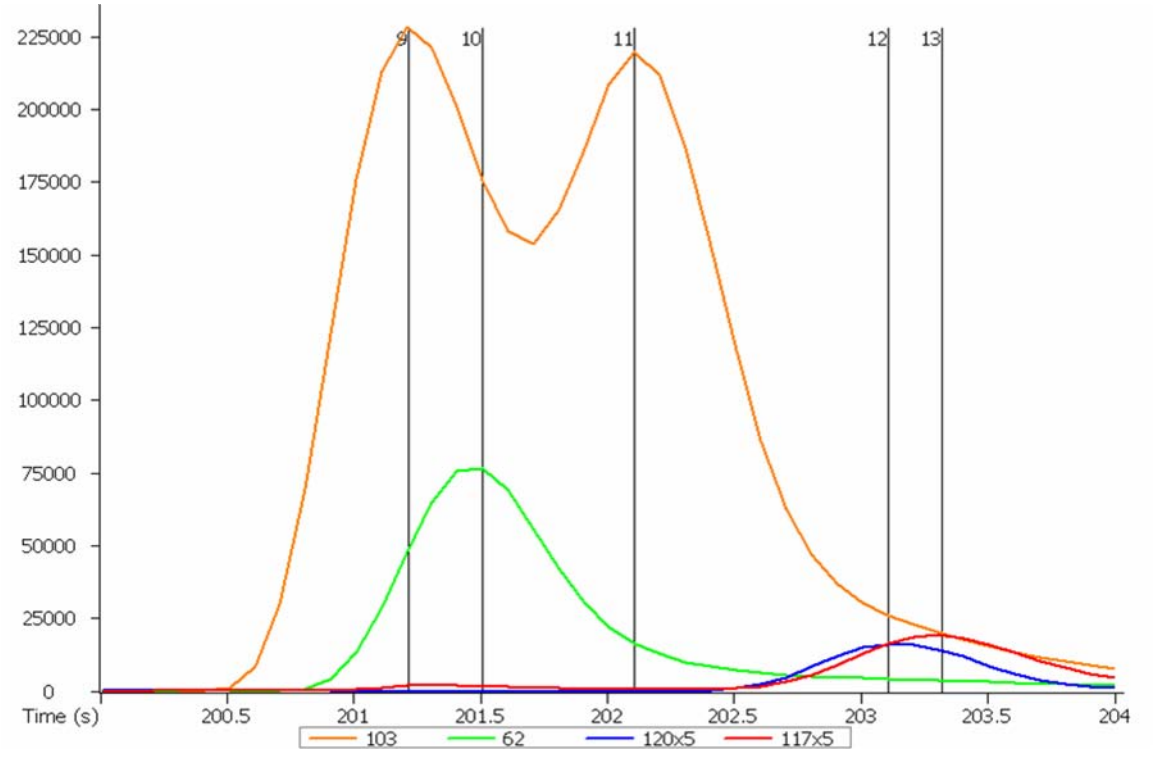


Figure 1. *Automated peak find with ChromaTOF. Each numbered vertical line is a peak marker. Even though there are obvious coelutions, ChromaTOF located the peaks.*

Just as important as locating the peaks in a complex sample, is producing deconvoluted mass spectra that are fully library searchable. Figures 2 and 3 demonstrate this for two of the peaks from Figure 1. When viewing the figures, notice that shared ions between the compounds, represented by extracted unique ions 103 (orange) and 62 (green), are properly proportioned by the ChromaTOF Spectral Deconvolution algorithm. This guarantees that library search results will be accurate.

