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Editorial

Quality Control in Metabolomics

Oliver Fiehn, UC Davis Genome Center

Comprehensive analysis of small molecule metabolites (30-1500 Da) is a challenging task for quality control. Metabolites are found in very different concentrations in complex biological matrices, from which they have to be extracted without compromising the structural integrity and relative abundances. There are metabolites which are transformed extremely rapidly if enzymatic activity is not stopped completely at the time of sample collection, such as the ratio of the energy metabolites ATP to ADP. Similarly, redox carriers such as NADH and NADPH are very sensitive to oxidative degradation during sample preparation. Consequently, quality control in metabolomics means more than just taking care of chromatographic or mass spectrometry parameters. Quality control is an attitude towards gaining reliable data, rather than an automatic procedure implemented in instrument software.

The first issue critical to obtaining valid metabolomic data is understanding the question behind a study. This means that communication with the partners of the metabolomic laboratory is an essential part of any metabolomic study. Most often, at least one other partner will be involved in a study (e.g. another laboratory focused on understanding the effect of a particular genetic alteration in an organism), and these partners may already have hypotheses on specific metabolic pathways that should be pursued. These hypotheses may then lead to suggestions for analytical procedures. For example, many secondary metabolites are easier to analyze by LC/MS methods whereas most primary metabolites can readily be quantified by GC/MS procedures. Therefore, communication with the partners should focus on the chemical classes of compounds that should be targeted. It is also critical for the analytical laboratory to understand that unbiased analysis of mass spectrometric data sets does not constitute metabolomics. A multivariate statistical differentiation of 'test' versus 'control' samples is meaningless if no identified metabolites can be reported that allow biological interpretation! Unidentified signals in metabolite analysis are as useless as unscored peptide peaks in proteomic experiments. Metabolomics is not a number game of detection of m/z features, but must be regarded as an extension of classical target-driven analytical chemistry. Only if the quantification and identification of known compounds empowers biological interpretations, can unknown peaks be further investigated and pulled into statistical tests.

> Metabolomics is not a numbers game of detection; it is an extension of classical target-driven analytical chemistry.

There is a fundamental problem associated with metabolomics analyses, that is, the lack of clean up steps. If metabolomics means a comprehensive analysis of a wide range of small molecules, varying in molecular size, functional moieties, lipophilicity, volatility, or other physicochemical parameters, then the analytical laboratory faces tough choices. One option is to employ a variety of fractionation steps, but this can cause biases in metabolite coverage, require a number of different analytical procedures (raising the subsequent challenge of integrating the data sets), and also may result in analyte loss or degradation. Alternatively, the whole extract is subjected to one or several analytical methods; however, certain matrix components may lead to deterioration of analytical quality. In such cases, literally dirt is injected into the instrument! It is critical, therefore, to acknowledge that each matrix type requires validation and that procedures that worked for microbial organisms may be very inadequate for more complex samples such as blood plasma. For example, nonvolatile material will remain in the liner and other parts of the injector in GC/MS systems, causing problems with cross-contamination, progressing pyrolysis of material, and ultimately the formation of adsorptive materials, or catalytically active sites, in the injector system. Therefore, frequent liner changes are highly recommended.

Correspondingly, for LC/MS procedures, matrix components may be irreversibly adsorbed onto stationary phases, giving rise to similar challenges as described for GC/MS. Additionally, the soft electrospray ionization in LC/MS is a more selective or vulnerable

Continued on page 23

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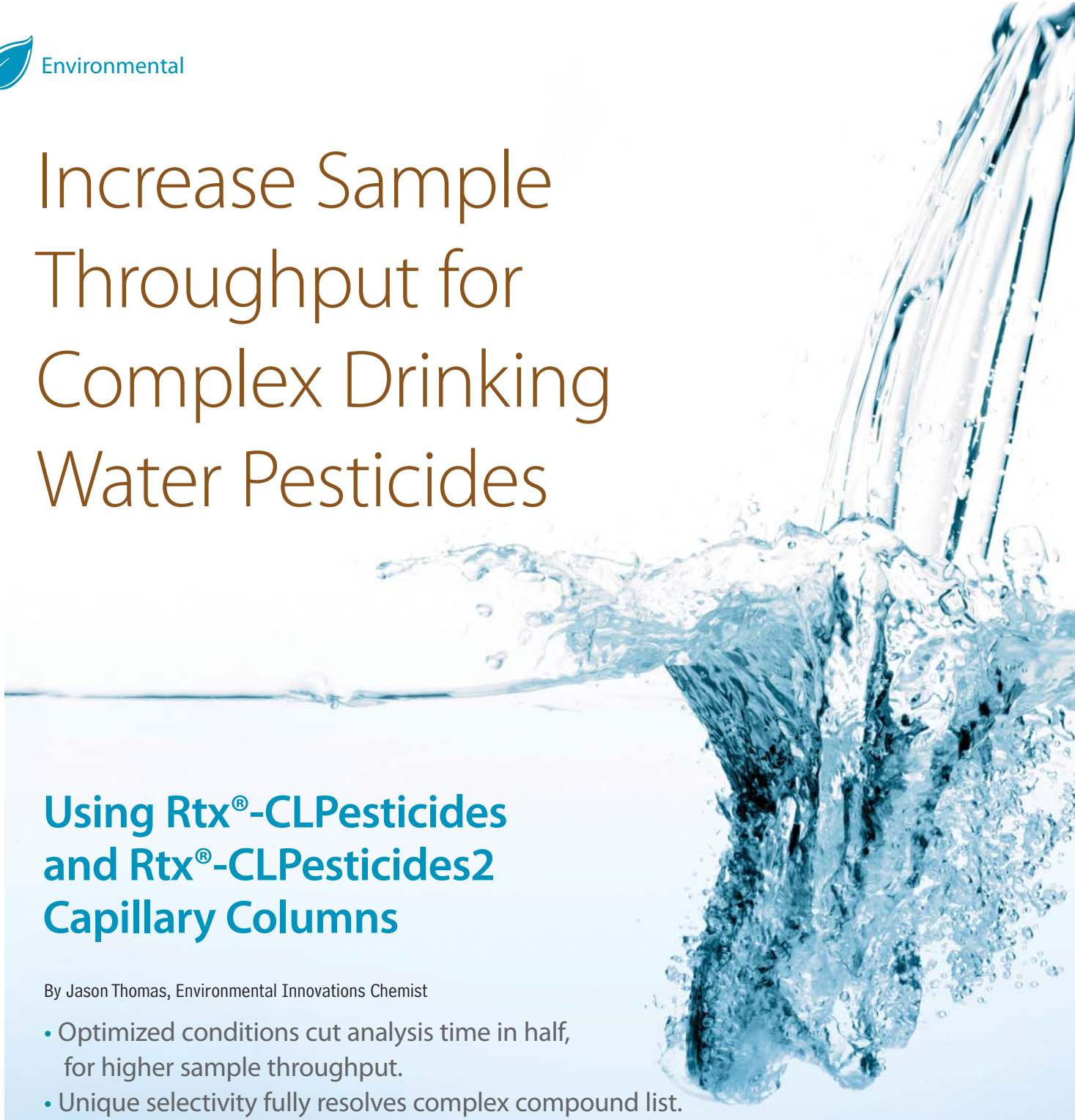
Increase Sample Throughput for Complex Drinking Water Pesticides



Using Rtx®-CLPesticides and Rtx®-CLPesticides2 Capillary Columns

By Jason Thomas, Environmental Innovations Chemist

- Optimized conditions cut analysis time in half, for higher sample throughput.
- Unique selectivity fully resolves complex compound list.
- Meets all method QA requirements, reducing rework.

With the advent of modern agriculture, and its vast selection of chemical pest control measures, the farming community has made significant increases in productivity and efficiency. Crop yield per acre is at an all time high, due in part to the role of pesticides and herbicides in mitigating the devastating effects of many plant and insect pests.[1] However, the use of these chemicals can have drawbacks, including surface and ground water contamination. EPA Methods, such as 508.1, are used to monitor pesticides and herbicides in drinking and ground water. The optimized dual column method shown here satisfies all method requirements in half the analysis time, significantly improving sample throughput.

Continued on page 4.

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Environmental

Increase Sample Throughput for Complex Drinking Water Pesticides

Continued from page 3.

EPA Method 508.1 includes many of the components as Method 505, a similar GC/ECD method, but also contains several others, expanding the list to 38 compounds. This method calls for solid phase extraction and extract concentration, followed by analysis using a GC/ECD system. In order to increase sample throughput, an optimized method was developed using a dual column configuration with the Rtx®-CLPesticides/Rtx®-CLPesticides2 column pair. These columns, used under the conditions shown, offer a unique selectivity that allows the target analytes to be resolved in approximately half the analysis time of the original method (Figure 1). There was one coelution on the primary column, but these compounds were separated on the second column. Both columns easily passed the comprehensive system performance criteria adapted from 508.1 (Table I).[2]

In conclusion, due to the complexity of the compound list in Method 508.1, a very high degree of selectivity is required of the capillary column in order to achieve adequate resolution of all target analytes in a reasonable time. The optimized dual column method shown here offers a significantly faster analysis time, while maintaining excellent resolution of challenging drinking water pesticides and herbicides.

References

1. http://www.usda.gov/nass/pubs/trackrec/track00a.htm#principal
2. US EPA Method 508.1, James W Eichelberger Rev 1.0 1994.

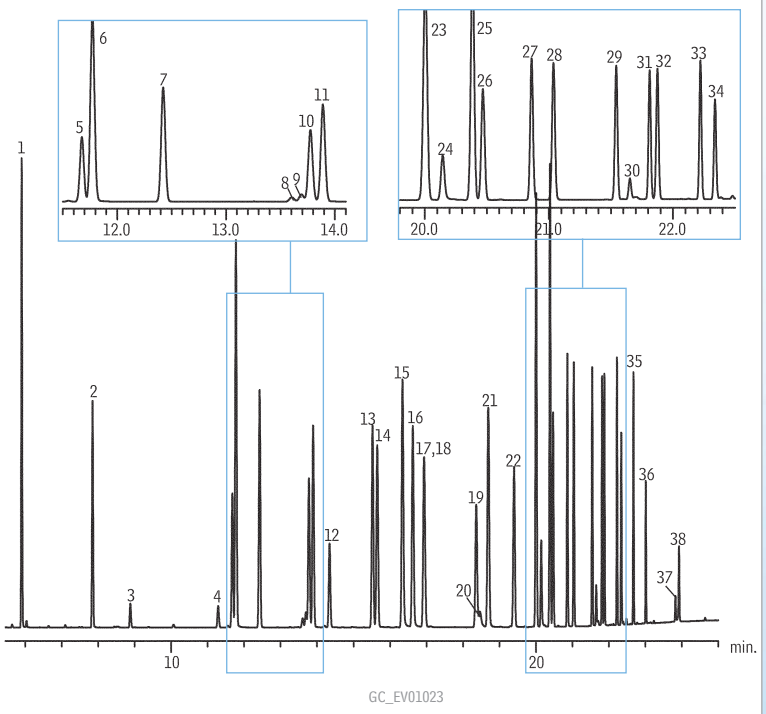
Conditions for Figure 1

Column:	Rtx®-CLPesticides2, 30m, 0.32mm ID, 0.25µm (cat.# 11324) and Rtx®-CLPesticides, 30m, 0.32mm ID, 0.32µm (cat.# 11141) with 5m x 0.32mm ID Rxi® deactivated guard tubing (cat.# 10039), connected using Universal "Y" Press-Tight® Connector (cat.# 20405-261)
Sample:	50ng/mL 508.1 Calibration Mix #1 (cat.# 32094), 100ng/mL 508.1 Calibration Mix #2 (cat.# 32095), 100ng/mL 508.1 Calibration Mix #3 (cat.# 32096), 50ng/mL 508.1 Internal Standard (cat.# 32091), 250ng/mL 508.1 Surrogate (cat.# 32092), 500ng/mL Atrazine (cat.# 32208), 500ng/mL Simazine (cat.# 32236) in ethyl acetate
Inj.:	2µL splitless (hold 0.75 min.), 4mm cyclo double gooseneck liner (cat.# 20896)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Linear velocity:	26cm/sec. @ 80°C
Oven temp.:	80°C (hold 0.5 min.) to 155°C (hold 1 min.) @ 19°C/min. to 210°C @ 4°C/min. to 310°C (hold 0.5 min.) @ 25°C/min.
Detector temp.:	ECD @ 325°C

Figure 1 Resolve all critical pairs using Rtx®-CLPesticides and Rtx®-CLPesticides2 columns.

