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Restek Connections

In This Issue

About Restek Corporation

A leading innovator of chromatography solutions for both LC and GC, Restek has been developing and manufacturing columns, reference standards, sample preparation materials, accessories, and more since 1985. We provide analysts around the world with products and services to monitor the quality of air, water, soil, food, pharmaceuticals, chemicals, and petroleum products. Our experts enjoy diverse areas of specialization in chemistry, chromatography, engineering, and related fields as well as close relationships with government agencies, international regulators, academia, and instrument manufacturers.

Letter from the Bench

Welcome to the new look for your *Restek Advantage*!

When we sat down to plan this issue, one of our goals was to share more chromatography news and better connect with you, our reader. That's how our new Hot Topics and Restek Connections departments came to be.

Of course, as always, much of this *Advantage* highlights the application work of our Innovations Lab, where we're lucky to have seasoned veterans working alongside young, enthusiastic chemists to solve your toughest problems. Rick Lake and Ty Kahler show you how to get the most selectivity for your LC separations. Their work employs the hydrophobic subtraction model to define a highly selective and orthogonal set of 4 USLC™ columns.

You will also be interested in reading our article on marijuana potency testing, PLOT columns in process GC, wool in GC inlet liners, large volume splitless injection... We have something inside for every analyst.

Finally, we also set up a new email address: **advantage@restek.com** Use it to let us know what you think of your new *Restek Advantage*. I say "*your*" because we create this technical document with your needs and interests in mind. Your feedback will be invaluable for assembling future issues.

Cheers!

Jack Cochran
Director of New Business & Technology

You Have Opinions... And We Want Them.

We chemists are an opinionated bunch, so the odds are good that you have some thoughts about the *Restek Advantage*. Love it? Hate it? Want to see something different in the next issue? Maybe you have a response to one of our technical articles? Whatever you have to say, let's hear it! Email your comments to advantage@restek.com and you may even see them in an upcoming issue.

Product Spotlight

Restek Introduces Secondary Columns for GCxGC

Restek now offers a full line of secondary columns with a wide range of polarities to help you accurately analyze highly complex samples using GCxGC. These new columns can be matched with any Restek Rxi® or Rtx® primary column to create the perfect orthogonal separation for your application—and our online column combination guide makes pairing simple. A 2 m length means greater convenience and reduced cost while 0.15, 0.18, and 0.25 mm ID formats accommodate varying sample capacities, speeds, and detectors. And, of course, because they're Restek columns, you know you're getting the high thermal stability and unrivaled inertness you've come to rely on. Our chemists have been performing comprehensive two-dimensional gas chromatography since its commercial inception, and now you can put our years of GCxGC experience to work in your lab, too.

www.restek.com/gcxgc

Have You Tried Our Reversible Inlet Seals?

Flip Seal™ inlet seals feature a patented design that lets you simply flip them and use them again instead of throwing them away, so you get twice the life for the same price. Soft Vespel® rings embedded in the top and bottom surfaces eliminate the need for a washer and require very little torque to make a reliable seal. Choose gold plating or Siltek® treatment to reduce breakdown and adsorption of active compounds for maximum transfer onto the GC column. For decreased costs and increased performance, you owe it to your data to try our reversible Flip Seal™ inlet seals today.

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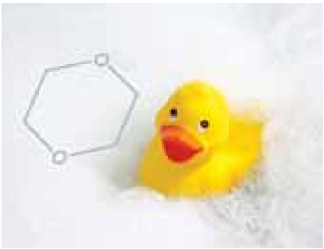
Chromatography in the News

1,4-Dioxane in Your Bathwater



Next time you take a bath, you might just be enjoying a nice, long soak in 1,4-dioxane. Dioxane is a by-product of the ethoxylation process, which is employed most notably to create sodium myreth sulfate and sodium laureth sulfate for the manufacture of soaps and cosmetics. Unfortunately, 1,4-dioxane is also a possible human carcinogen and has also been classified by the World Health Organization's International Agency for Research on Cancer (IARC) as a Group 2B compound. Global concern has prompted companies to begin eliminating it from their products and has also led to regulatory changes. For example, in the U.S., the recently signed third Unregulated Contaminant Monitoring Regulation (UCMR 3) will require monitoring using newly promulgated methods. 1,4-dioxane will be analyzed according to U.S. EPA Method 522, which concentrates the sample using solid phase extraction (SPE) instead of the most common technique previously used for this compound: purge and trap. Restek offers dioxane reference standards specifically formulated for Method 522, and you can find them at **www.restek.com/epa522**

Questions From You

Our Technical Service specialists field an astounding variety of questions from our customers. Today's featured topic is the flowmeter.

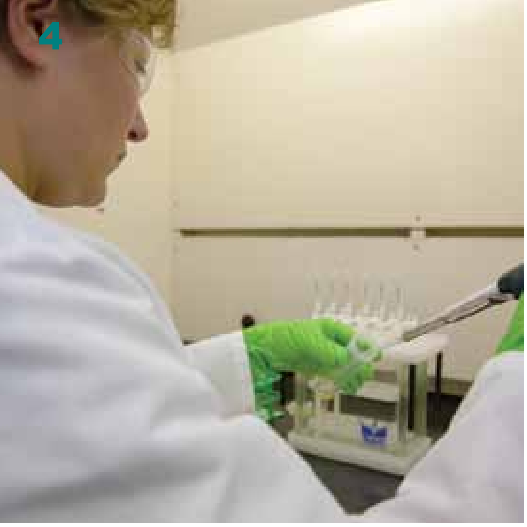
Q: Why do I see a difference in readings from different flowmeters?

A: All flowmeters present some level of flow impedance, but the amount differs among meters. When any meter is connected to a flow source, the system is loaded which will usually result in a change of flow from the source. The amount of change in flow depends on the level of impedance. While each meter will display the correct current flow, they may have different readings because the actual flow changes based on the degree of impedance. For this reason, it is inappropriate to "check" the flow measurement of one volumetric flowmeter against that of another.

We just released a full FAQ on the ProFLOW 6000 flowmeter! Find answers to your questions at **www.restek.com/FAQFlow**

- **Brandon Tarr**
Product Development Engineer

Wrestling with a question of your own?
Call 1-814-353-1300, ext. 4, or email **support@restek.com** today!



Marijuana Potency Testing—Quick and Easy by GC or LC

By Amanda Rigdon and Jack Cochran

- Single extraction for both GC and LC.
- Fast results on Rxi®-5Sil MS GC or Ultra Aqueous C18 LC columns.
- Convenient standards for potency testing.

Although marijuana is illegal at the federal level in the United States, the use of medicinal marijuana is currently legal in many states. In some areas, it is widely used, and demand is rising for potency data for medicinal products purchased at dispensaries. Potency testing is more straightforward than impurity testing because the active compounds are present in much higher concentrations relative to matrix. Currently, GC is the most popular method for potency testing due to its ease of use and the availability of relatively inexpensive instrumentation. However, LC is also a viable technique for medical cannabis potency testing. As shown in this article, the same straightforward sample preparation technique can be used for cannabis potency testing by either GC or LC.

Simple Sample Prep

Cannabinoids were extracted from 7 different marijuana samples under the supervision of local law enforcement personnel. The extraction procedure consisted of weighing 0.2 g of sample into a 40 mL VOA vial, adding 40 mL of isopropyl alcohol, shaking for 5 minutes, and then allowing the sample to settle. The procedure was very quick and produced extracts that were compatible with both GC and LC analysis.

GC Analysis

The 3 compounds of interest for GC potency testing are Δ^9-tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD). While THC is primarily responsible for the hypnotic effects of marijuana, CBD acts to attenuate these effects. Since CBD has been shown to have medicinal properties, it is desired at higher concentrations in medical marijuana. Because the samples that were extracted were illicit samples seized by local law enforcement, the CBD levels were very low. In general, higher CBD levels are observed in medicinal marijuana strains. CBN is an indicator of sample breakdown due to age or poor storage conditions.