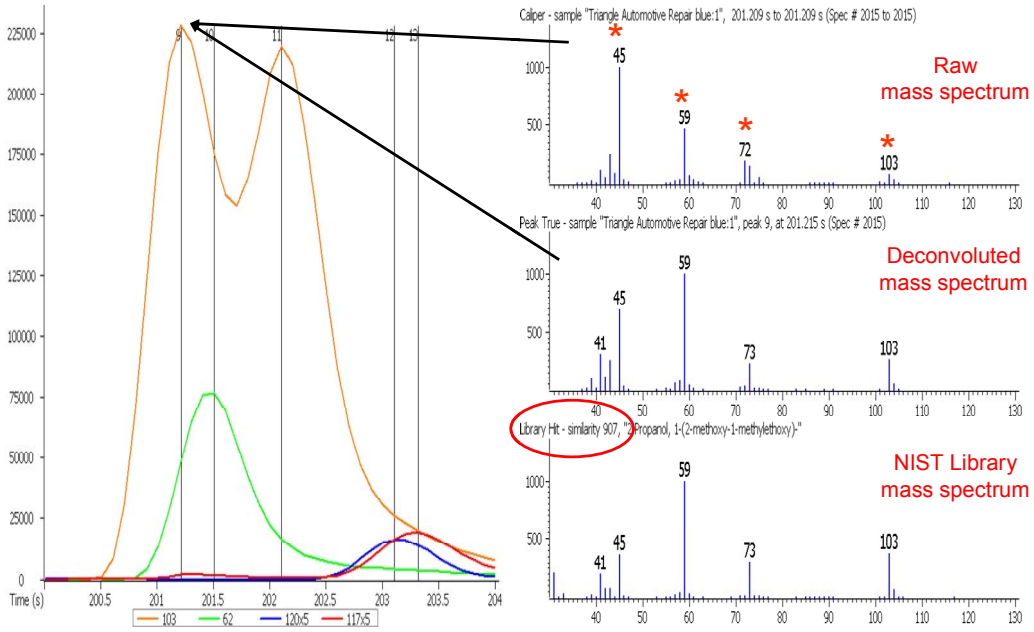


Figure 2. *Automated peak find and spectral deconvolution for methoxy methylethoxy propanol in a blue ballpoint pen ink. The raw mass spectrum is a combination of ions (including those that are shared, marked with red asterisks) from the coelutions. The deconvoluted spectrum has a similarity of 907 (out of 999) with the library spectrum.*

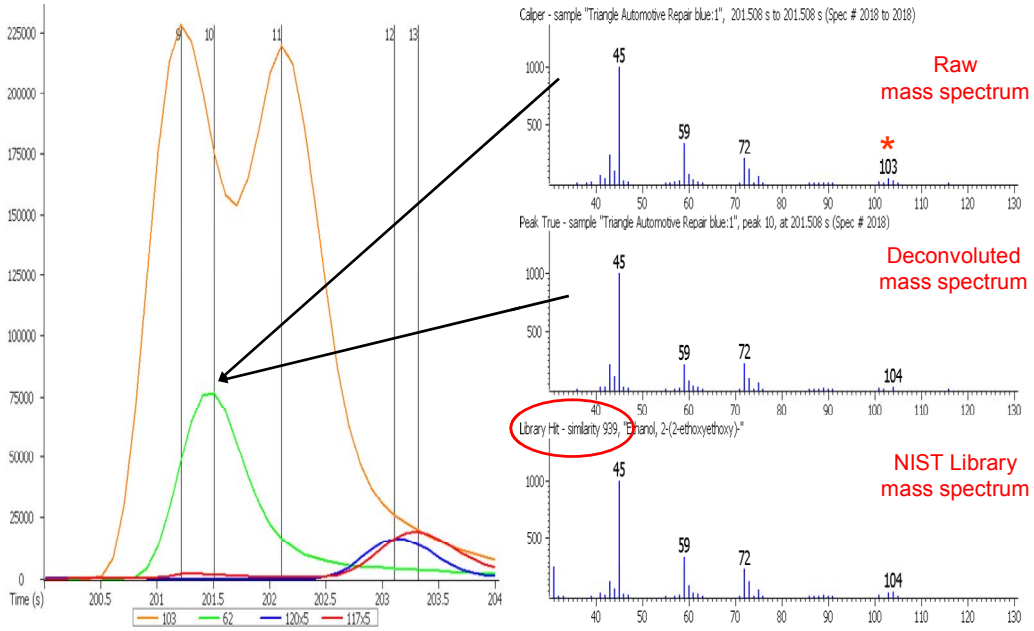


Figure 3. *Automated peak find and spectral deconvolution for ethoxy ethoxyethanol in a blue ballpoint pen ink. The deconvoluted spectrum has a similarity of 939 (out of 999) with the library spectrum.*