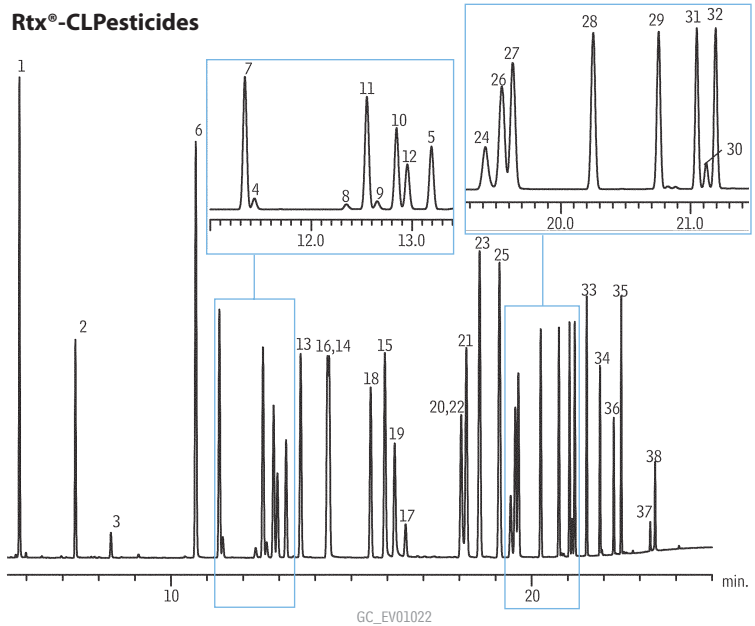
1. hexachlorocyclopentadiene
2. etridiazole
3. chlorneb
4. propachlor
5. trifluralin
6. hexachlorobenzene
7. α-BHC
8. simazine
9. atrazine
10. pentachloronitrobenzene (IS)
11. γ-BHC
12. β-BHC
13. δ-BHC
14. heptachlor
15. chlorothalonil
16. metribuzin
17. alachlor
18. aldrin
19. 4,4'-dibromobiphenyl (SS)
20. metachlor
21. DCPA
22. heptachlor epoxide
23. γ-chlordane
24. cyanazine
25. α-chlordane
26. endosulfan I
27. 4,4'-DDE
28. dieldrin
29. endrin
30. chlorobenzilate
31. 4,4'-DDD
32. endosulfan II
33. 4,4'-DDT
34. endrin aldehyde
35. endosulfan sulfate
36. methoxychlor
37. *cis*-permethrin
38. *trans*-permethrin

Rtx®-CLPesticides2



GC_EV01023

Rtx®-CLPesticides



GC_EV01022

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Satisfy all method requirements in half the time!

Table I Rtx®-CLPesticides and Rtx®-CLPesticides2 columns easily pass EPA Method 508.1 performance criteria.

Test/Requirement	Analyte	Concentration (ppb)	Rtx®-CLPesticides2	Rtx®-CLPesticides
Inertness (breakdown <20%)	endrin	50	0.9%	1.4%
Inertness (breakdown <20%)	4,4'-DDE	100	1.0%	1.1%
Sensitivity (S/N>3)	chlorpyrifos	2	12.0	6.2
Chromatographic performance (0.8<PGF<1.15)	DCPA	50	1.03	1.06
Column performance (resolution>0.50)	chlorothalonil	50	9.9	26.8
Column performance (resolution>0.50)	gamma-BHC	40	9.9	26.8

Rxi® Guard/Retention Gap Columns (fused silica)

Nominal ID	Nominal OD	5-Meter	5-Meter/6-pk.	10-Meter	10-Meter/6-pk.
0.25mm	0.37 ± 0.04mm	10029	10029-600	10059	10059-600
0.32mm	0.45 ± 0.04mm	10039	10039-600	10064	10064-600
0.53mm	0.69 ± 0.05mm	10054	10054-600	10073	10073-600

Universal "Y" Press-Tight® Connectors

Description	ea.	3-pk.
Universal "Y" Press-Tight Connector	20405	20406
Deactivated Universal "Y" Press-Tight Connector	20405-261	20406-261
Siltek Treated Universal "Y" Press-Tight Connector	20485	20486

Rtx®-CLPesticides Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.32	-60 to 320/340°C	30-Meter	11141

508.1 Calibration Mix #1 (17 components)

aldrin
α-BHC
β-BHC
δ-BHC
γ-BHC (lindane)
4,4'-DDD
4,4'-DDE
4,4'-DDT
dieldrin
endosulfan I
endosulfan II
endosulfan sulfate
endrin
endrin aldehyde
heptachlor
heptachlor epoxide (isomer B)
methoxychlor

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32094

508.1 Calibration Mix #2 (11 components)

chlorobenzilate
α-chlordane
γ-chlordane
chlorneb
DCPA (Dacthal®)
etridiazole
hexachlorobenzene
cis-permethrin*
trans-permethrin*
propachlor
trifluralin

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32095

*1000µg/mL total permethrin. Exact content of each isomer listed on certificate of analysis.

508.1 Calibration Mix #3 (8 components)

alachlor
atrazine
chlorthalonil
cyanazine
hexachlorocyclopentadiene
metolachlor
metribuzin
simazine

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32096

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	-60 to 320/340°C	30-Meter	11324

508.1 Internal Standard

pentachloronitrobenzene
100µg/mL in ethyl acetate, 1mL/ampul
cat. # 32091

508.1 Surrogate

4,4'-dibromobiphenyl
500µg/mL in ethyl acetate, 1mL/ampul
cat. # 32092

Atrazine

1,000µg/mL in acetone, 1mL/ampul
cat. # 32208

Simazine

1,000µg/mL in acetone, 1mL/ampul
cat. # 32236

Splitless Liners for Agilent GC

ID* x OD & Length	qty.	cat.#
Cyclo Double Gooseneck (4mm)		
4.0mm x 6.5mm x 78.5mm	5-pk.	20896

*Nominal ID at syringe needle expulsion point.

Resprep™-C18 SPE Disks

Description	qty.	cat.#
Resprep-C18 47mm SPE Disks	20-pk.	24004

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One Stop Shop for EPA Method 535

By Jason Thomas, Innovations Chemist, Julie Kowalski, Ph.D., Innovations Chemist, and Christopher Borton, Applied Biosystems

- Full package: reference standards, SPE cartridges, and HPLC columns.
- Chromatographic resolution of alachlor ESA and acetochlor ESA isomers.
- Meet method requirements, with superior sensitivity.

Acetamide herbicides are used in large quantities to suppress weed growth in corn and soybean fields. However, due to the polar nature of ethanesulfonic acid (ESA) and oxanilic acid (OA) degradation products, contamination of drinking water sources is a concern. EPA Method 535 is designed to monitor drinking water for ESA and OA breakdown products of these herbicides. Chromatographic analysis is extremely important for this method because two analytes, alachlor ESA and acetochlor ESA, are isomers that share the same mass spectral multiple reaction monitoring (MRM) transitions, and thus must be separated chromatographically.

Resolution of all Method 535 analytes, including alachlor ESA and acetochlor ESA isomers, can easily be accomplished using Restek's full line of Method 535 products, which includes reference standards, solid phase extraction (SPE) cartridges, and HPLC columns that meet method guidelines. In the procedure shown here, 6mL CarboPrep™ 90 SPE cartridges were used for sample preparation, both to help extend the lifetime of the analytical column as well as to prevent matrix enhancement or suppression. LC/MS/MS analysis was performed on an Ultra C18 column coupled to an Applied Biosystems API 3200™ LC/MS/MS system equipped with a TurboIonSpray® probe for the Turbo V™ source.

Figure 1 Easily resolve Method 535 herbicide degradates on an Ultra C18 HPLC column.

Sample: acetamide herbicides
Inj.: 25µL
Conc.: 50ng/mL
Sample diluent: 5mM ammonium acetate in water

Column: Ultra C18
Cat.#: 9174312
Dimensions: 100mm x 2.1mm
Particle size: 3µm
Pore size: 100Å

Conditions:
Mobile phase: A: 5mM ammonium acetate
B: methanol

Time:	%B
0.0	20%
4.0	30%
10.0	30%
15.0	50%
17.0	85%
18.0	85%
18.1	20%
28.0	20%

Flow: 250µL/min.
Temp.: ambient

Analytes	RT (min)	Quantifer MRM	Qualifier MRM
dimethachlor ESA	7.9	300/80	300/121
alachlor OA	14.5	264/160	264/158
acetochlor OA	14.8	264/146	264/144
1. alachlor ESA	16.1	314/80	314/121
2. metolachlor OA	16.4	278/206	278/174
3. acetochlor ESA	16.4	314/80	314/121
4. metolachlor ESA	16.9	328/80	328/121
butachlor ESA	19.7	356/80	356/121

Det.: API 3200™ LC/MS/MS system
Ion Source: TurboIonSpray® probe for Turbo V™ source (negative)
Mode: MRM
Curtain gas: 25
Collision gas: medium/5
IonSpray voltage: -4,500
Temperature: 500°C
Ion source gas 1: 50
Ion source gas 2: 50
Interface heater: on
Dwell time: 50
Vertical probe position: 2

Excellent response at low concentrations! Fully resolve alachlor ESA and acetochlor ESA isomers.