For GC potency testing, 1 µL of prepared extract was manually injected onto a 5890 GC equipped with a flame ionization detector and analyzed on a 15 m Rxi®-5Sil MS column (cat.# 13620). To ensure accurate and reproducible manual injections, a Merlin Microshot injector (cat.# 22229) was used. Figure 1 shows an overlay of a cannabinoid standard (cat.# 34014) that contains the 3 target analytes (blue trace) and a representative chromatogram of a marijuana sample (red trace). The use of a narrow-bore, thin-film analytical column resulted in sharp peaks, which improve sensitivity and allow a split injection to be used to reduce column contamination.

LC Analysis

LC potency testing requires the analysis of the 3 components discussed above, but also includes Δ^9- tetrahydrocannabolic acid (THCA). While THCA is not hallucinogenic, all THC in the marijuana plant exists as THCA, and only converts to THC upon heating (i.e., smoking, vaporizing, cooking, or injecting into a hot GC inlet). Since the sample extraction and LC analysis employ no heat, potency must be determined based on THCA when using LC, rather than with THC as is used in GC analysis.

For LC potency testing, extracts were diluted 10x with isopropyl alcohol, and 10 µL of extract was injected onto a 3 µm Ultra Aqueous C18 column (cat.# 9178312). Figure 2 shows an overlay of the cannabinoid standard described above with the addition of THCA (blue trace) and a representative chromatogram of the same marijuana sample (red trace).

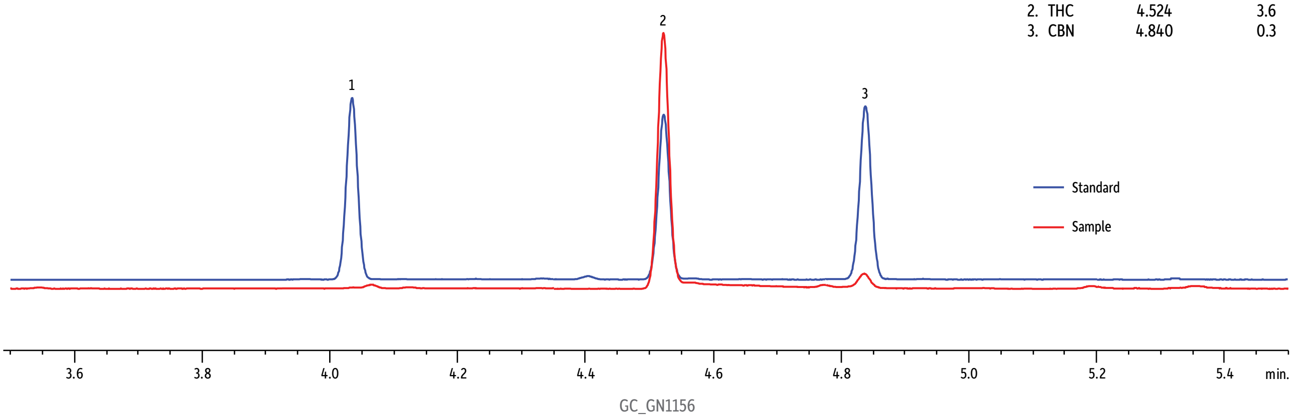
Summary

Both the GC and LC methods shown here for determining medical marijuana potency employ a straightforward and cost-effective extraction procedure and fast analysis times. This allows reliable potency analyses at a reasonable cost per sample.

For further details, visit our technical blog at **www.restek.com/potpotency**

Figure 1: Potency testing of marijuana using an Rxi®-5Sil MS GC column results in higher sensitivity for all target analytes.

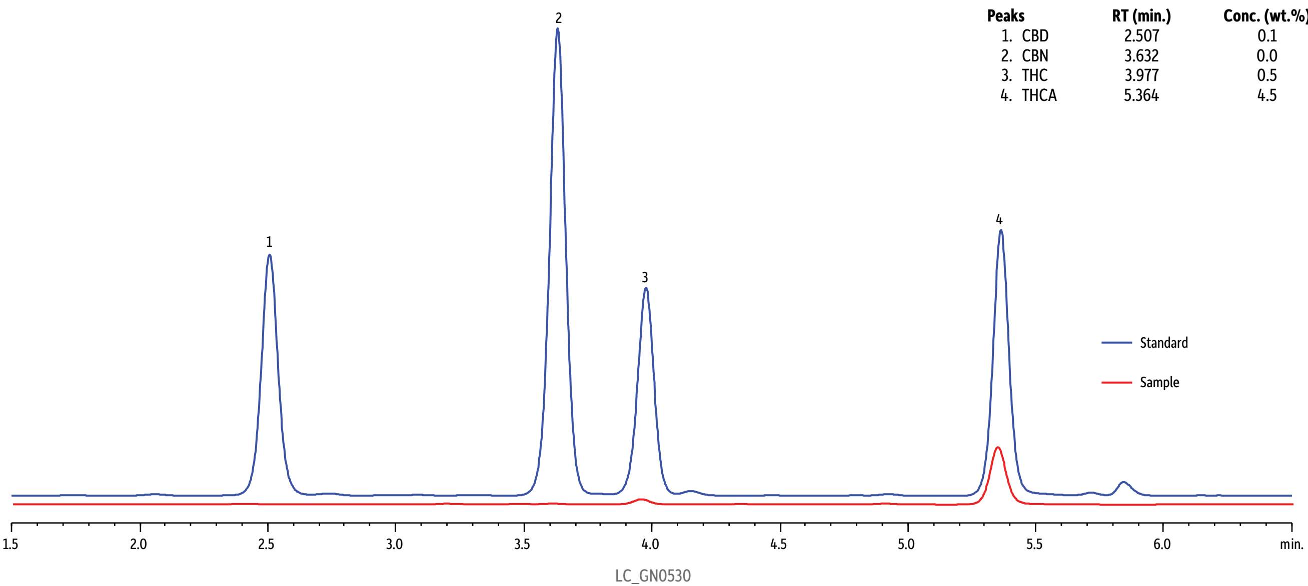
Peaks	RT (min.)	Conc. (wt.%)
1. CBD	4.035	0.0
2. THC	4.524	3.6
3. CBN	4.840	0.3



Column: Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620); **Injection**: Inj. Vol.: 1 µL split (split ratio 20:1); Liner: Sky™ 4.0 mm ID single taper/gooseneck inlet liner w/wool (cat.# 23303.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp: 200 °C (hold 0 min.) to 300 °C at 15 °C/min. (hold 0 min.); **Carrier Gas**: H_2, constant pressure (7 psi, 48.3 kPa); Temp.: 200 °C; Dead Time: 0.6 min. @ 200 °C; **Detector**: FID @ 300 °C; Make-up Gas Flow Rate: 45 mL/min.; Make-up Gas Type: N_2; **Instrument**: HP5890 GC; **Notes**: Blue trace = cannabinoids standard (cat.# 34014) diluted to 100 µg/mL in isopropyl alcohol.; Red trace = extracted marijuana sample; Sample extraction: Weigh 0.2 g of sample into a 40 mL VOA vial, add 40 mL of isopropyl alcohol, shake for 5 minutes, and allow sample to settle.; Quantification: Potency values (weight%) were based on a 1-point standard curve using the standard show above.

Figure 2: Ultra Aqueous C18 columns easily separate THCA, which is used to determine marijuana potency when testing by LC.