A more dramatic example of automated peak find and spectral deconvolution is seen in Figure 4 where a minor peak (diethylaniline) is buried underneath a huge peak. Not only was this minor compound located underneath the large 2-phenoxyethanol peak, but a deconvoluted mass spectrum was produced that had excellent similarity with a library mass spectrum.

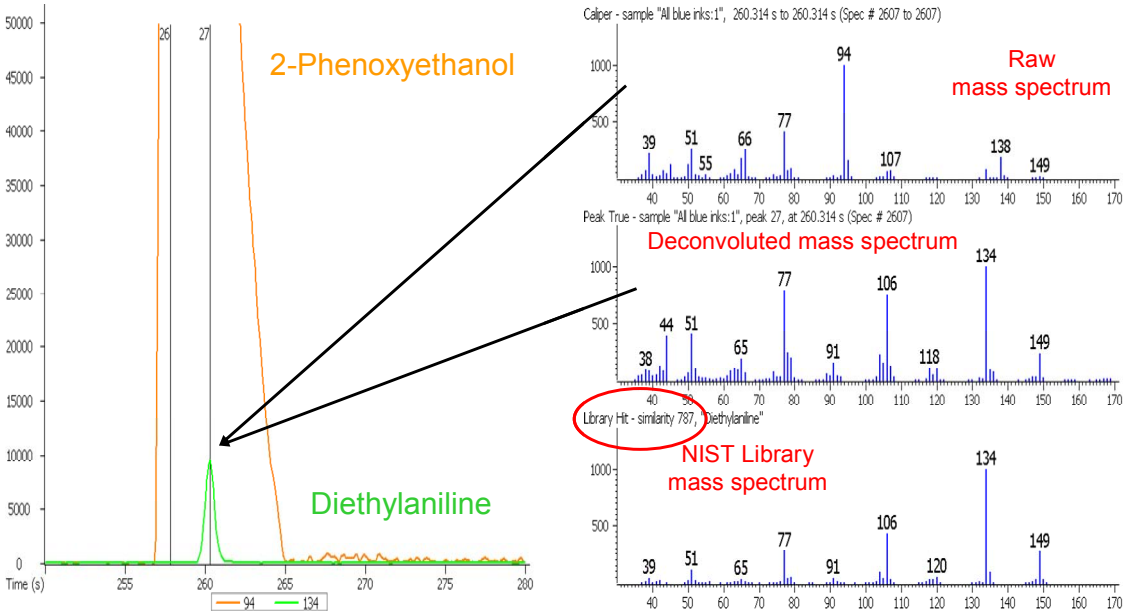


Figure 4. *Automated peak find and spectral deconvolution for diethylaniline in the presence of 2-phenoxyethanol in a mix of blue ballpoint pen inks. Note that the raw mass spectrum taken at the peak apex for diethylaniline is characteristic of 2-phenoxyethanol due to its high concentration. The deconvoluted spectrum for diethylaniline matches well with the library spectrum.*

The aging of documents created or signed with ballpoint pens is often done by "calibrating" how certain ink components decrease over time. For the aged ink experiment in this work, 2-phenoxyethanol was chosen for monitoring because it is the principal solvent in many ballpoint ink formulations. Preliminary experiments showed that the 2-phenoxyethanol concentration in an ink scribble (as sampled by ambient temperature SPME) dropped rapidly and stabilized (Figure 5). This observation was confirmed with older ink samples also, as shown in Figure 6.