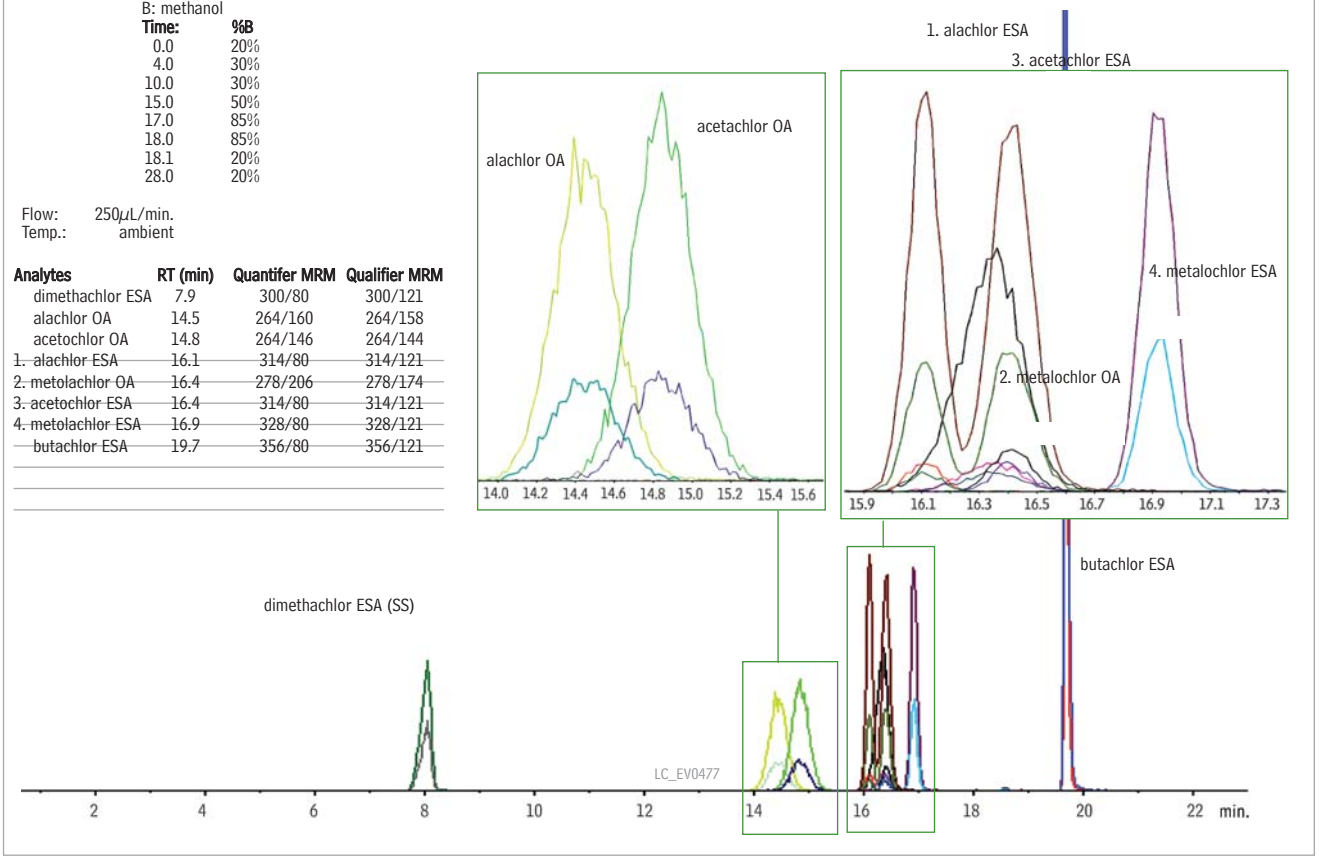

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Table I Reliably achieve minimum detection limits of 0.004µg/L or less.

Analyte	LCMRL (µg/L)	Standard Deviation	Calculated Detection Limit in Matrix (µg/L)
alachlor OA	0.013	0.28	0.003
acetochlor OA	0.014	0.27	0.003
alachlor ESA	0.013	0.18	0.002
metolachlor OA	0.013	0.21	0.003
acetochlor ESA	0.012	0.29	0.004
metolachlor ESA	0.012	0.18	0.002

Seven matrix spikes prepared at 0.013µg/L (proposed MRL).

Table II Outstanding accuracy and precision using Ultra C18 HPLC columns.

Analytes	Average Recovery (%)	%RSD
dimethachlor ESA	100.1	9.2
metolachlor OA	95.0	8.5
metolachlor ESA	94.8	8.9
alachlor OA	96.6	8.5
acetochlor OA	97.0	8.9
alachlor ESA	92.5	8.6
acetochlor ESA	94.3	8.0

Four lab fortified blanks spiked at 0.2µg/L.
Method requirements: average recovery ±30% of the true value, %RSD ≤20%.

CarboPrep™ SPE Cartridges
Nonporous graphitized carbon

SPE Cartridge	Tube Volume, Bed Weight	qty.	cat#
CarboPrep 90	6mL, 500mg	30-pk.	26092

Ultra C18 Columns (USP L1)
Excellent for a wide range of analyses

Physical Characteristics:

particle size: 3µm, spherical
pore size: 100Å
carbon load: 20%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

3µm Column, 2.1mm	cat. #
100mm	9174312

Method 535 Individual Compounds

Volume is 1mL/ampul. Concentration is µg/mL.

Compound	Solvent	Conc.	cat.#
acetochlor ESA sodium salt	M	100	33092
acetochlor OA	M	100	33094
alachlor ESA sodium salt	M	100	33096
alachlor OA	M	100	33099
metolachlor ESA sodium salt	M	100	33200
metolachlor OA	M	100	33201

M=methanol

Method 535 Internal Standard

butachlor ESA sodium salt
100µg/mL in methanol, 1mL/ampul
cat. # 33202

Method 535 Surrogate Standard

dimethachlor ESA sodium salt
100µg/mL in methanol, 1mL/ampul
cat. # 33203

Consistent chromatographic resolution of 3.5 or greater for alachlor ESA and acetochlor ESA was easily achieved as shown in Figure 1. Surrogate recoveries, matrix spikes, minimum detection limits, and internal standard recoveries produced

Resolution of all target analytes, including alachlor ESA and acetochlor ESA isomers, can easily be achieved.

consistently acceptable results at low concentrations and showed no interferences from the drinking water matrix. The method reporting limits (MRL) listed in Table I are based on seven replicate fortified blanks prepared at the proposed MRL of 0.013µg/L in drinking water. An LCMRL of 0.012 to 0.014µg/L was established and validated with a calculated detection limit of 0.004µg/L or less. Precision and accuracy were demonstrated using four replicate fortified blanks at 0.2µg/L; recovery and RSD values easily met method requirements (Table II). All analytes were detected in laboratory blanks at ≤1/3 MRL values demonstrating low system background levels.

The optimized method developed here shows superior sensitivity for the ESA and OA degradates of chloroacetanilide herbicides alachlor, acetochlor, and metolachlor, as well as reliable resolution between isomers alachlor ESA and acetochlor ESA. This method is simplified by Restek's suite of Method 535 products. All of the reference materials, sample preparation products, and HPLC columns needed are now available from a single source, to facilitate successful Method 535 analysis.

References

1. C. Borton. EPA Method 535; Detection of Degradates of Chloroacetanilides and other Acetamide Herbicides in Water by LC/MS/MS. Applied Biosystems, Foster City, CA, 2008.

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Breaking Down? Improve BDE-209 Response

Using a New Rtx®-1614 Column for PBDE Analysis

By Jason Thomas, Environmental Innovations Chemist, and Jack Cochran, Director of New Business and Technology

- Higher sensitivity and inertness for BDE-209 than the method-specified column, for more accurate, reproducible results.
- Meets all method requirements for resolution, tailing factors, and retention.
- Optimized short column conditions give improved BDE-209 response 3 times faster.

Polybrominated diphenyl ethers (PBDEs) are ubiquitous in humans and in the environment. Rapid and accurate PBDE methods are increasingly in demand as adverse effects have been associated with PBDE exposure. EPA Draft Method 1614 presents a considerable challenge to the analytical column due to the large number of PBDE compounds and stringent activity guidelines. One target compound, decabromodiphenyl ether (BDE-209), is of particular concern as it is frequently encountered and is the primary component in the only remaining commercial PBDE mixture. Column inertness is critical for BDE-209 analysis, as the breakdown mechanism is predominately column-related.

EPA Draft Method 1614 stipulates a 5% phenyl methyl column in a 30m x 0.25mm x 0.10µm format with a shorter 15m column option. Here we compare the performance of a method-specified column (DB-5HT) to the new Rtx®-1614 column, a 5% phenyl methyl column with a unique deactivation for maximum inertness to BDE-209. Although this method requires analysis on a high-resolution mass spectrometer, the columns were evaluated first on an Agilent 6890 GC with µ-ECD to assess inertness and general chromatographic performance. Columns were then analyzed on an Agilent 7890/5975 GC/MS to verify separation requirements under vacuum outlet conditions.

Table I Maximize BDE-209 response with an Rtx®-1614 column, in 15 or 30m lengths!

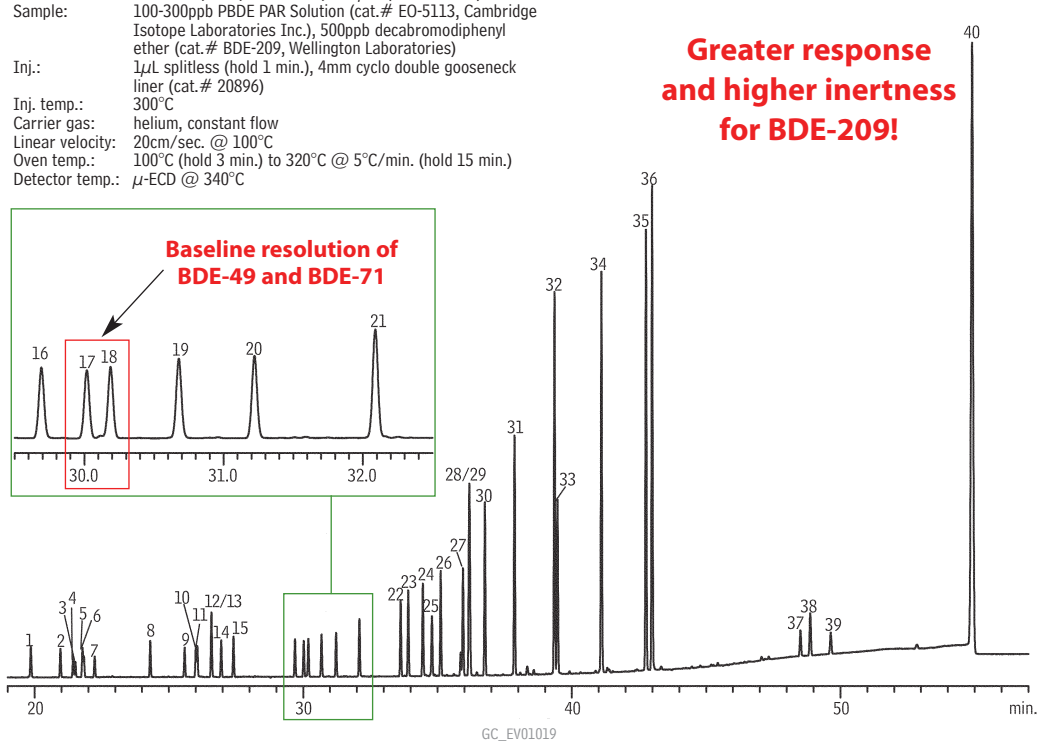
Column	BDE-209 Average RRF*
Rtx®-1614 (15m)	0.681
Rtx®-1614 (30m)	0.636
DB-5HT (30m)	0.502

*Relative response factors based on internal standard hexabromobiphenyl (n=5). Analyses run under optimized conditions.

The Rtx®-1614 column meets the method requirements for the resolution of critical pairs, tailing factors, and retention. The data in Figure 1 demonstrate the separation of a large list of PBDEs on the Rtx®-1614 column; note the baseline resolution of congeners 49 and 71, which are required to have a 40% valley height of the smallest peak. The Rtx®-1614 column also performed exceptionally well for inertness to BDE-209 (Table 1). Compared to the performance of the DB-5HT, shown in Figure 2, the Rtx®-1614 column

Figure 1 Separate PBDEs accurately and reliably on an Rtx®-1614 column.

Column: Rtx®-1614, 30m, 0.25mm ID, 0.10µm (cat.# 10295)
Sample: 100-300ppb PBDE PAR Solution (cat.# EO-5113, Cambridge Isotope Laboratories Inc.), 500ppb decabromodiphenyl ether (cat.# BDE-209, Wellington Laboratories)
Inj.: 1µL splitless (hold 1 min.), 4mm cyclo double gooseneck liner (cat.# 20896)
Inj. temp.: 300°C
Carrier gas: helium, constant flow
Linear velocity: 20cm/sec. @ 100°C
Oven temp.: 100°C (hold 3 min.) to 320°C @ 5°C/min. (hold 15 min.)
Detector temp.: µ-ECD @ 340°C



1. BDE-10
2. BDE-7
3. BDE-8
4. BDE-11
5. BDE-12
6. BDE-13
7. BDE-15
8. BDE-30
9. BDE-32
10. BDE-17
11. BDE-25
12. BDE-28
13. BDE-33
14. BDE-35
15. BDE-37
16. BDE-75
17. BDE-49
18. BDE-71
19. BDE-47
20. BDE-66
21. BDE-77
22. BDE-100
23. BDE-119
24. BDE-99
25. BDE-116
26. BDE-118
27. BDE-85
28. BDE-155
29. BDE-126
30. BDE-154
31. BDE-153
32. BDE-138
33. BDE-166
34. BDE-183
35. BDE-181
36. BDE-190
37. BDE-208
38. BDE-207
39. BDE-206
40. BDE-209

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shows a greater response for BDE-209 and less peak fronting, indicating less on-column breakdown.