Peaks	RT (min.)	Conc. (wt.%)
1. CBD	2.507	0.1
2. CBN	3.632	0.0
3. THC	3.977	0.5
4. THCA	5.364	4.5



Column: Ultra Aqueous C18 (cat.# 9178312); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 3 µm; Pore Size: 100 Å; Temp.: 30 °C; **Sample:** Inj. Vol.: 10 µL; **Mobile Phase:** A: Water + 10 mM potassium phosphate (pH = 2.5), B: Methanol; Flow: 0.4 mL/min.; Gradient (%B): 0 min. (80%), 1.0 min. (80%), 5.0 min. (95%), 6.0 min. (95%), 6.1 min. (80%), 8.0 min. (80%); **Detector**: UV/Vis @ 220, 4 nm; Cell Temp: 40 °C; **Instrument**: Shimadzu UFLCXR; **Notes**: Blue trace = cannabinoids standards (cat.#s 34014 and 34093) diluted to 100 µg/mL in isopropyl alcohol; Red trace = extracted marijuana sample; Sample extraction: Weigh 0.2 g of sample into a 40 mL VOA vial, add 40 mL of isopropyl alcohol, shake for 5 minutes, and allow sample to settle. Dilute extract 10x with isopropyl alcohol.; Quantification: Potency values (weight%) were based on a 1-point standard curve using the standard show above.

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; similar to 5% phenyl/95% dimethyl polysiloxane)

Description	temp. limits	cat.#
15m, 0.25mm ID, 0.25µm	-60 to 330/350°C	13620

similar **phases**

DB-5ms, VF-5ms, CP-Sil 8 Low-Bleed/MS, DB-5ms UI, Rtx-5Sil MS, ZB-5ms, Optima 5ms, AT-5ms, SLB-5ms, BPX-5

Ultra Aqueous C18 Columns (USP L1)

Description	cat.#
3µm Columns	
100mm, 2.1mm ID	9178312
3µm Columns	
100mm, 2.1mm ID (with Trident Inlet Fitting)	9178312-700

similar **phases**

AQUA C18, Aquasil C18, Hypersil Gold AQ, YMC ODS-Aq

Acknowledgment

Randy Hoffman, a Police Evidence Technician at The Pennsylvania State University (PSU), supplied the seized marijuana samples while overseeing their handling. Frank Dorman at PSU provided access to the samples and assisted with prep.

Simplify HPLC and UHPLC Method Development

With the Restek USLC™ Column Set

By Rick Lake and Ty Kahler

- Column selectivity has the most significant influence on chromatographic peak separation (i.e., resolution).
- Initially focusing on columns instead of mobile phases will drastically speed up method development.
- Restek's USLC™ column set boasts the widest range of selectivity available—using just 4 stationary phases!

Equation 1: Selectivity is the driving parameter of resolution, as it affects peak separation to the greatest degree.

$$R = \frac{1}{4}\sqrt{N} \times (k/(k+1)) \times (\alpha-1)$$

Efficiency *Retention Factor* *Selectivity*

Wasted effort. Lost time. Frustration. Making the wrong decisions can needlessly complicate and delay successful method development. By understanding selectivity's impact on resolution and focusing on column choice to create ***alternate*** selectivity, you can drastically speed up LC method development. Enter the new Restek Ultra Selective Liquid Chromatography™ (USLC™) columns.

Change Your Habits—and Your Columns—to Optimize Resolution

Resolution is the result of 3 cumulative terms: efficiency (N), retention capacity (k), and selectivity (α). How well and how quickly we resolve our analytes depends upon our ability to control these factors. Of the 3, selectivity affects resolution to the greatest degree (Equation 1). For that reason, any discussion about resolution in method development should focus on selectivity.

All too often, HPLC method developers use C18 columns and rely on adjusting mobile phases to alter selectivity and reach a desired separation. While it is true that mobile phase adjustments may alter selectivity, it is a laborious task that typically creates only marginal differences. In addition, some mobile phases are not practical with certain detection modes, including mass spectrometry (MS) and refractive index (RI). To save time and work, you should first focus on choosing the right stationary phases (i.e., columns). Columns pose fewer issues with MS and RI, change easily, and offer alternate and even orthogonal separations for maximum effect with each change.

Choosing columns can be incredibly difficult, but by characterizing stationary phase selectivity, we created new guidelines for easily making the right choice.

The Highest Range of Alternate Selectivity

Using the hydrophobic subtraction model (H-S model) [1], we quantified the selectivity of our stationary phases and determined which phases produce the greatest degree of dissimilarity compared to a C18 benchmark. We then matched these phases with specific solute types based on molecular interactions commonly encountered in reversed phase chromatography. By doing so, we were able to (1) find a small set of columns with the widest range of ***alternate*** selectivity available and (2) recommend columns based on the chemical properties of target analytes.

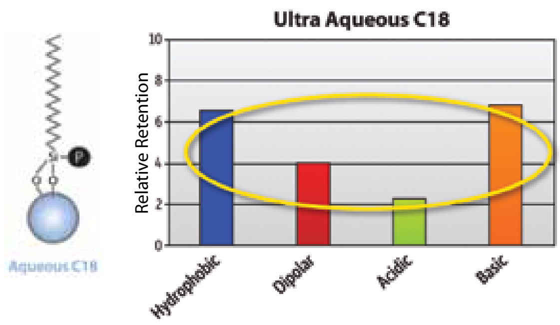
Figure 1 illustrates the retention profile of a C18 compared with those of the 4 Restek USLC™ columns. USLC™ phases are highly selective and exhibit significantly different retention profiles based on specific solute chemical properties, so you can match USLC™ columns to specific analytes and accelerate method development!

To confirm the orthogonality of the Restek USLC™ column set, we also quantified its selectivity (S) as described by Neue et al. [2] by looking at the degree of scatter along a regression line when compared to a conventional C18 (Figure 2). USLC™ phases produce the highest range of alternate selectivity available today—using only 4 columns.

Summary

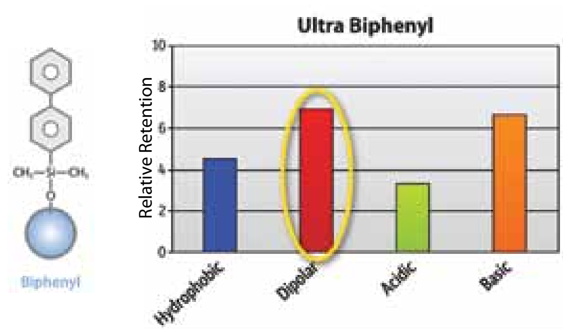
The Restek USLC™ column set has a profile that encompasses the widest range of reversed phase selectivity available today. Instead of manually altering mobile phases, operational parameters, or instrument settings—often with minimal effect on resolution—take advantage of the Restek USLC™ column set. These 4 orthogonal stationary phases and their defined retention profiles let you quickly determine the best column for almost any reversed phase situation.

Figure 1: Stationary phase selectivity can be characterized by looking for column types with varying retention profiles. When compared to a C18, the 4 Restek USLC™ phases offer diverse retention profiles—that is, a true range in selectivity.



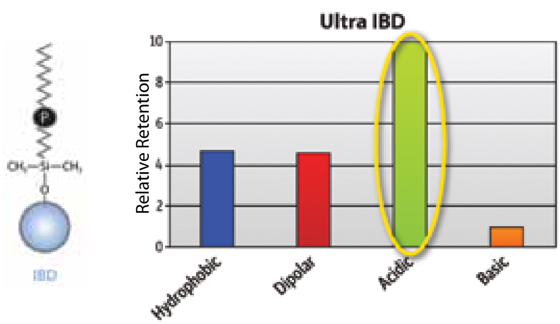
Restek USLC™ Phase: **Aqueous C18**

- **General purpose with a well-balanced retention profile.**
- Increased retention for acids and bases.
- Resistant to dewetting—compatible with 100% aqueous mobile phases.