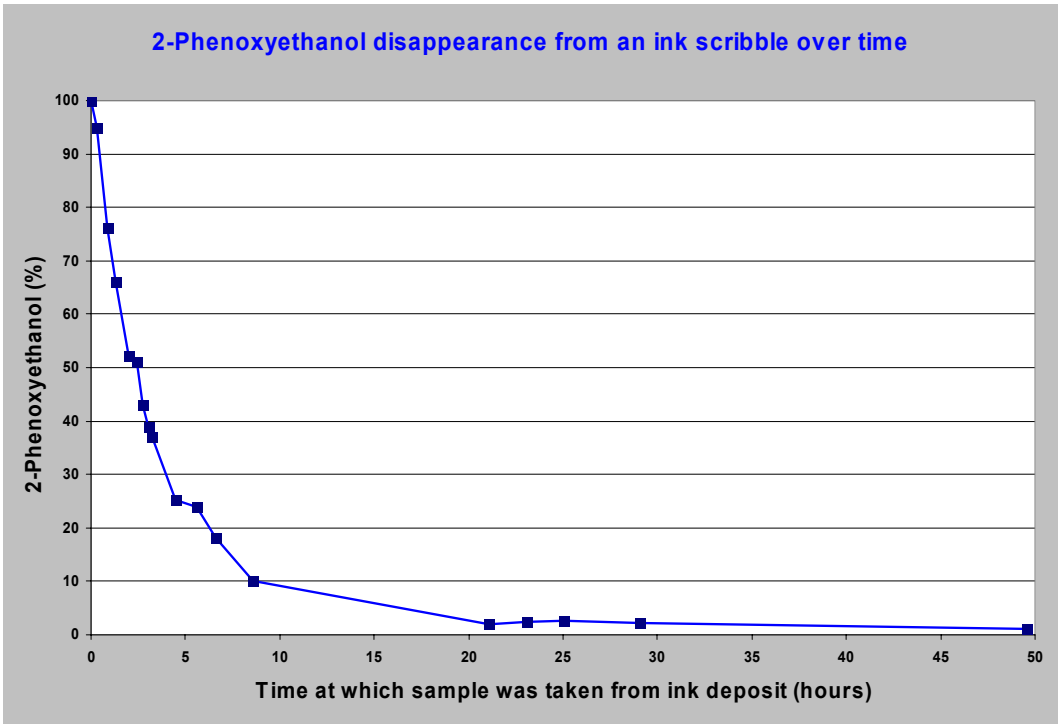


Figure 5. *2-Phenoxyethanol disappearance from an ink scribble over time as determined by SPME and fast GC-TOFMS. The concentration of 2-phenoxyethanol that can be determined by SPME GC-TOFMS stabilizes after about 20 hours.*