Although the method originally stipulated that BDE-209 must elute at least 48 minutes from injection, eliminating the possibility of much method optimization, a new revision provides a short column option which can greatly improve analysis time and BDE-209 response. Since BDE-209 breaks down primarily in the column, reducing column residence time by using a shorter 15m column, in combination with higher flows and quicker ramp rates, dramatically improves performance. Even applying optimized parameters to a 30m column results in greatly enhanced analyses, relative to the original method-stipulated operating conditions. To further optimize this method, BDE-209 degradation was reduced by using a maximum oven temperature of less than 300°C and setting the injection temperature at 340°C, to ensure complete vaporization, resulting in a consistent and high response (Figure 3).

In conclusion, the Rtx®-1614 is an excellent column choice for analyzing EPA Draft Method 1614, as well as any routine screening analysis of PBDEs, due to its selectivity, sensitivity, and inertness, specifically with respect to BDE-209.

Rtx®-1614 Columns (fused silica)

(5% phenyl methyl)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.10	-60 to 330/360°C	15-Meter	10296
0.25mm	0.10	-60 to 330/360°C	30-Meter	10295

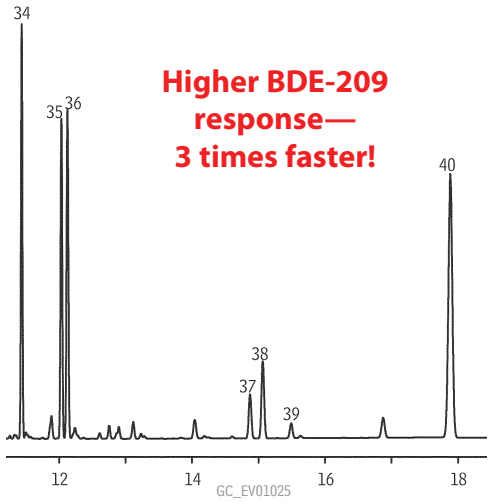
Splitless Liners for Agilent

ID* x OD & Length	qty.	cat.#
Cyclo Double Gooseneck (4mm)		
4.0mm x 6.5mm x 78.5mm	5-pk.	20896

*Nominal ID at syringe needle expulsion point.

Figure 3 Improve BDE-209 response and analysis times with optimized conditions using the short column option.

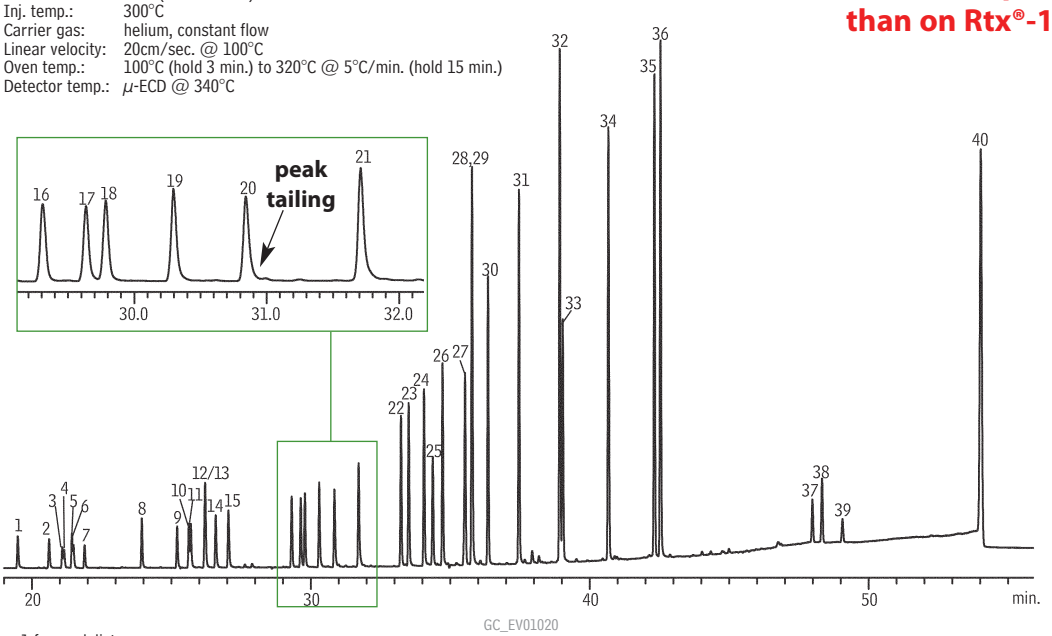
Column: Rtx®-1614, 15m, 0.25mm ID, 0.10µm (cat.# 10295)
Sample: 100-300ppb PBDE PAR Solution (cat.# EO-5113, Cambridge Isotope Laboratories Inc.), 500ppb decabromodiphenyl ether(cat.# BDE-209, Wellington Laboratories)
Inj.: 1µL splitless (hold 1 min.), 4mm cyclo double gooseneck liner (cat.# 20896)
Inj. temp.: 340°C
Carrier gas: helium, constant flow
Linear velocity: 60cm/sec. @ 120°C
Oven temp.: 120°C (hold 1 min.) to 275°C @ 15°C/min. to 300°C @ 5°C/min. (hold 5 min.)
Detector temp.: µ-ECD @ 345°C



See Figure 1 for peak list.

Figure 2 More BDE-209 peak fronting on the method-specific column indicates greater on-column breakdown.

Column: DB-5HT, 30m, 0.25mm ID, 0.10µm
Sample: 100-300ppb PBDE PAR Solution (cat.# EO-5113, Cambridge Isotope Laboratories Inc.), 500ppb decabromodiphenyl ether (cat.# BDE-209, Wellington Laboratories)
Inj.: 1µL splitless (hold 1 min.), 4mm cyclo double gooseneck liner (cat.# 20896)
Inj. temp.: 300°C
Carrier gas: helium, constant flow
Linear velocity: 20cm/sec. @ 100°C
Oven temp.: 100°C (hold 3 min.) to 320°C @ 5°C/min. (hold 15 min.)
Detector temp.: µ-ECD @ 340°C



See Figure 1 for peak list.

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Environmental

Increase Polycyclic Aromatic Hydrocarbon Sample Throughput

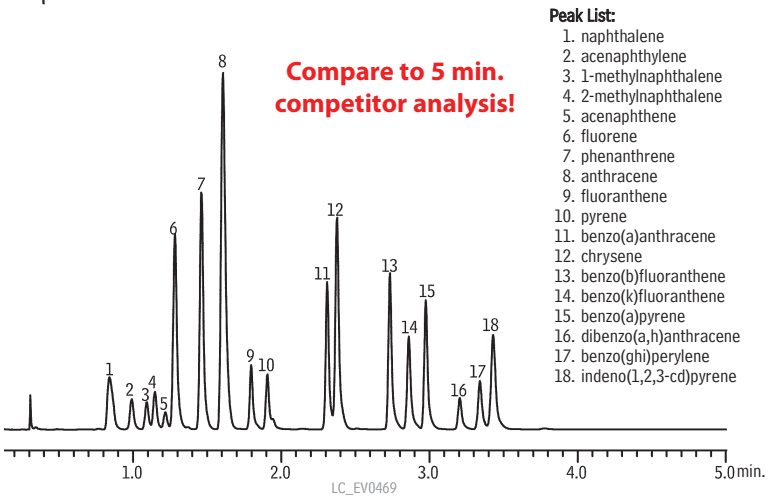
With UHPLC and HPLC Column Options

By Michelle Long, Environmental Innovations Chemist

- Two stationary phases optimized for PAH resolution.
- 3.5 minute EPA 610 and 6 minute EU PAH analyses by UHPLC.
- Portugal PAHs resolved by isocratic HPLC in 4 minutes.

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants resulting primarily from the incomplete combustion of organic materials. PAHs are an increasing human health concern, as this group of chemicals includes several known or suspected carcinogens. Exposure usually occurs by eating charbroiled foods, inhaling fumes from automobile or industrial emissions, or from other sources such as burning coal, wood, and tobacco. PAHs are also present in some medicines, plastics, and pesticides. National and international regulatory agencies provide target analyte lists and, although these lists are not identical, a number of compounds are common across the recommended lists. Here we analyze target compounds from the United States Environmental Protection Agency (EPA), European Union (EU), and Portugal lists by UHPLC and HPLC. Procedures shown use optimized stationary phases and provide analysis times of 3.5 to 6 minutes, allowing labs to achieve significantly faster sample throughput.

Figure 1 Baseline resolve EPA 610 PAHs in less than 3.5 minutes on 1.9µm Pinnacle™ DB PAH columns.



Sample:		Conditions:	
Inj.:	2µL	Mobile phase:	A: water
Conc.:	20µg/mL each component		B: acetonitrile
Sample diluent:	acetonitrile		Time (min.) %B
			0 50
Column:	Pinnacle™ DB PAH		1 60
Cat.#:	9470252		3 100
Dimensions:	50mm x 2.1mm		5 100
Particle size:	1.9µm		
Pore size:	140Å	Flow:	0.6mL/min.
		Temp.:	30°C
Instrument:	JASCO X-LC	Det.:	UV @ 264nm

Two Phases Optimized for PAHs

Although most HPLC methods recommend a C18 column, the Pinnacle™ II PAH and Pinnacle™ DB PAH stationary phases both have been optimized specifically for polycyclic aromatic hydrocarbons and offer greater selectivity for these compounds. Pinnacle™ II PAH columns are available in standard formats, while the Pinnacle™ DB PAH columns are offered on 1.9µm silica. To demonstrate the fast analysis times and optimal selectivity of these phases, US, EU, and Portugal lists were analyzed on 1.9µm Pinnacle™ DB PAH columns using ultra-high pressure liquid chromatography (UHPLC). Portugal PAHs were also analyzed isocratically on a Pinnacle™ II PAH (50mm x 3.2mm, 4µm) column. Conventional HPLC was used for the Portugal list because, since only five analytes are included on the target list, fast analysis times and high sample throughput can be achieved without the high backpressures associated with UHPLC.

Fully Resolve PAHs in 3.5 to 6 Minutes

The 1.9µm Pinnacle™ DB PAH column resolved all 18 US EPA 610 analytes in less than 3.5 minutes (Figure1). The column was held at a constant temperature of 30°C to improve overall peak shape. The priority PAHs included in EU recommendation 256/2005 were also analyzed on the 1.9µm Pinnacle™ DB PAH column and were separated in less than 6 minutes (Figure 2). Using the 1.9µm Pinnacle™ DB PAH column pairs the stationary phase's high selectivity for PAHs with the increased efficiency and fast analysis times of UHPLC. The Portugal PAH list was analyzed by UHPLC (data not shown), but was also analyzed by conventional HPLC using a 4µm Pinnacle™ II PAH column. All target analytes were resolved in less than 4 minutes (Figure 3).

For the analysis of polycyclic aromatic hydrocarbons, two stationary phases provide optimum results. The Pinnacle™ II PAH phase is available in standard column dimensions while the Pinnacle™ DB PAH phase is available in 1.9µm particle size dimensions. Both alkyl phases have been optimized specifically for PAHs and offer exceptionally fast analysis times, providing a significant opportunity to labs interested in increasing sample throughput.

Acknowledgement
Thanks to JASCO for supplying the JASCO X-LC system used for this work.