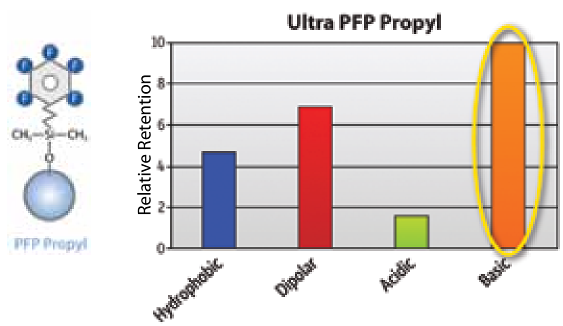
Restek USLC™ Phase: **Biphenyl**

- **Increased retention for dipolar, unsaturated, or conjugated solutes.**
- Increased retention for fused-ring solutes containing electron withdrawing ring substituents.
- Enhanced selectivity when used with methanolic mobile phase.



Restek USLC™ Phase: **IBD**

- **Increased retention for acids.**
- Moderate retention for hydrophobic and dipolar solutes.
- Resistant to dewetting—compatible with 100% aqueous mobile phases.
- Capable of multi-mode mechanisms.

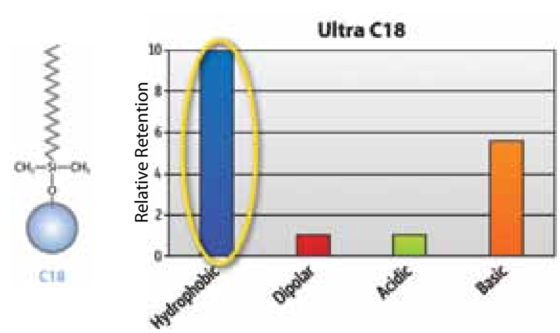


Restek USLC™ Phase: **PFP Propyl**

Properties:

- **Increased retention for protonated bases.**
- Increased retention for solutes containing dipolar moieties.
- Capable of multi-mode mechanisms.

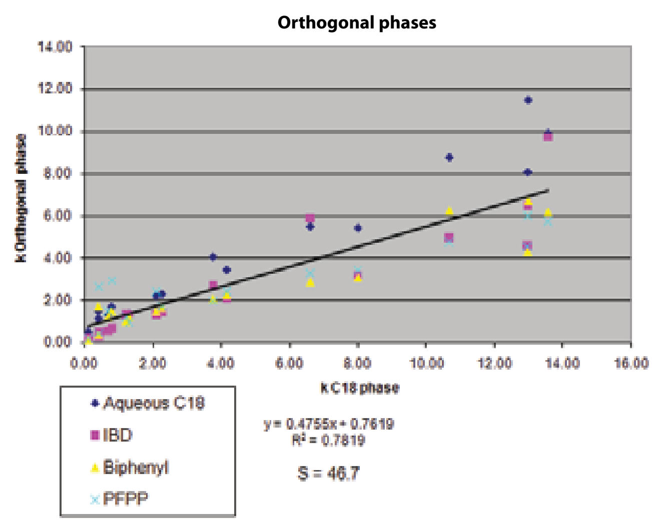
C18 BENCHMARK



Restek Phase: **C18 Benchmark**

- General purpose.
- Strong hydrophobic retention.

Figure 2: Restek has extended the selectivity (S) for a range of columns and defined a set—the 4 USLC™ phases—that is ideal for fast column selection and faster method development.



All columns in Figures 1 and 2 were tested using the same silica support.

For a detailed analysis of USLC™ column selectivity data, visit **www.restek.com/USLCarticle**

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[1] L.R. Snyder, J.W. Dolan, P.W. Carr, *The Hydrophobic-Subtraction Model of Reversed-Phase Column Selectivity,* J. Chromatogr. A 1060 (2004) 77.

[2] U.D. Neue, J.E. O'Gara, A. Mendez, *Selectivity in Reversed-Phase Separations Influence of the Stationary Phase,* J. Chromatogr. A 1127 (2006) 161.

Acknowledgements

The authors gratefully acknowledge the contributions of Dr. Lloyd Snyder from LC Resources and Dr. Frank Dorman from The Pennsylvania State University. The authors also wish to thank the contributing team of researchers Randy Romesberg, Bruce Albright, Mike Wittrig, Brian Jones, and Vernon Bartlett.

Large Volume Splitless Injection With an Unmodified GC Inlet

Lets You Skip Sample Concentration for Pesticides and BFRs in Drinking Water

By Michelle Misselwitz and Jack Cochran

- Eliminate time-consuming extract concentration without sacrificing sensitivity.
- Simplified approach uses standard injection port—no specialized equipment.
- Analyze at sub-ppb levels with faster, less labor-intensive procedure.

Using large volume splitless injection is advantageous when trying to analyze trace-level contaminants in clean matrices like drinking water because greater levels of target compounds are introduced onto the analytical column. A special injection port is generally required for large volume injection, which has limited its application. A concurrent solvent recondensation–large volume splitless injection (CSR-LVSI) technique described by Magni and Porzano [1,2] offered a more practical alternative, but involved some modification of a split/splitless injection port.

We have used CSR-LVSI successfully with a completely unmodified Agilent split/splitless GC inlet. The setup utilizes a pre-column (e.g., 5 m x 0.53 mm) press-fitted to the analytical column and a starting GC oven temperature below the boiling point of the solvent. A fast autosampler injection with liquid band formation into a liner containing glass wool is used to prevent backflash in the injection port. Here we investigated the applicability of this approach to analyzing pesticides and brominated flame retardants (BFRs) in drinking water according to U.S. EPA Method 527 [3].

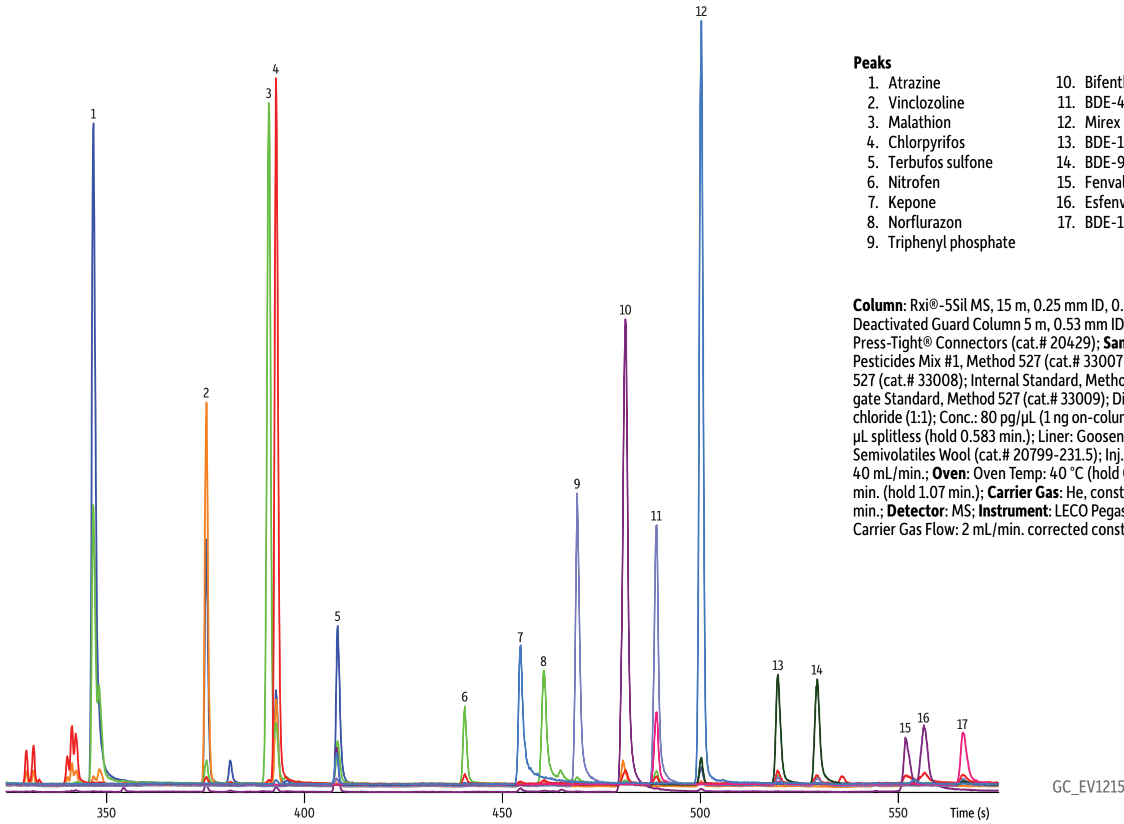
Table I: Calibration standards and concentration equivalents.