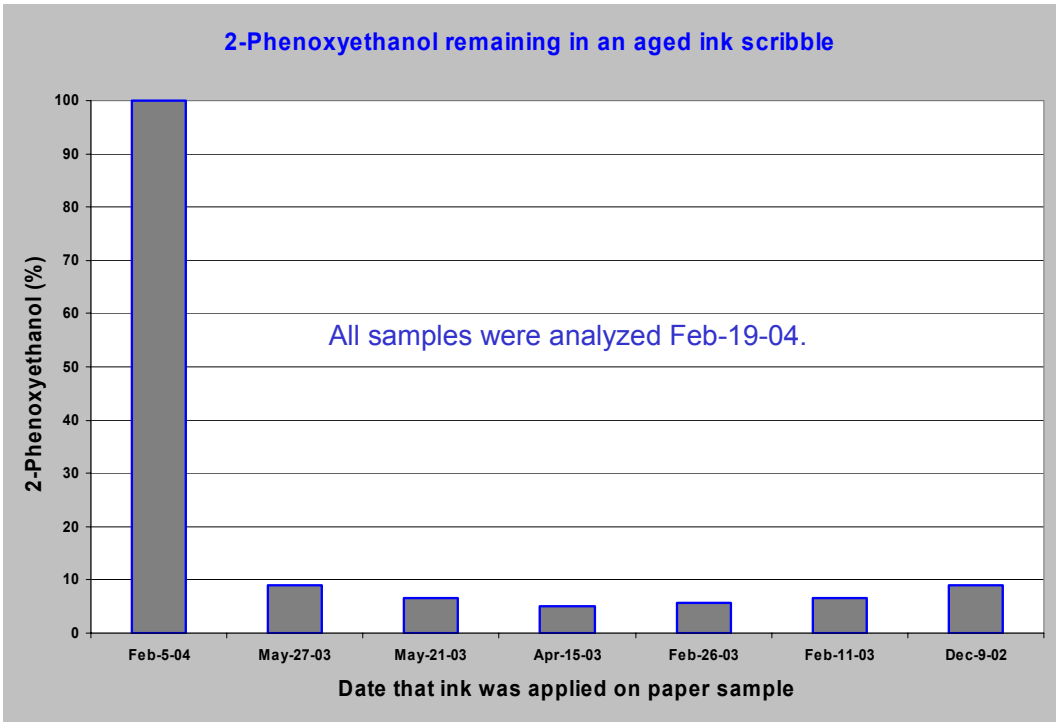


Figure 6. *2-Phenoxyethanol disappearance from aged ink scribbles as determined by SPME and fast GC-TOFMS. The SPME vial was heated to 70°C prior to sampling. 2-Phenoxyethanol concentration differences as determined by SPME GC-TOFMS are indistinguishable for 2003 and 2002 samples.*

Based on the results seen in Figure 6, it was concluded that documents could not be aged past 8 months using SPME GC-TOFMS and 2-phenoxyethanol concentrations. It was theorized that using a less volatile component, one that might stay in the paper longer than 2-phenoxyethanol, could be used to age ballpoint pen ink documents. Unfortunately, diphenylamine gave similar results to 2-phenoxyethanol (Figure 7). Although the results are not shown here, the ratio of less- to more-volatile compounds, as analyzed by SPME GC-TOFMS, was no help in determining ballpoint pen ink age either.