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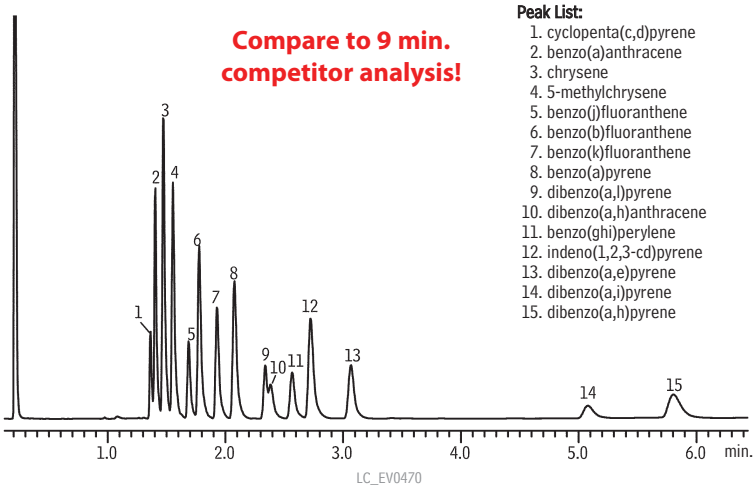
Figure 2 Excellent resolution of EU PAHs on 1.9µm Pinnacle™ DB PAH columns.



Sample:
Inj.: 2µL
Conc.: 20µg/mL each component
Sample diluent: acetonitrile

Column: Pinnacle™ DB PAH
Cat.#: 9470252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm
Pore size: 140Å

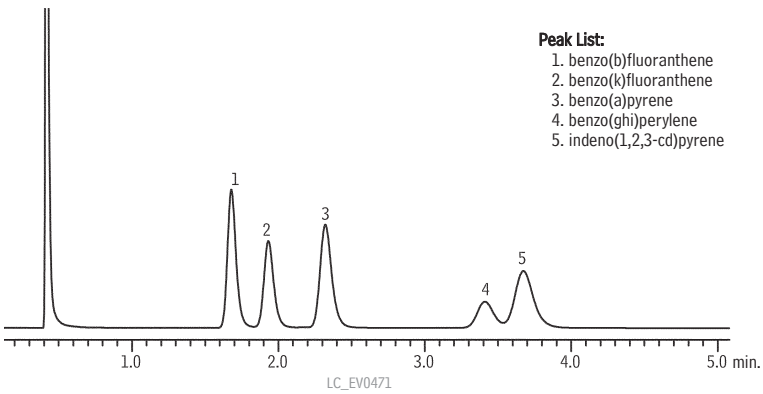
Instrument: JASCO X-LC

Conditions:
Mobile phase: A: water
B: acetonitrile

Time (min.)	%B
0	50
1	90
2	95
5	100
7	100

Flow: 0.6mL/min.
Temp.: 30°C
Det.: UV @ 264nm

Figure 3 Simple, isocratic separation of Portugal PAHs on 4µm Pinnacle™ II PAH columns.



Sample:
Inj.: 2µL
Conc.: 20µg/mL each component
Sample diluent: acetonitrile

Column: Pinnacle™ II PAH
Cat.#: 9219453
Dimensions: 50mm x 3.2mm
Particle size: 4µm
Pore size: 110Å

Instrument: JASCO X-LC

Conditions:
Mobile phase: water:acetonitrile, 5:95 (v/v)
Flow: 0.6mL/min.
Temp.: 30°C
Det.: UV @ 264nm

Pinnacle™ II PAH Columns

Physical Characteristics:

particle size: 4µm, spherical
pore size: 110Å
endcap: fully endcapped
pH range: 2.5 to 10
temperature limit: 80°C

4µm Column, 3.2mm	cat. #
50mm	9219453

Pinnacle™ DB PAH UHPLC Columns

Physical Characteristics:

particle size: 1.9µm
pore size: 140Å
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

1.9µm Column, 2.1mm	cat. #
50mm	9470252

ordering note

For guard cartridges for these columns, visit our website at **www.restek.com**.

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Characterizing all 136 Tetra- to Octachlorinated Dioxins and Furans

Using the Rtx®-Dioxin2 Column

By Jack Cochran, Director of New Business and Technology

- Known elution orders for all tetra- through octachlorinated dioxin and furan congeners.
- Resolve 14 of 17 tetra- through octachlorine 2,3,7,8-substituted dioxins and furans.
- TCDD and TCDF specificity, with a column stable up to 340°C.

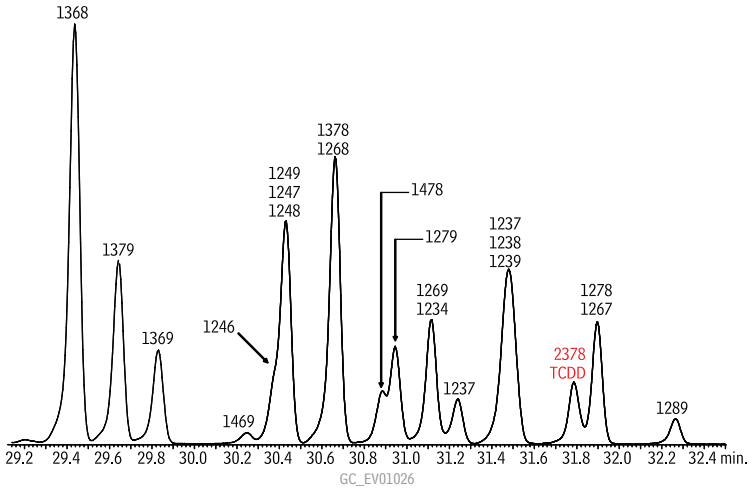
Successful analyses of dioxins and furans are critical because of the extremely toxic nature of these compounds. However, confidently resolving the most toxic congeners, 2,3,7,8-substituted tetrachlorinated dibenzodioxin (TCDD) and tetrachlorinated dibenzofuran (TCDF), is often complicated by the presence of the many other possible congeners. Even with high resolution GC/high resolution MS methods, the proper choice of chromatographic column is essential for separating 2,3,7,8-substituted dioxins and furans from the less toxic congeners and matrix-related compounds.

Complete Column Characterization

It is rare that a column's performance is characterized against all possible 136 tetra- through octachlorinated dioxins and furans. These standards are difficult to obtain, and testing can be time consuming. However, here the Rtx®-Dioxin2 column is characterized against all 136 compounds using standards from Cambridge Isotope Laboratories, Inc. When compared to industry standard stationary phases, a unique selectivity is observed for the Rtx®-Dioxin2 column, and specific resolutions and coelutions are noted. Very few coelutions involving the toxic 2,3,7,8-substituted congeners are observed, making the Rtx®-Dioxin2 column an excellent choice for single column analyses of dioxins and furans (Tables I and II.)

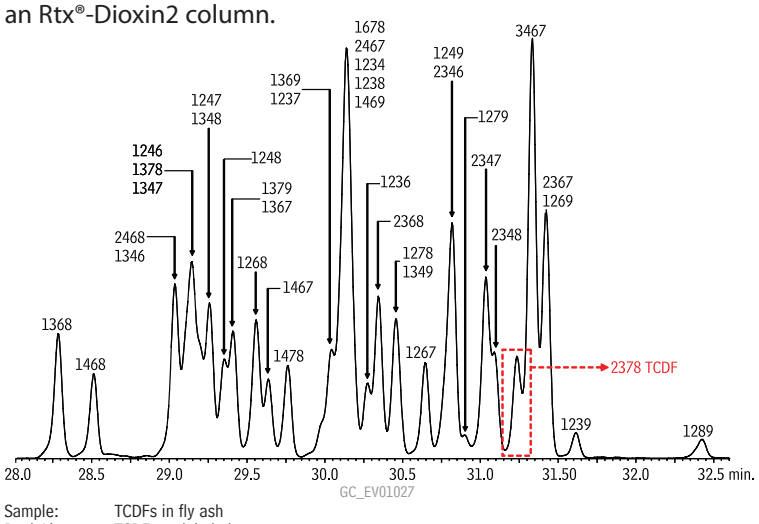
Figure 1 shows fly ash samples, run under the same chromatographic conditions used to characterize the column. 2,3,7,8-tetrachlorodibenzofuran is not resolved under these conditions. However, the characterization study used simple linear temperature programming, and additional work exploring nonlinear oven programs and different flow parameters yielded better resolution between some congeners, especially 2,3,7,8-TCDF (data available upon request). The value in this work is not necessarily to show complete separation of all the congeners on a single column, but to show where all of the 136 compounds of interest elute, making all possible coelutions known.

Figure 1 GC/HR-MS analysis of tetrachlorinated dioxins in fly ash on an Rtx®-Dioxin2 column.



Column:	Rtx®-Dioxin2, 40m, 0.18mm ID, 0.18μm (cat.# 10759)
Sample:	TCDDs in fly ash
Inj.:	1μL splitless
Inj. temp.:	280°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	120°C (hold 1 min.) to 160°C @ 10°C/min. to 320°C @ 4°C/min. (hold 4 min.)
Det.:	Waters AutoSpec Ultima HRMS
Ionization:	EI
Mode:	SIM
Peak List:	TCDDs as labeled

Figure 2 GC/HR-MS analysis of tetrachlorinated furans in fly ash on an Rtx®-Dioxin2 column.



Sample: TCDFs in fly ash
Peak List: TCDFs as labeled
See Figure 1 for conditions

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The Rtx®-Dioxin2 column is an excellent column for the analysis of dioxin and furan congeners. It has a unique selectivity for the toxic congeners, including specificity for 2,3,7,8-TCDD and 2,3,7,8-TCDF. Here we characterized all 136 tetra- through octachlorine dioxins and furans and defined all possible coeutions. While commonly used cyanopropyl columns are limited by a low maximum operating temperature of 240°C, the Rtx®-Dioxin2 column is stable up to 340°C, extending column lifetime and improving analyses of dioxins and furans.

Rtx®-Dioxin2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.18	20°C to 340°C	40-Meter	10759
0.25mm	0.25	20°C to 340°C	60-Meter	10758

Stable up to 340° for extended column lifetime!

Table I Retention times (RT) and relative retention times (RRT) for all tetra- through octachlorinated dioxins on an Rtx®-Dioxin2 column.

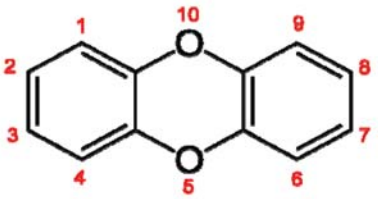
tetrachlorinated			pentachlorinated			hexachlorinated			heptachlorinated			octachlorinated		
Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)
1368	29.43	0.8198	12468	33.75	0.9401	124679	37.89	1.0554	1234679	42.44	1.1822	12346789	46.93	1.3069
1379	29.64	0.8256	12479	33.80	0.9415	124689	37.89	1.0554	1234678	43.34	1.2072			
1369	29.84	0.8312	12469	34.17	0.9515	123468	38.53	1.0730						
1469	30.25	0.8424	12368	34.67	0.9657	123679	38.79	1.0805						
1246	30.38	0.8462	12478	34.83	0.9702	123689	38.82	1.0813						
1249	30.42	0.8474	12379	34.92	0.9727	123469	38.90	1.0833						
1247	30.43	0.8476	12467	35.02	0.9755	123478	39.55	1.1017						
1248	30.44	0.8479	12369	35.08	0.9769	123678	39.66	1.1047						
1378	30.64	0.8535	12489	35.08	0.9772	123467	39.78	1.1081						
1268	30.68	0.8546	12346	35.36	0.9850	123789	39.98	1.1136						
1478	30.88	0.8599	12347	35.40	0.9858									
1279	30.95	0.8621	12367	35.89	0.9997									
1269	31.12	0.8669	12378	35.91	1.0003									
1234	31.15	0.8677	12389	36.21	1.0086									
1236	31.25	0.8705												
1237	31.47	0.8766												
1238	31.50	0.8774												
1239	31.51	0.8777												
2378	31.79	0.8855												
1278	31.90	0.8883												
1267	31.90	0.8886												
1289	32.27	0.8989												

Blue boxes represent coelutions

Red boxes represent coelutions with 2378-substituted congeners

Bold red text indicates congeners with 2378 substitution

Number Sequence for Polychlorinated Dibenzo Dioxins



RRTs were calculated versus 12378 ^{13}C-labeled dioxin.