Level	Prepared Standard (pg/µL)	On-Column Amount Injected (pg/12.5 µL)	Equivalent Concentration in 1 L Samples (ug/L)
1	2	25	0.05
2	4	50	0.1
3	10	125	0.25
4	20	250	0.5
5	40	500	1
6	80	1,000	2

Table II: Average percent recoveries and relative standard deviations for 1 µg/L and 0.1 µg/L laboratory fortified blank samples analyzed using disk extraction with no extract concentration and CSR-LVSI GC-TOFMS (n = 3).

	1.0 µg/L % Recovery		0.1 µg/L % Recovery	
Compounds	AVG (n = 3)	%RSD	AVG (n = 3)	%RSD
Dimethoate	73	2.4	75	9.3
Atrazine	96	1.8	84	13
Propazine	93	3.3	92	8.5
Vinclozoline	97	4.0	97	8.0
Prometryne	179	3.0	113	7.9
Bromacil	78	2.2	66	3.1
Malathion	98	2.7	85	6.5
Thiobencarb	93	3.9	70	1.9
Chlorpyrifos	92	3.1	84	1.7
Parathion	94	0.7	92	4.6
Terbufos sulfone	88	2.8	105	11
Oxychlordane	75	8.5	74	10
Esbiol	88	2.7	79	6.5
Nitrofen	91	3.0	77	5.3
Kepone	102	18	56	32
Norflurazon	91	7.2	105	10
Hexazinone	87	0.8	68	2.1
Bifenthrin	100	3.0	81	3.2
BDE-47	96	4.4	87	15
Mirex	93	4.5	76	2.3
BDE-100	93	3.8	89	11
BDE-99	93	2.9	79	33
Perylene-D12	103	1.6	98	3.3
Fenvalerate	92	0.4	59	16
BB-153	88	3.4	45	14
Esfenvalerate	89	3.7	69	20
BDE-153	88	13	54	49

Figure 1: Extracted ion chromatogram of 80 pg/µL standard from 12.5 µL CSR-LVSI injections.



Peaks

1. Atrazine	10. Bifenthrin
2. Vinclozoline	11. BDE-47
3. Malathion	12. Mirex
4. Chlorpyrifos	13. BDE-100
5. Terbufos sulfone	14. BDE-99
6. Nitrofen	15. Fenvalerate
7. Kepone	16. Esfenvalerate
8. Norflurazon	17. BDE-153
9. Triphenyl phosphate	

Column: Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620) using IP Deactivated Guard Column 5 m, 0.53 mm ID (cat.# 10045) with Universal Press-Tight® Connectors (cat.# 20429); **Sample**: PBDE Mix (cat.# 33098); Pesticides Mix #1, Method 527 (cat.# 33007); Pesticides Mix #2, Method 527 (cat.# 33008); Internal Standard, Method 527 (cat.# 33010); Surrogate Standard, Method 527 (cat.# 33009); Diluent: ethyl acetate:methylene chloride (1:1); Conc.: 80 pg/µL (1 ng on-column); **Injection**: Inj. Vol.: 12.5 µL splitless (hold 0.583 min.); Liner: Gooseneck Splitless (4 mm) w/ Semivolatiles Wool (cat.# 20799-231.5); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min.; **Oven**: Oven Temp: 40 °C (hold 0.60 min.) to 320 °C at 30 °C/min. (hold 1.07 min.); **Carrier Gas**: He, constant flow; Flow Rate: 2 mL/min.; **Detector**: MS; **Instrument**: LECO Pegasus 4D GCxGC-TOFMS; **Notes**: Carrier Gas Flow: 2 mL/min. corrected constant flow via pressure ramps

The typical procedure for preparing samples according to EPA Method 527 involves extracting a 1 L water sample, drying the extract, and concentrating it down to a final volume of 1 mL. To determine if using CSR-LVSI could eliminate the need for extract concentration, linearity and recovery were assessed. Water samples were fortified at 0.1 µg/L and 1 µg/L levels and then extracted using Resprep® resin SPE disks, dried with anhydrous sodium sulfate, and diluted to 25 mL with methylene chloride:ethyl acetate (1:1). This differs from the method, which calls for the samples to be concentrated to 1 mL after drying. In order to achieve the detection limits described in the method, a 12.5 µL injection volume was used.

Linear Responses for Challenging Compounds Using CSR-LVSI

Calibration curves were built using duplicate 12.5 µL injections of 2, 4, 10, 20, 40, and 80 pg/µL standards. All compounds exhibited good linearity down to 2 pg/µL, which is equivalent to 25 pg on-column and 0.05 µg/L in the original water sample (Table I). Results for Kepone (r = 0.995) are especially notable, as it can be problematic due to the formation of a hemiacetal that chromatographs poorly. Good chromatographic separations were obtained using a 15 m x 0.25 mm x 0.25 µm Rxi®-5Sil MS column, and the fast oven program resulted in an analysis time of less than 10 minutes (Figure 1).

Determine Sub-ppb Levels Without Extract Concentration

The average recovery for all compounds for the 1 µg/L (500 pg on-column) and 0.1 µg/L (50 pg on-column) spikes were quite good at 94% and 80%, respectively (Table II). Individual recoveries met EPA Method 527 criteria, except for the 0.1 µg/L value for hexabromobiphenyl 153 (BB-153) and the 1.0 µg/L value for prometryne. Recovery results demonstrated that employing CSR-LVSI and eliminating the concentration step can be an effective way to meet detection limits while reducing sample preparation time by more than an hour.

Summary

When the extract concentration step was eliminated, good linearity and recovery results were obtained while sample preparation time was significantly reduced. CSR-LVSI with an unmodified Agilent split/splitless GC inlet has been shown to be a technically viable approach that has the advantage of speeding up sample preparation without compromising sensitivity for pesticides and BFRs in drinking water.

For the complete version of this technical article, visit **www.restek.com/LVSI**

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[1] P. Magni, T. Porzano, J. Sep. Sci. 26 (2003) 1491.
[2] Patent No: US 6,955,709 B2.
[3] U.S. Environmental Protection Agency, Method 527, Determination of Selected Pesticides and Flame Retardants in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS), April 2005.