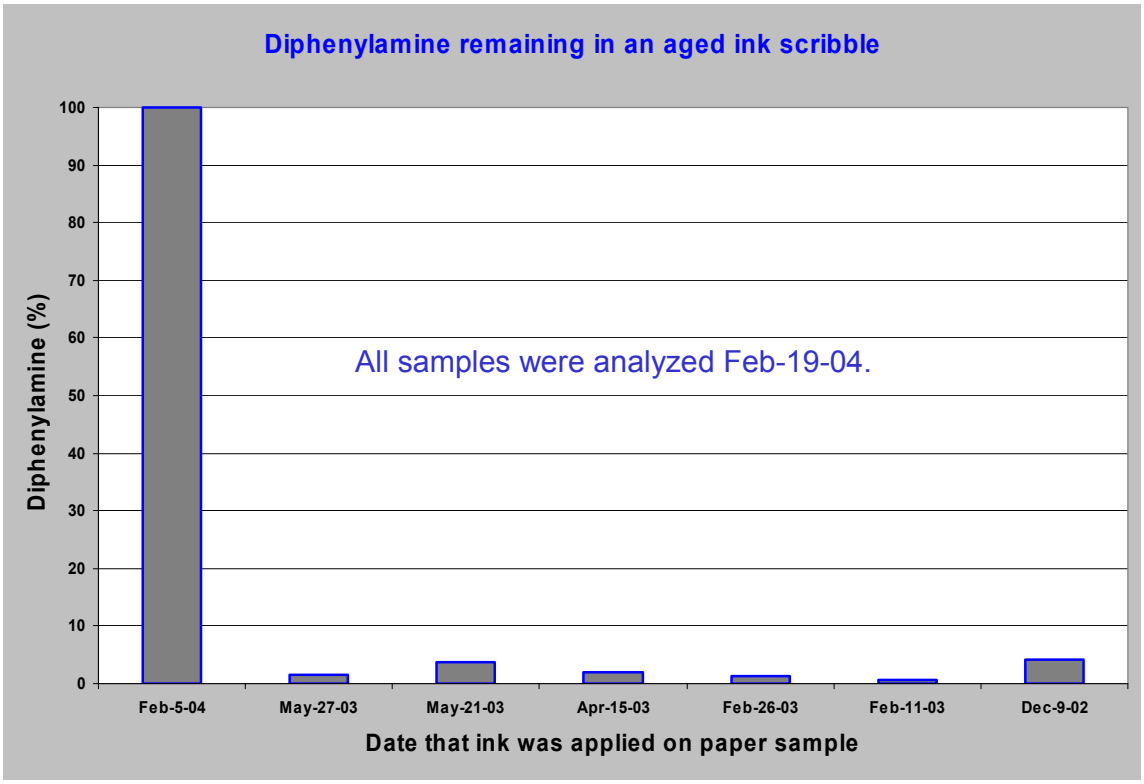


Figure 7. *Diphenylamine disappearance from aged ink scribbles as determined by SPME and fast GC-TOFMS. The SPME vial was heated to 70°C prior to sampling. Diphenylamine concentration differences as determined by SPME GC-TOFMS are indistinguishable for 2003 and 2002 samples.*

When heating the ink scribbles to 70°C for headspace sampling by SPME, most of the volatile contribution is from the paper, and the chromatogram is quite complex. It becomes a challenge to determine which compounds can be attributed to the ballpoint ink. To simplify the data processing for these samples, a feature specific to ChromaTOF, "Compare", was used. This algorithm provides a quick and fully automated way to compare two samples on a GC peak-by-GC peak, mass spectrum-by-mass spectrum basis. As an example in this work, a Reference was created using blank paper (that with no ink line on it) from the February 5, 2004 sample, and then Compare was used to analyze the same sample paper that contained ink lines.

The Compare procedure starts with automated peak find, spectral deconvolution, library searching of the Peak Table, and calculating peak area/height for each compound (for the Reference sample). For the paper used here, over 200 compounds were located and library searched in less than 2 minutes! When Compare is used against the sample (ink scribbles), a Peak Table containing four analyte types is generated.

- Match (compound found and within user-defined concentration range)
- Out of Tolerance (compound found but outside user-defined concentration range)
- Contaminant (compound not in Reference)
- Not Found (compound not in sample, versus Reference)

A Peak Table can be filtered such that any or all of the analyte types is displayed in the table. In the present case, the Contaminants, which can be defined as ink compounds, are of highest interest. Table 3 shows the Contaminants (ink compounds) illuminated through the use of Compare that had spectra in the NIST library. Another five compounds were located, but their spectra were not in the library, and are therefore not included in the table. The Compare data processing time, which included automated peak find, spectral deconvolution, calculation of area/height, matching of results, and library searching of unmatched compounds, took less than a minute.

Table 3. Ink compounds (Contaminants from Compare) in an aged ink scribble.

Retention time (sec.)	Compound Name
175.4	2-Butoxyethanol
192.0	Benzaldehyde
195.5	Phenol
201.6	2-(2-Ethoxyethoxy)-ethanol
205.5	2-Ethoxyethanol
219.4	Phenylethanol
258.4	2-Phenoxyethanol
264.3	Phenoxypropanol
342.1	Diphenylamine

Figure 8 graphically illustrates the Compare results by overlaying chromatograms for the paper and the paper/ink scribble samples. Diphenylamine, marked with a blue asterisk, is one ballpoint ink compound located with Compare. Although it may be possible to visually locate major compounds of interest through inspecting overlaid total ion chromatograms (TICs), the automated way is faster, and often leads to location of important compounds that may be buried beneath the TIC.