Table II Retention times (RT) and relative retention times (RRT) for all tetra- through octachlorinated furans on an Rtx®-Dioxin2 column.

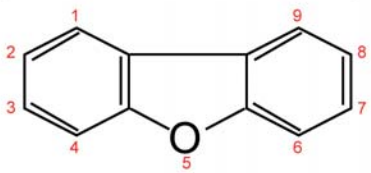
tetrachlorinated			pentachlorinated			hexachlorinated			heptachlorinated			octachlorinated		
Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)
1368	28.29	0.8181	13468	32.38	0.9364	123468	37.23	1.0766	1234678	41.99	1.2143	12346789	47.07	1.3604
1468	28.52	0.8243	12468	32.44	0.9378	134678	37.38	1.0807	1234679	42.36	1.2243			
2468	29.03	0.8393	13678	33.53	0.9694	124678	37.40	1.0812	1234689	42.60	1.2319			
1346	29.03	0.8393	13467	33.58	0.9705	134679	37.62	1.0873	1234789	43.92	1.2697			
1246	29.11	0.8413	12467	33.61	0.9717	124679	37.83	1.0876						
1378	29.15	0.8427	14678	33.70	0.9717	124689	38.08	1.1009						
1347	29.19	0.8441	13478	33.69	0.9743	123467	38.45	1.1116						
1247	29.26	0.8459	12368	33.71	0.9746	123478	38.58	1.1154						
1348	29.27	0.8459	12478	33.76	0.9760	123678	38.70	1.1191						
1248	29.35	0.8485	13479	33.85	0.9783	123479	38.86	1.1234						
1379	29.40	0.8497	13469	34.00	0.9829	123469	38.96	1.1263						
1367	29.42	0.8503	12479	34.09	0.9858	123679	39.14	1.1315						
1268	29.56	0.8546	12346	34.14	0.9870	123689	39.40	1.1387						
1467	29.64	0.8569	12469	34.25	0.9902	234678	39.42	1.1400						
1478	29.76	0.8604	23468	34.35	0.9928	123489	40.29	1.1651						
1369	29.97	0.8664	12347	34.36	0.9931	123789	40.31	1.1654						
1237	30.03	0.8684	12348	34.39	0.9945									
1678	30.10	0.8702	12378	34.61	1.0006									
2467	30.14	0.8714	12678	34.85	1.0075									
1234	30.16	0.8719	12367	34.86	1.0075									
1238	30.18	0.8725	12379	34.99	1.0116									
1469	30.19	0.8725	12679	35.27	1.0197									
1236	30.27	0.8754	23467	35.48	1.0257									
2368	30.35	0.8772	12369	35.51	1.0266									
1278	30.45	0.8803	12489	35.56	1.0277									
1349	30.48	0.8812	23478	35.68	1.0318									
1267	30.66	0.8864	12349	35.74	1.0335									
1249	30.78	0.8864	12389	36.47	1.0544									
2346	30.83	0.8910												
1279	30.89	0.8930												
2347	31.03	0.8968												
2348	31.10	0.8991												
2378	31.22	0.9028												
3467	31.33	0.9058												
2367	31.41	0.9081												
1269	31.44	0.9089												
1239	31.61	0.9141												
1289*	32.43	0.9376												

Blue boxes represent coelutions

Red boxes represent coelutions with 2378-substituted congeners

Bold red text indicates congeners with 2378 substitution

Number Sequence for Polychlorinated Dibenzo Furans



RRTs were calculated versus 12378 ^{13}C-labeled furan.

*Note that the 1289 tetra chlorinated congener elutes after the 13468 penta chlorinated congener.

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Assure LC/MS/MS System Performance for Drug Analyses

Using a System Suitability Test Mix

By Kristi Sellers, Clinical/Forensic Innovations Chemist and Houssain El Aribi, Ph.D., LC/MS Product Specialist, MDS Sciex

- Increase sample throughput and data quality with easy, reliable verification of LC/MS/MS performance.
- Extensively documented standard preparation assures accurate, consistent solutions.
- Method included in Cliquid® Drug Screen & Quant Software—automatically generates test reports.

Sample throughput is a critical issue in drug toxicology, and it can be adversely affected by inferior system performance. Poor system performance can produce unreliable data, increase downtime, and necessitate sample reanalysis, which ultimately decreases sample throughput. To ensure that your LC/MS/MS system is running properly, a system suitability mix should be analyzed on a regular basis before case samples are analyzed.

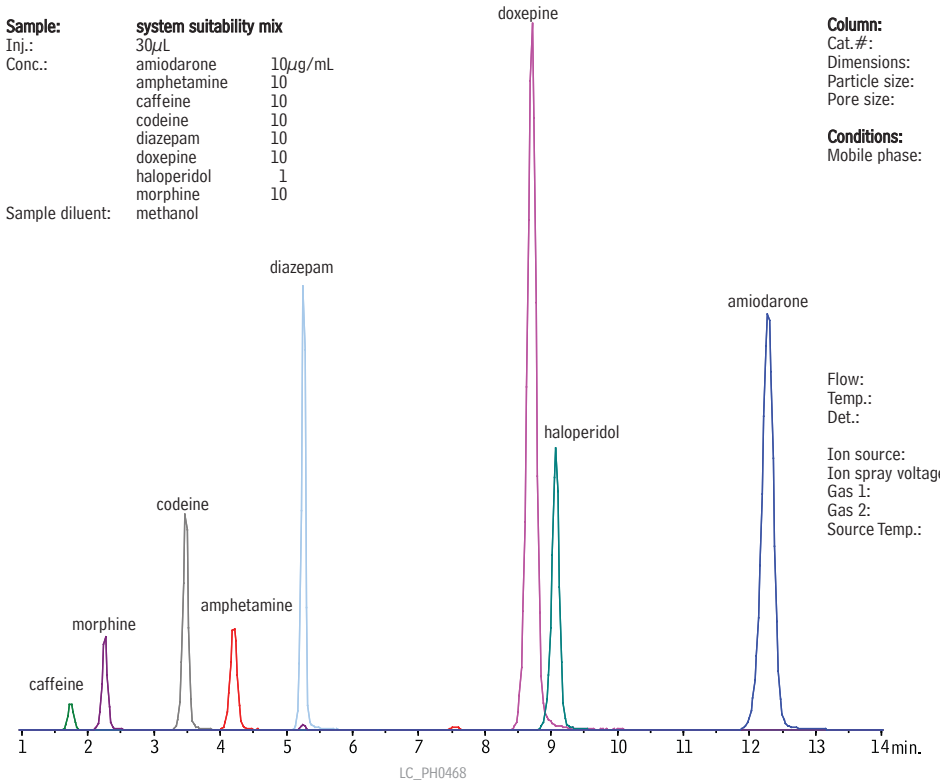
Restek and Applied Biosystems have developed a system suitability mix specifically for drug testing that contains compounds covering a wide range of molecular weights, polarities, and retention times (Table I). This standards mix is designed to verify system performance and identify system problems. Figure 1 shows a representative chromatogram (+MRM transitions) of this suitability mix analyzed on an Applied Biosystems API 3200™ LC/MS/MS system. This simple test evaluates the entire analytical system, including the autosampler, column, HPLC pumps, and mass spectrometer. The data is automatically compared to expected results by Applied Biosystem's Cliquid® Drug Screen & Quant Software to identify system problems.

Table I Mix components vary in chemical properties, providing a rigorous system performance test.

Mass Spectrometer Conditions:

Analyte	MW	RT (min)	Q1	Q3
Amiodarone	645	12.30	646.0	58.0
Amphetamine	135	4.21	136.1	91.1
Caffeine	194	1.72	195.1	122.9
Codeine	299	3.47	300.2	165.2
Diazepam	284	5.25	285.1	193.2
Doxepin	279	8.72	280.2	107.1
Haloperidol	375	9.08	376.1	123.0
Morphine	285	2.24	286.1	165.1

Figure 1 Increase sample throughput by verifying system readiness with a drug standard system suitability mix. (MRM transitions)



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The Cliquid® Drug Screen & Quant Software automates this test and generates a verification report which highlights failures. Peak area, peak shape, retention time reproducibility, fragmentation, and library search function all are evaluated through the software by comparing the test mix data to expected results. For example, full scan linear ion trap MS/MS data for diazepam and caffeine are compared to the library to assess fragmentation. A mass spectral match of 80% or more must be achieved to pass this portion of the system suitability test. Otherwise, the failure will be highlighted on the automated report.

Use this system suitability mix for drug analyses to assure system performance and simplify troubleshooting.

Analyzing this system suitability mix for drug analysis on a regular basis assures system performance, improves data quality, increases sample throughput, and simplifies troubleshooting. Moreover, the Cliquid® Drug Screen & Quant Software for Routine Forensic Toxicology enables nonexpert LC/MS/MS users to employ this system suitability test with little effort.

Acknowledgement
Method and data supplied by Applied Biosystems.

References

H. El Arbi, T. Sasaki, A. Schreiber, K. Sellers, K. Herwehe. Development of an LC/MS/MS System Suitability Test for Forensic Toxicology Applications. Applied Biosystems/MDS Sciex, 2007.

Allure® PFP Propyl Columns (USP L43)
Excellent Columns for LC/MS and ELSD

Physical Characteristics:

particle size: 5µm, spherical
pore size: 60Å
carbon load: 17%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

5µm Column, 2.1mm	cat. #
30mm	9169532
50mm	9169552
5µm Column, 3.2mm	**cat. #**
30mm	9169533
50mm	9169553
5µm Column, 2.1mm	**cat. #**
30mm (with Trident Inlet Fitting)	9169532-700
50mm (with Trident Inlet Fitting)	9169552-700
5µm Column, 3.2mm	**cat. #**
30mm (with Trident Inlet Fitting)	9169533-700
50mm (with Trident Inlet Fitting)	9169553-700

Allure® PFP Propyl Guard Cartridges	qty.	cat. #
10 x 2.1mm	3-pk.	916950212
10 x 4.0mm	3-pk.	916950210
20 x 2.1mm	2-pk.	916950222
20 x 4.0mm	2-pk.	916950220

ordering note

For other dimensions of these columns, visit our website at **www.restek.com**.

ABI/SCIEX Cliquid® Drug Screen Mix

Forensic Drug Screen Test Mixture

amiodarone	10µg/mL	diazepam	10
amphetamine	10	doxepine	10
caffeine	10	haloperidol	1
codeine	10	morphine	10

In P&T methanol, 1mL/ampul
cat. # 36340

Forensic Drug Screen Internal Standard

D5-diazepam D5-doxepine

10µg/mL each in P&T methanol, 10mL/ampul
cat. # 36341

Trident Direct Guard Cartridge System

Easy to Use, Low Dead Volume—The Ultimate Combination of Convenience and Column Protection

Trident Direct 20mm guard cartridge holder with filter
Protection against particulate matter and maximum protection against irreversibly adsorbed compounds.

Trident Direct 10mm guard cartridge holder with filter
Protection against particulate matter and moderate protection against irreversibly adsorbed compounds.