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; similar to 5% phenyl/95% dimethyl polysiloxane)

Description	temp. limits	cat.#
15m, 0.25mm ID, 0.25µm	-60 to 330/350°C	13620

Resprep® Resin SPE Disks

Description	qty.	cat.#
Resprep Resin SPE Disks	20-pk.	26023

Extending the Power of Stabilized PLOT Column Technology to Process GC Analyzers

By Jaap de Zeeuw, Rick Morehead, and Tom Vezza

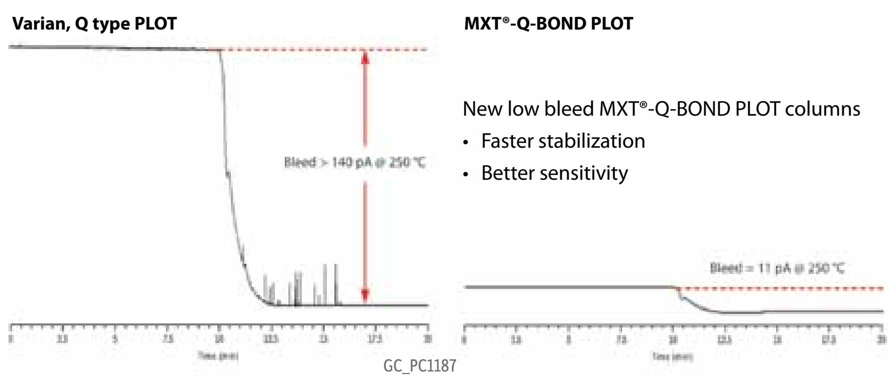
- New technology ensures consistent flows and predictable retention times.
- Rugged metal MXT® tubing stands up to process GC analyzer conditions.
- Available with all major adsorbents in 3.5" coils or on 7" 11-pin cages.

Porous layer open tubular (PLOT) columns are useful for analyzing volatiles in petrochemical product streams, as the specialized adsorbents provide good resolution and fast analysis times. However, conventional PLOT columns suffer from poor mechanical stability, limiting their use in process analyzers, which require robust columns for continual operation. Recently Restek developed new PLOT column bonding techniques that result in improved layer stability, consistent flow behavior, and more reproducible retention times. This technology, which was first developed for fused silica columns, has now been transferred to metal MXT® tubing, resulting in rugged columns that outperform typical metal PLOT columns and are ideal for process GC analyzers.

New Technology Improves Column Stability

Restek's PLOT columns are stabilized through a proprietary process that is based on concentric adsorption layers and improved particle bonding. New MXT® PLOT columns show greater thermal stability and much less phase bleed than the comparable competitor product (Figure 1). Lower bleed improves sensitivity and ensures faster stabilization times.

Figure 1: The bonding technology used in new MXT® PLOT columns increases thermal tolerance, resulting in lower bleed, faster stabilization times, and higher sensitivity.



Bleed comparison: Q type porous polymer columns were conditioned at 250 °C for equivalent periods and then tested to evaluate temperature stability. Split vent flow rate: 150 mL/min.; Oven: 250 °C (hold 10 min.) to 40 °C at 50 °C/min.; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

Figure 2: Conventional PLOT columns show continuous spiking resulting from particle generation. In contrast, the Restek column showed spikes during only the 2 initial analyses out of 240.

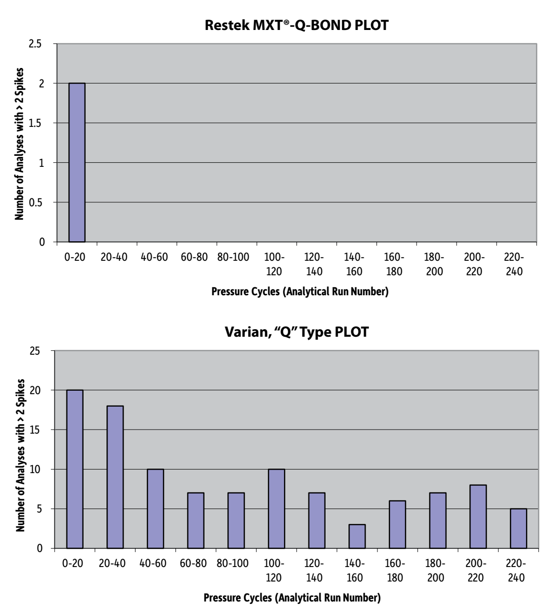
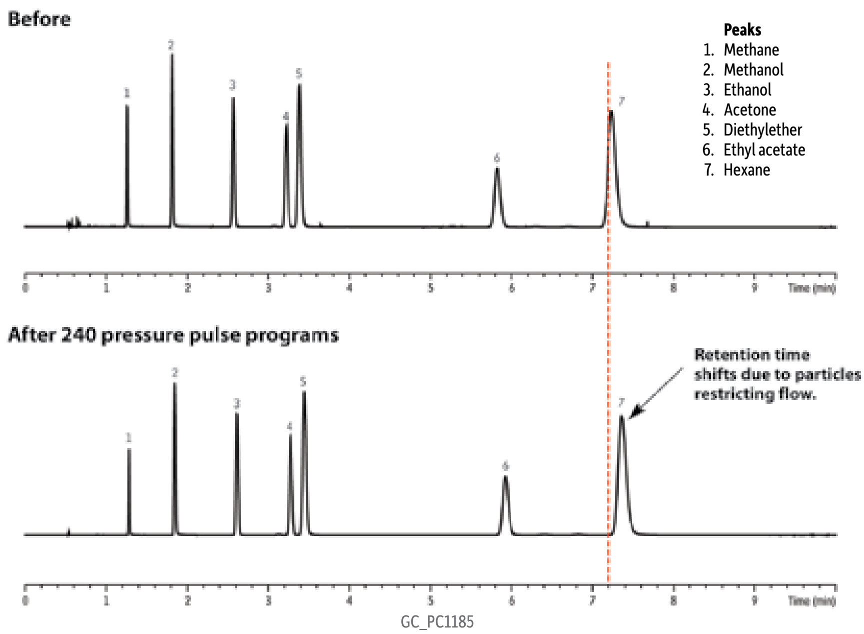
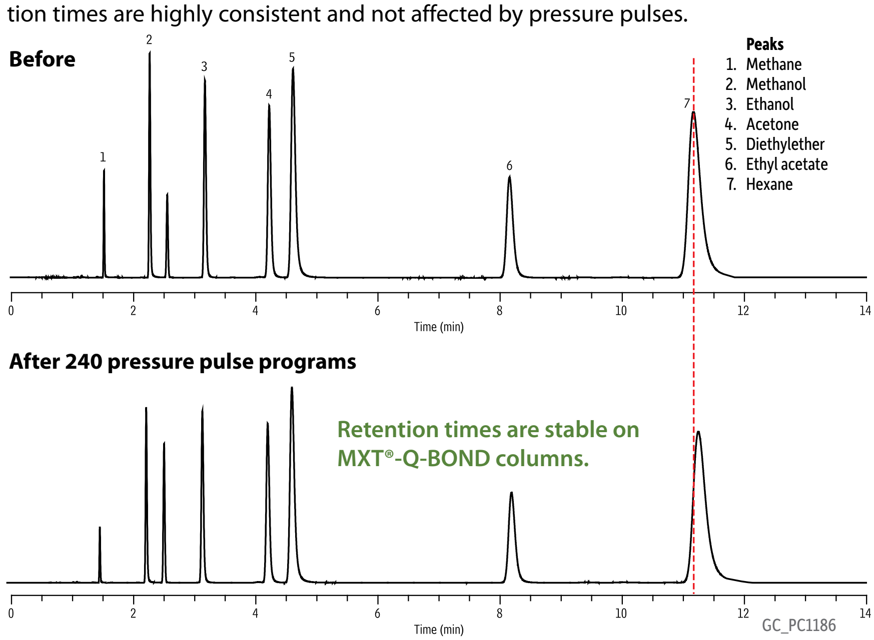


Figure 3: A conventional PLOT column releases particles following pressure pulsing, forming restrictions in the column that affect flow behavior and change retention time.



Isothermal testing before and after 240 pressure pulse cycles. Column: Varian Q type PLOT, 25 m x 0.53 mm ID; Sample: solvent mix; Injection: 1 µL split, 250 °C; Split vent flow rate: 150 mL/min.; Oven: 150 °C; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

Figure 4: MXT® PLOT columns are exceptionally stable; flow characteristics and retention times are highly consistent and not affected by pressure pulses.