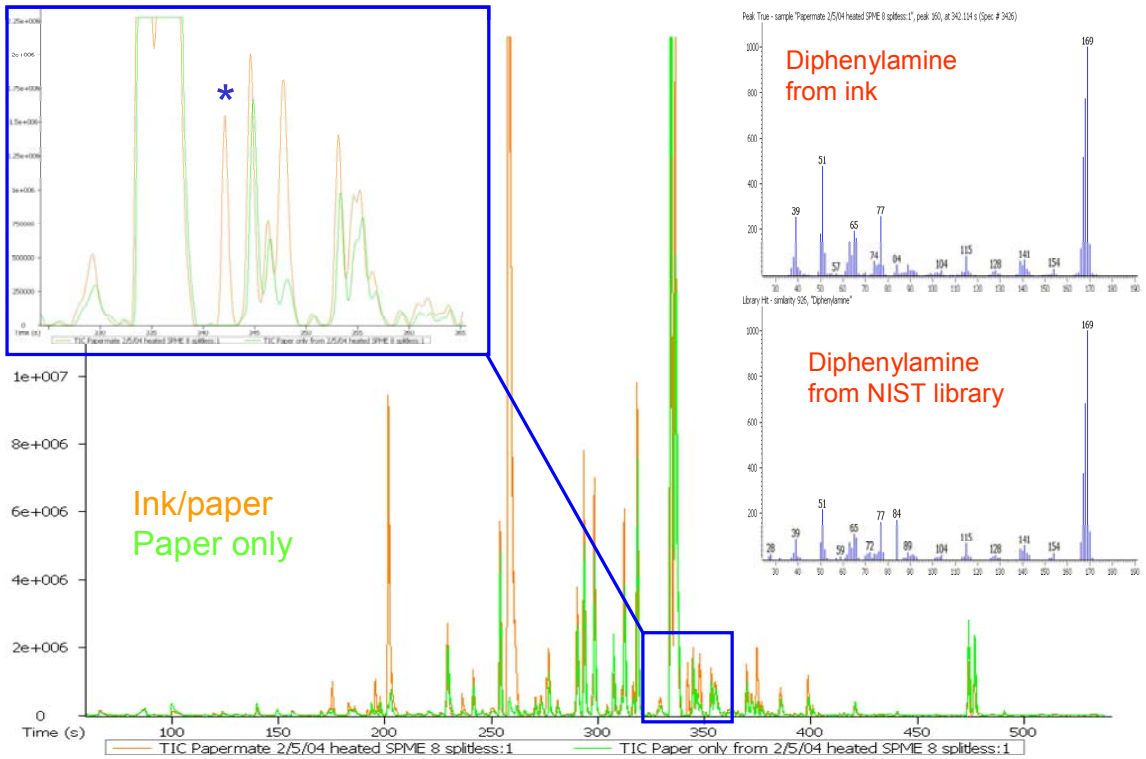


Figure 8. *Overlaid TICs of paper, and paper with ink scribbles, as analyzed using SPME GC-TOFMS. Diphenylamine (blue asterisk, inset chromatogram), which was located automatically with a Compare feature of ChromaTOF, can be attributed to the ballpoint pen ink.*

Conclusions

SPME GC-TOFMS is a fast and powerful way to characterize the volatile organic composition of ink samples, especially due to automated peak find and spectral deconvolution. Compare, a feature unique to ChromaTOF software, simplifies data reduction for complex samples, and quickly highlights (and identifies) compounds that can be considered as "Contaminants" when compared to a Reference. SPME needs further investigation as an ink dating technique for samples more than eight months old.

References

G.M. LaPorte, J.D. Wilson, A.A. Cantu, S.A. Mancke, and S.L. Fortunato, The Identification of 2-Phenoxyethanol in Ballpoint Inks Using Gas Chromatography/Mass Spectrometry—Relevance to Ink Dating*, J. Forensic Sci.*, Jan. 2004, Vol. 49, No. 1

V.N. Aginsky, Current Methods for Dating Ink on Documents

http://www.rileywelch.com/Aginsky_article.pdf

Acknowledgment

Gerald LaPorte at the United States Secret Service in Washington, DC provided the aged ink samples. Chris Cox at Restek Corporation provided the Rtx-TNT column used for this work.