Description	qty.	cat.#
10mm guard cartridge holder with filter	ea.	25084
20mm guard cartridge holder with filter	ea.	25086
Connection tip for Waters-style end fittings	ea.	25088
PEEK tip standard fittings	ea.	25087

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Separating NSAIDs through Aromatic Selectivity

Improve Retention by Using An Allure® Biphenyl HPLC Column

By Rick Lake, Pharmaceutical Innovations Chemist, and Benjamin Smith, Applications Technician

- Optimize retention and selectivity of non-steroidal anti-inflammatory drugs, for better separations.
- Orthogonal separations with simple mobile phase changes
- Increased retention requires higher organic content, increasing desolvation efficiency in LC/MS.

Non-steroidal anti-inflammatory drugs (NSAIDs), in either prescribed or over-the-counter formulations, are widely used to treat pain, fever, and inflammation. While steroidal anti-inflammatory drugs all share a similar, four-ring chemical structure, NSAIDs have more diverse chemical structures, complicating their analysis. The work we report here is based on three common classes of NSAIDs: arylalkanoic acids, 2-arylpropionic acids (profens), and oxicams.

NSAIDs have a high carbon to heteroatom ratio and, therefore, historically have been separated through reversed phase HPLC on C18 columns. A conventional C18 stationary phase separates compounds based mainly on their overall hydrophobicity. Considering the carbon to heteroatom ratio, this is an effective separation mechanism for NSAIDs. Newer stationary phases are available, however, and we set out to determine if other phases, using other separation mechanisms, such as π-π interactions, could be more effective for assaying NSAIDs.

When selecting a stationary phase, it is advantageous to exploit inherent differences in the target analytes' chemical structures. Among these three classes of NSAIDS, there are some common functional groups, like halogens, amines, and carboxylic acids, but no one group is shared across the entire list of analytes (Figure 1). However, all of the target analytes do share one basic structural component – the six-carbon aromatic ring. Aromatic rings are common components of drug molecules, and they can be targeted using a phenyl-based stationary phase.

As a retention mechanism, phenyl stationary phases employ π-π interactions between the phenyl groups in the stationary phase and any unsaturated bonds in the analyte. The use of conventional phenyl phases has been somewhat limited due to their moderate retention capacity, relative to that of a C18 phase. Figure 2 illustrates the relative retention capacities of NSAID test probes on an Allure® Biphenyl column, a conventional phenyl column and a C18 column. Note that, in all cases, as commonly seen in practice, the conventional phenyl phase yields only moderate retention compared to that of a C18 column. However, the Allure® Biphenyl phase, which is a stationary

Figure 1 Aromatic rings make NSAIDs candidates for separation through π-π interactions.

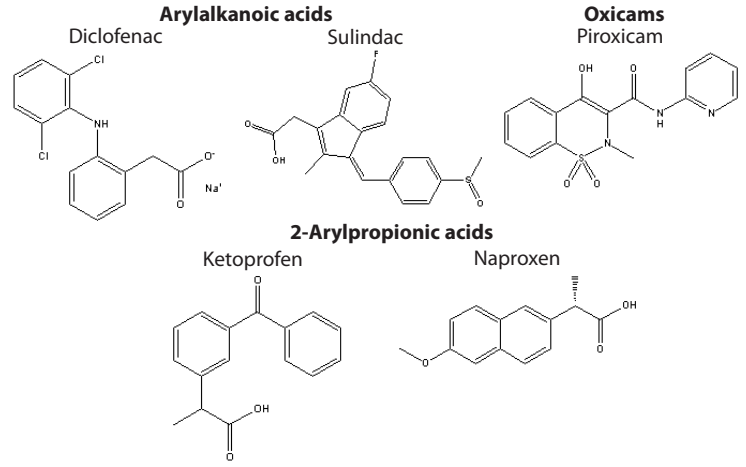
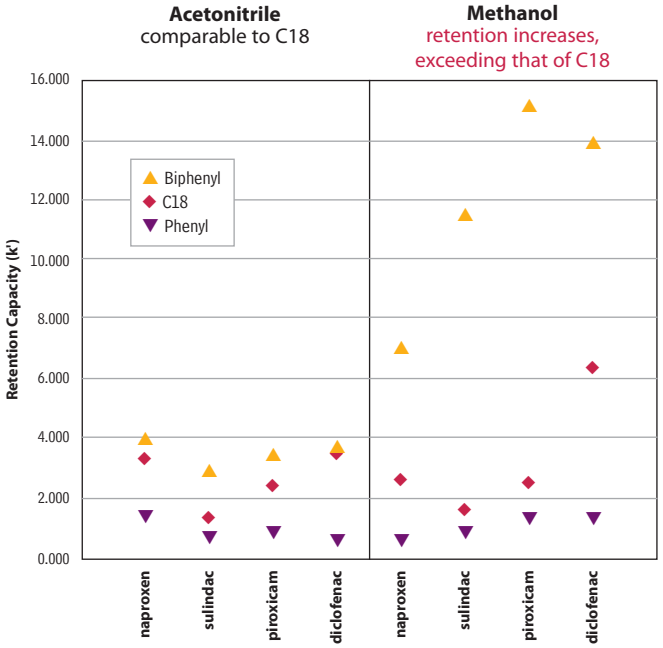


Figure 2 The retention capacity of the Allure® Biphenyl phase far exceeds that of conventional phenyl phases.



For each analyte all columns were assayed under identical isocratic conditions. The equivalent elutropic strength between acetonitrile and methanol was determined by the relative retention capacities of the C18 phase.

Columns:	5μm, 4.6mm x 150mm
Mobile Phase:	10mM potassium phosphate (pH 2.5): acetonitrile or methanol
Det.:	UV @ 254nm
Flow:	1.0 mL/min.

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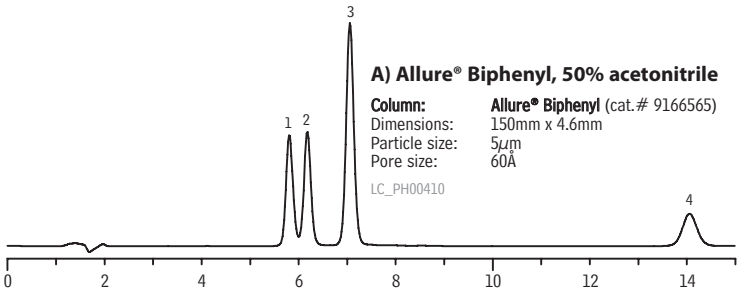
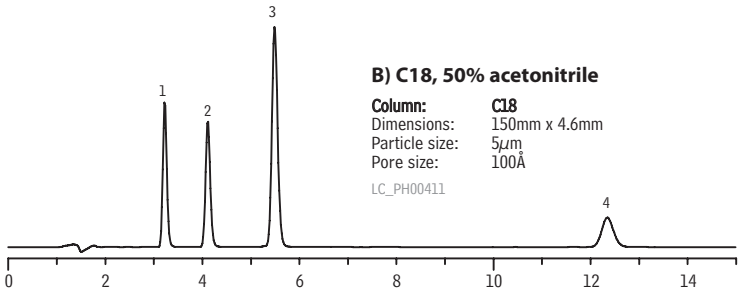
Figure 3 The versatility of the Allure® Biphenyl phase makes it a great alternative to conventional phenyl phase columns, especially in method development.

Sample:
Inj.: 5µL
Conc.: ~300µg/mL each component
Sample diluent: mobile phase

1. sulindac
2. piroxicam
3. ketoprofen
4. diclofenac

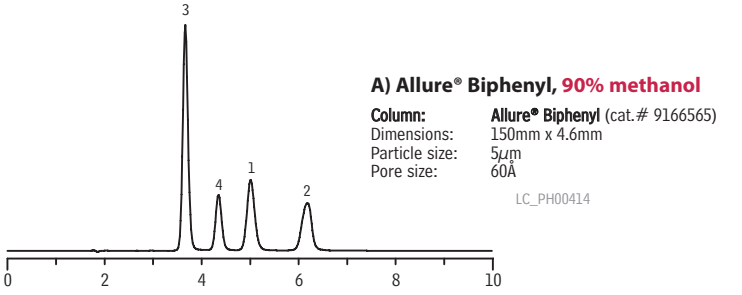
In acetonitrile, retention of NSAIDs on an Allure® Biphenyl column is comparable to retention on a C18 column and elution order is the same.

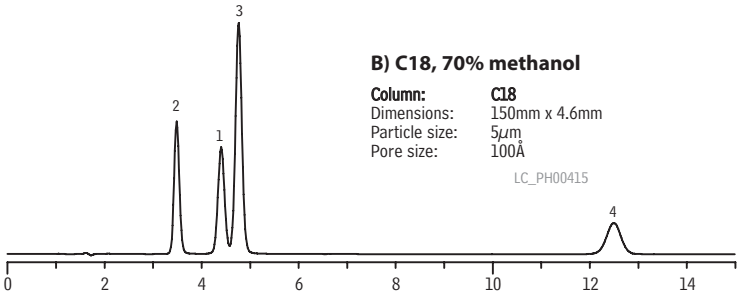




Conditions:
Mobile phase: 0.5% formic acid in water (pH 2.25):0.1% formic acid in acetonitrile, 50:50 (v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

In methanol, retention capacity & selectivity of NSAIDs are much greater on an Allure® Biphenyl column, compared to a C18 column, and elution order changes.





Conditions:
Mobile phase: 0.5% formic acid in water (pH 2.25):0.1% formic acid in methanol, 30:70 or 10:90 (v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

phase composed of two phenyl groups bonded end-to-end, easily achieves retention capacities similar to, and even greater than, those of a C18 column when used with a highly organic mobile phase. For this reason, we evaluated the enhanced retention of the Allure® Biphenyl column for assaying NSAIDs through aromatic selectivity.

First, we compared the retention characteristics of a conventional C18 column and an Allure® Biphenyl column, using acetonitrile as the organic modifier. As expected, the Allure® Biphenyl column exhibited similar retention under equivalent analytical conditions (Figure 3). But, when we assayed the same analytes, using methanol as the organic modifier, we found retention on the Allure® Biphenyl column was *greatly increased*. To maintain the same retention capacities (k') between the columns, we had to increase the organic content by 20% (Figure 3). In addition, selectivity between the two columns became dramatically different. Based on these results, we conclude that methanol in the mobile phase enhances π-π interactions between aromatic compounds and the biphenyl stationary phase, leading to greater retention and superior selectivity.

An Allure® Biphenyl column, in combination with a methanol-containing mobile phase, significantly improves separations of NSAIDs, or other aromatic drug compounds. Increased retention capacity creates a need for a higher percentage of organic solvent in the mobile phase, to elute the analytes in a timely manner. Increasing the organic content, in turn, increases sensitivity in LC/MS methods, because it optimizes the desolvation efficiency in electrospray interfaces. And this, in turn, makes an Allure® Biphenyl column the best choice for separating aromatics.