Isothermal testing before and after 240 pressure pulse cycles. Column: MXT®-Q-BOND PLOT, 30 m x 0.53 mm ID x 20 µm (cat.# 79716); Sample: solvent mix; Injection: 1 µL split, 250 °C; Split vent flow rate: 150 mL/min.; Oven: 150 °C; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

MXT®-Q-BOND Columns

(Siltek®-treated stainless steel PLOT)

ID	df	temp. limits	3.5" coil 15-Meter	7" 11-pin cage 15-Meter	3.5" coil 30-Meter	7" 11-pin cage 30-Meter
0.25mm	8µm	to 280/300°C	79718-273	79718		
0.53mm	20µm	to 280/300°C			79716-273	79716

Other phases available, visit www.restek.com/metalPLOT for details.

Stable Flow Ensures Predictable Retention Times

To demonstrate the superior stability of MXT® PLOT columns, an MXT®-Q-BOND column and a competitor's Q type column were subjected to 240 pressure pulse cycles and the spiking observed in each analytical run was used as an indicator of particle generation, or phase instability. Results demonstrate that particle generation on the Varian column was significantly higher (Figure 2), resulting in restrictions in the column that caused a shift in retention time (Figure 3). In contrast, the MXT®-Q-BOND column showed little spiking. Greater phase stability resulted in consistent flow behavior and predictable retention times (Figure 4).

Key Phases Available for Optimized Separations

New metal MXT® columns are available for all major adsorbent types: porous polymer, molecular sieve, and alumina. Porous polymer MXT® columns, such as the MXT®-Q-BOND column, are highly inert and effective at separating both polar and nonpolar compounds. Volatiles are strongly retained, making these columns extremely useful for determining solvents. Molecular sieve columns provide efficient separation of argon and oxygen, as well as other permanent gases. Metal MXT® alumina columns are recommended for light hydrocarbon analysis, as alumina is one of the most selective adsorbents available and allows all C1-C5 isomers to be separated with the highest degree of resolution.

Summary

MXT® PLOT columns from Restek offer greater stability than conventional PLOT columns, making them a better choice for process monitoring. New bonding techniques produce columns with highly reproducible flow characteristics, improved layer stability, and excellent separation efficiencies. These robust columns produce exceptionally reproducible chromatography, providing the reliable performance needed for process GC analyzer applications.

For the complete version of this technical article, visit
www.restek.com/metalPLOT

Rethinking the Use of Wool With Splitless GC

By Scott Grossman

- An obstruction like wool is a must for efficient vaporization under split conditions.
- Wool is also necessary under splitless conditions to minimize sample loss and improve transfer onto column.
- With exceptionally inert Sky™ inlet liners, you can use wool with confidence.

When running a split injection with an autosampler, few would challenge that you need a liner with an obstacle like wool to achieve accurate, precise results. After all, when you combine a fast injection with a high split flow rate, your sample simply needs more time to vaporize or else it may be lost out the split vent. Wool stops the sample and gives it the time it needs to efficiently and completely vaporize, presenting a homogenous mixture to the column and split vent. Unlike in split injections, conventional wisdom has long held that you do not need wool under splitless conditions. However, a highly recommended paper by Bieri et al. argues that wool is just as important in splitless work. [1]

Should Splitless Mean Wool-Free?

Why do so many chromatographers believe that wool is not necessary to get accurate and representative sample transfer in a splitless run? The only flow out of the inlet (other than the septum purge) is through the column, so the thinking is that, since the flow will be so much slower than it is under split conditions, the sample will have ample time to vaporize and transfer onto the column without assistance. But, could autoinjecting the sample using a fast plunger speed pose a problem? And can't the sample still become trapped or be lost? The visualization and chromatographic experiments Bieri et al. outlined were very effective in supporting their claim that wool is a must for split ***and*** splitless runs alike. So, I decided to expand upon their work using common styles of splitless liners.

Putting Wool Through the Wringer

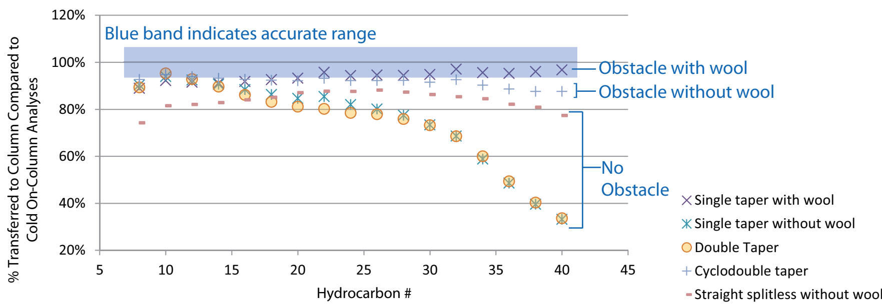
Since the integral question is whether you lose sample when performing splitless injections without wool, I opted to benchmark with cold on-column injections to force 100% of the sample onto the column. My sample was a 17-component mixture of straight-chain hydrocarbons spanning a molecular weight range from C8 to C40. In addition to cold on-column capability, my GC also had a split/splitless inlet, so I collected all response data using the same FID.

Figure 1 shows the data from a series of splitless analyses using the same sample but different liners. Results clearly illustrate that, for a wide molecular weight range, the use of wool—or to a lesser degree another obstacle like a cyclo double gooseneck—is necessary for accurate sample transfer and a reduction of molecular weight discrimination. You can also see that the only time the entire mass of analytes was transferred to the column under splitless conditions was when we employed a single gooseneck with wool. The liners with no obstruction had much less desirable results.

Use Wool With Confidence

Of course, there is a reason why one may prefer not to use wool: It is a common source of activity that can break down and trap sensitive analytes. In that case, how do you avoid counteracting wool's advantage in improving vaporization? The wool in a Sky™ inlet liner is made of fused quartz and is deactivated after packing, reducing the loss of sensitive analytes (Figure 2). By using Sky™ liners with exceptionally inert wool, you can help ensure efficient vaporization and improved transfer onto your column for more accurate results and lower detection limits. With Restek Sky™ inlet liners, you can use wool with confidence—and should under split ***and*** splitless conditions.

Figure 1: Only the liners with an obstruction were able to produce even 90% sample transfer with splitless injections—and only the liner with wool offered full accuracy.

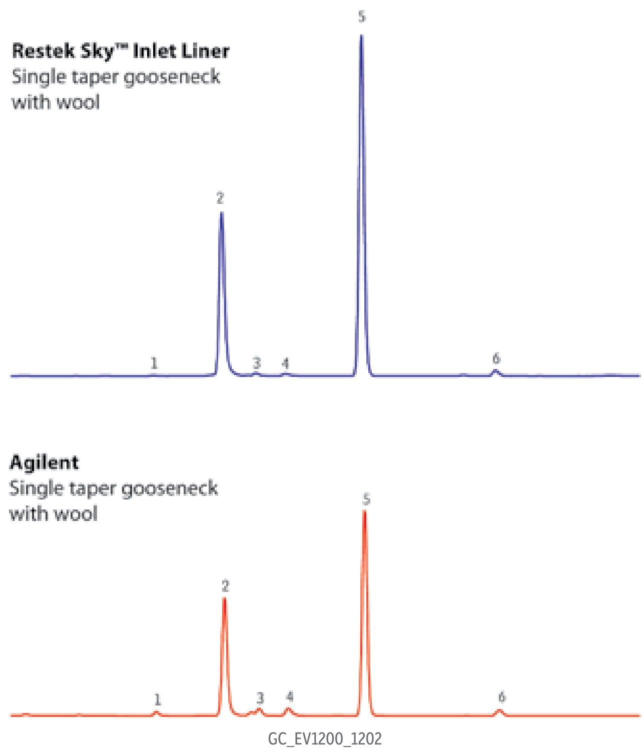


References

[1] Stefan Bieri, Philippe Christen, Maurus Biedermann, and Koni Grob, *Inability of Unpacked Gooseneck Liners to Stop the Sample Liquid After Injection With Band Formation (Fast Autosampler) Into Hot GC Injectors*, Anal. Chem. 76 (2004) 1696.

For a closer look at the form and function of GC inlet liners, view Scott's webinar at **www.restek.com/linerwebinar**

Figure 2: Endrin and DDT breakdown is significantly reduced with Sky™ liners, due to higher inertness and lower activity—even when using wool.



Inert Sky™ liners reduce analyte breakdown, giving you more accurate results.

	% Breakdown	
	Endrin	DDT
Restek	**4.8**	**1.3**
Agilent	12	5.2

Peaks

1. DDE*
2. Endrin
3. DDD*
4. Endrin aldehyde*
5. DDT
6. Endrin ketone*

*breakdown products

Column Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620); **Sample** endrin (50 ng/mL) and DDT (100 ng/mL) in hexane; **Injection** Inj. Vol.: 1 µL splitless (hold 0.75 min.); Liner: Comparison of Sky™ Single Taper Gooseneck Liner with Wool (cat.# 23303.5) and Agilent Single Taper Gooseneck Liner with Wool (cat.# 5062-3587); Inj. Temp.: 250 °C.; **Detector:** µ-ECD @ 300 °C.

Innovators in Chromatography

A continuing series of guest editorials contributed by collaborators and internationally recognized leaders in chromatography.

Analysis of Brominated Flame Retardants by Liquid Chromatography Mass Spectrometry

By Dr. Chris Marvin, Environment Canada

Dr. Chris Marvin is a Research Scientist for Environment Canada, Burlington, Ontario. His research interests include new and emerging environmental contaminants, occurrence and fate of contaminants in the Great Lakes, and LC-MS methods development.

A wide variety of brominated flame retardants (BFRs) are currently used in industry and commerce. Use of these compounds has increased exponentially in the past 50 years as a result of strict regulations regarding the flame retardancy of consumer products. Roughly 40% of all flame retardants on the market are brominated. Some of these compounds have the potential to be persistent, toxic, bioaccumulative, and are amenable to long range transport. In addition, the occurrence, distribution, and fate of many of these compounds in the environment remain largely unknown.

Polybrominated diphenyl ethers (PBDEs) remain the most widely studied of the BFRs, despite the penta- and octa-formulations being banned in Europe and voluntary cessation of production in North America. With the exception of the fully-substituted decabromodiphenyl ether (BDE-209), the PBDEs are easily determined by gas chromatography-mass spectrometry (GC-MS) and are now routinely measured in a wide range of environmental matrices. Due to its unique chemical and physical properties, including high molecular weight, poor solubility, and sensitivity to heat and light, accurate determination of BDE-209 remains a significant challenge. A host of other BFRs are not readily amenable to analysis by GC-MS and pose an analytical challenge as a result of their physical properties. Although their chemical structures appear quite simple, BFRs such as hexabromocyclododecane (HBCD), 1,2,5,6-tetrabromocycloctane (TBCO) and tetrabromoethylcyclohexane (TBECH) thermally isomerize and partition poorly on GC stationary phases. HBCD is one of the most widely used BFRs with production globally in excess of 20,000 tons; HBCD is the primary flame retardant used in the extruded and expanded polystyrene foams used as thermal insulation in buildings, as well as in upholstery fabrics. Some laboratories continue to report HBCD concentrations as the sum of the three predominant isomers based on analysis by GC, i.e., the sum of α-, β- and γ-HBCD. These nonisomer specific analyses preclude thorough investigation of environmental pathways, and potential shifting of isomer profiles during manufacture or cycling in the environment. Differences in pathways of HBCD in the environment are evidenced by the predominance of γ-HBCD in the technical mixture and in sediment, while α-HBCD is dominant in biota (typically >90%). In addition, an inherent property of aliphatic BFRs is that they exist as diastereomers. Therefore, the study of enantioselective accumulation of BFRs in food chains requires separation of the individual enantiomers.

The last decade has been a period of extraordinary progress in development of LC-MS technology. As a result, detection limits of some LC-MS methods are on a par with those of gas chromatography-high resolution mass spectrometry (GC-HRMS) methods. These technological advances allow the resolving power of contemporary LC stationary phases to be coupled with the sensitivity and specificity of state-of-the-art mass spectrometers. In addition, electrospray ionization (ESI), one of the most commonly used ionization mechanisms, is softer than electron ionization (EI) used in GC-MS. Robust LC-MS methods for analysis of BFRs, including HBCD and tetrabromobisphenol-A (TBBPA), are now routinely used in analytical laboratories. Most methods for analysis of BFRs are based on negative ion mass spectrometry. Despite these advances, significant analytical challenges remain in LC-MS methods development. LC-MS continues to be susceptible to matrix effects, and the technique still generally lacks the retention time reproducibility of GC-MS methods. The use of isotopically-labeled internal standards is effective in minimizing matrix effects, but investigations of new chemicals continue to be plagued by a paucity not only of labeled compounds, but authentic native standards.

Other challenges of LC-MS analysis of BFRs can include poor ionization efficiency and limited fragmentation. In the case of TBCO and TBECH, both ESI and atmospheric pressure chemical ionization (APCI) result in weak molecular ions or molecular ion adducts. Adequate detectability of the compounds can be achieved by monitoring the Br- ions in selected ion monitoring (SIM) mode; however, this approach negates the advantages of a triple quadrupole mass spectrometer, in that the power of tandem MS techniques cannot be exploited. Atmospheric pressure photoionization (APPI) is the latest ionization technique developed for LC-MS; in fact, the impetus behind development of APPI was the need to extend the range of compounds beyond those only amenable to ESI or APCI. Typical variations of the technique are based on vaporization of the liquid sample (similar to APCI), combination with a dopant, and subsequent ionization resulting from gas phase reactions initiated by photons from a krypton discharge lamp. APPI has shown great potential for analysis of compounds across a broad range of polarities, but particularly for nonpolar analytes. The method is also reportedly less susceptible to matrix effects than ESI and APCI.

Progress in LC-MS methods development continues as lessons learned from investigations of individual compounds are applied to subsequent generations of BFRs. A new challenge in the evolution of LC-MS methods for BFRs is the development of comprehensive methods for concurrent analysis of multiple compound classes. The primary challenge in development of comprehensive methods is identification of suitable LC stationary phases coupled with MS ionization techniques applicable to compounds exhibiting a broad range of chemical and physical characteristics. The LC stationary phase must provide adequate separation among compounds that can exhibit dramatically different retention behaviors; key factors include particle size, pore size, and stationary phase chemistry. In addition, even individual isomers within the same compound class can exhibit significantly different mass spectrometric response factors. A further convoluting factor is the limited solubility of BFRs in typical reversed phase (RP) HPLC mobile phases. Many BFR standards are marketed in nonpolar solvents such as toluene, necessitating a solvent exchange step prior to analysis. The same issue arises for BFRs isolated from environmental samples using conventional column cleanup methods, in that these techniques frequently culminate in the extracts being concentrated in nonpolar solvents amenable to analysis by GC.

The primary challenge in development of comprehensive methods is identification of suitable LC stationary phases coupled with MS ionization techniques applicable to compounds exhibiting a broad range of chemical and physical characteristics.

Ultimately, partnerships among experts in the field of analytical standards, separation science, and mass spectrometry will yield viable comprehensive methods for BFRs. In the past few years, suppliers of analytical standards and manufacturers of LC stationary phases and mass spectrometers have been astute in recognizing trends in analysis of compounds of potential environmental concern, and correspondingly have been proactive in developing technologies of great value to the toxics research and monitoring community.

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