Allure® Biphenyl Columns (USP L11)

Physical Characteristics:

particle size: 5µm, spherical
pore size: 60Å
carbon load: 23%
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

5µm Column, 4.6mm	cat. #
150mm	9166565

For other dimensions of these columns, visit our website at **www.restek.com**

Allure® Guard Cartridges

Allure Biphenyl	qty.	cat. #
10 x 2.1mm	3-pk.	916650212
10 x 4.0mm	3-pk.	916650210
20 x 2.1mm	2-pk.	916650222

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Easily Resolve Oxytocin PEGylation Reaction Products

Using Viva Wide Pore HPLC Columns

Julie Kowalski, Ph.D., Bioanalytical Innovations Chemist

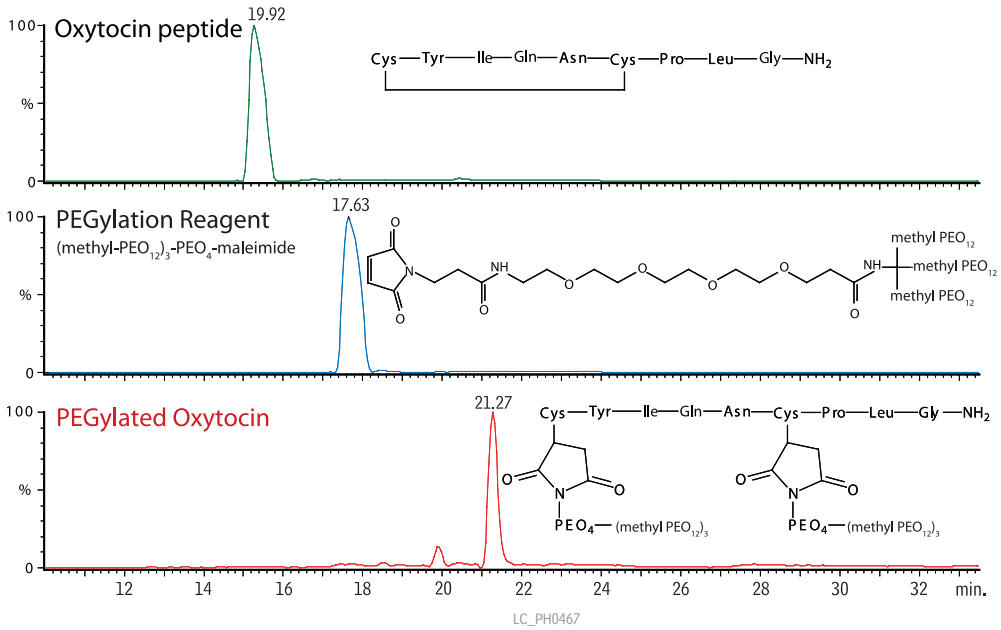
- Ideal for PEGylation reaction monitoring.
- Easy isocratic method saves time, eliminating column equilibration time between injections.
- Largest available surface area in 250-350Å pores; engineered for proteins, peptides, and other large biomolecules.

PEGylation, the covalent attachment of polyethylene glycol (PEG) units to proteins and peptides, is an important tool in drug discovery. PEGylation is used to enhance drug delivery, while maintaining the therapeutic function of the active compound. Viva Wide Pore HPLC columns are ideal for the separation of large molecules, such as oxytocin PEGylation reaction products, as the target analytes can enter the larger pores and access more surface area, increasing retention and overall resolution. For analytes with molecular weights larger than 3,000, pore diameters of 250-350Å offer the best combination of retention and pressure stability, and Viva Wide Pore silica has the greatest available surface area in 250-350Å pores. Here we demonstrate the suitability of Viva Wide Pore HPLC columns for PEGylation reaction monitoring.

Viva columns reliably separate large, closely related compounds.

The PEGylation reaction mixture consisted of oxytocin with an excess of reducing agent tris(2-carboxyethyl)phosphine (TCEP) and (methyl-PEO_{12})$_3$-PEO_4-maleimide. The oxytocin solution was mixed with ammonium bicarbonate buffer to pH 8. Excess TCEP was added and the resulting solution incubated at 60°C for 1 hour. The test solution was cooled to room temperature and a molar excess of (methyl-PEO_{12})$_3$-PEO_4-maleimide was added, followed by incubation in a water bath at 40°C for 1 hour. Approximately 6 nmoles of oxytocin was injected in 20µL of deionized water with 0.1% formic acid. The extracted ion chromatograms in Figure 1 show excellent resolution for the three compounds of interest. The added retention power of Viva columns allows separation of large, closely related compounds, making it an ideal column for monitoring PEGylation reactions.

Figure 1 Excellent resolution of oxytocin PEGylation reaction products on Viva Wide Pore HPLC columns.



Conditions:

Mobile phase:	0.1% formic acid in water:0.1%formic acid in acetonitrile, 60:40
Flow:	0.1mL/min.
Temp.:	ambient
Det.:	Micromass Quattro II
Interface:	ESI
Ion mode:	positive
Temp.:	200°C
Capillary:	2.25kV
Cone:	40V

Sample:	**oxytocin PEGylation reaction products**
Inj.:	20µL
Conc.:	300pmoles/µL
Sample diluent:	0.1% formic acid in water (v:v)

Column:	**Viva Wide Pore C18**
Cat.#:	9514561
Dimensions:	150mm x 1.0mm
Particle size:	5µm
Pore size:	300Å

Viva C18 Columns (USP L1)

5µm Column, 1.0mm	cat. #
150mm	9514561

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Rapid Screening Method for Carbamates in Orange Oil

Using an Ultra Carbamate HPLC Column

Julie Kowalski, Ph.D., Innovations Chemist

- Fast analysis times, for increased sample throughput.
- Simple methodology saves time—no sample preparation.
- Accurate mass identification, for definitive results.

Concern over the presence of pesticides in food products, particularly citrus, is growing, resulting in an increasing number of countries regulating insecticides such as carbamates. EPA Method 531.1 describes a method for the analysis of carbamates in water, but not in other commodities. Matrices like citrus oil contain numerous interferences and often require time-consuming sample preparation. However, the method described here requires no sample preparation and provides fast analysis times, significantly increasing sample throughput.

Carbamates are most easily determined via HPLC analysis because derivatization is required for GC analysis. The rapid screening method shown here uses the Ultra Carbamate HPLC column, which is designed specifically for analyzing carbamates and is compatible with both traditional detectors and mass spectrometry. This column works well with mass spectrometry amenable buffers and allows an initial mobile phase composition of 20% organic, which promotes complete ionization at the electrospray source.

Orange oil was spiked at 10ppm with a carbamate mix and analyzed (Figures 1-2). The monoisotopic masses and retention times were compared to an injected standard and found to match closely (Table I). The high mass accuracy of the Leco Unique TOF-MS allowed positive analyte identification, even in a complex mixture containing compounds with the same nominal mass (within 1 amu) as the target carbamate. By using the Ultra Carbamate column in conjunction with the Leco Unique TOF-MS, we were able to develop a quick, easy, and accurate screening method for carbamates in a complex matrix such as orange oil.

References:

B. Mayer-Helm, L. Hofbauer, J. Muller. Rapid Communications in Mass Spectrometry, 20 (2006), page 529-536

Ultra Carbamate Column

3µm Column, 2.1mm	cat. #
50mm	9177352

Figure 1 Reference standard carbamates resolve quickly on an Ultra Carbamate HPLC column. (extracted ion chromatograms)

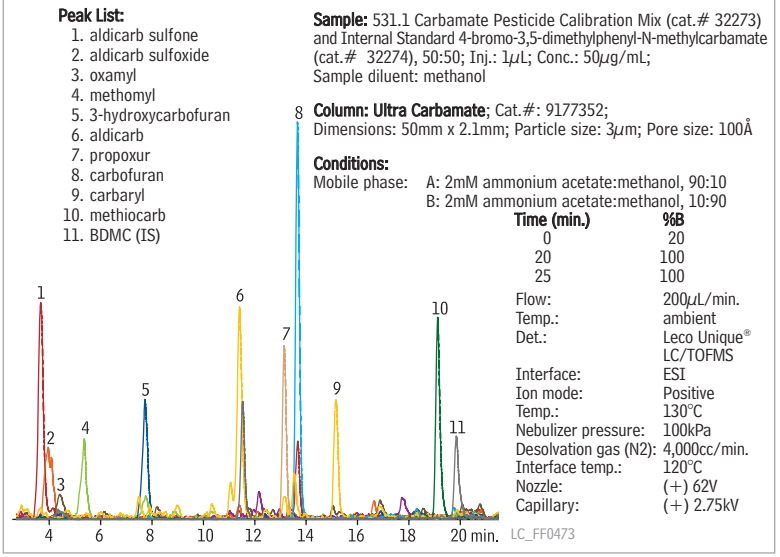

Figure 2 Positive identification of carbamates in orange oil injected with no sample preparation. (extracted ion chromatograms)

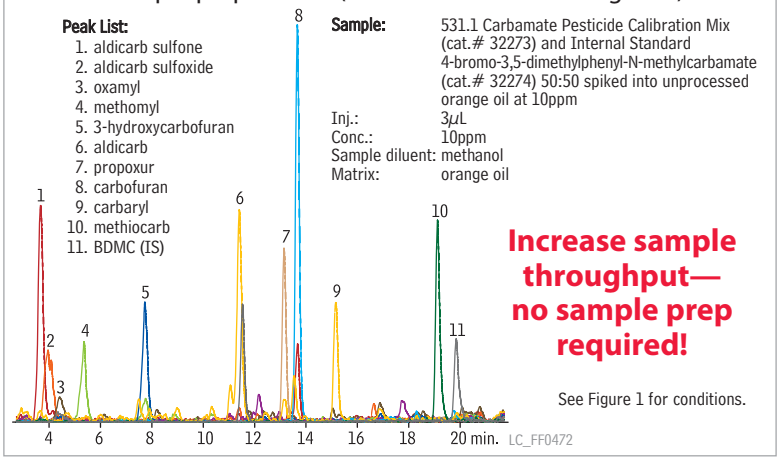

Table I Carbamates were positively identified in matrix using both retention time and mass.

		calculated ion monoisotopic mass	standard ion monoisotopic mass	standard retention time (min.)	orange oil ion monoisotopic mass	orange oil retention time (min.)
aldicarb sulfone	[M+H]+	223.075	223.099	3.81	223.142	3.67
aldicarb sulfoxide	[M+H]+	207.080	207.103	4.31	207.122	4.09
oxamyl	[M+NH4]+	237.102	237.085	4.97	237.110	4.41
methomyl	[M+H]+	163.054	163.074	5.84	163.086	5.36
3-hydroxycarbofuran	[M+H]+	238.108	238.121	8.32	238.128	7.73
aldicarb	[M+H]+	191.085	191.0728 116.0751*	11.92	116.052*	11.53
propoxur	[M+H]+	210.113	210.152	13.53	210.153	13.14
carbofuran	[M+H]+	222.113	222.140	13.98	222.120	13.66
carbaryl	[M+H]+	202.087	202.084	15.48	202.101	15.17
methiocarb	[M+H]+	226.090	226.097	19.22	226.060	19.12
BDMC	[M+H]+	258.013	258.042	19.89	258.005	19.84

* m/z 116.052 is a fragment ion with higher intensity than the [M+H]+ ion and was used for identification in orange oil

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Using Thermal Desorption to Enhance Aroma Profiling by GC/MS

Lower Detection Limits with Latest Technology

By Irene DeGraff, Product Marketing Manager, Lara Kelly, Markes International, and Liz Woolfenden, Markes International

- Accommodates a wide range of sampling methods.
- Allows sample re-collection, for repeat analysis and result verification.
- Eliminates extraction solvents, purges volatile interferences, and concentrates sample vapors, for enhanced low-level detection.

Flavor and fragrance profiling by GC/MS presents significant analytical challenges, as profiles typically comprise hundreds of volatile organic compounds (VOCs), often with the lowest concentration analytes having the most profound effects on perceived aroma. Conventional sample preparation methods (solvent extraction, steam distillation, etc.) do not meet sensitivity requirements and often distort the vapor profile so that it is not representative of what the consumer experiences. Recently, thermal desorption (TD) has emerged as a useful complement to GC/MS, enabling more aroma profiling applications to be carried out using quantitative, automatic instrumentation. TD combines automated sample preparation with selective analyte enrichment, allowing VOCs to be injected into the GC/MS as a narrow concentrated band, free of most or all sample matrix effects.

Many Sampling Options, No Extraction Interferences

One of the strengths of thermal desorption for food, flavor, and fragrance profiling is that it offers a versatile range of sampling methodologies including sorbent tubes/traps, on-line sampling, direct desorption, and off-line thermal extraction (dynamic headspace) sampling. Whichever of these approaches is used, the compounds of interest are separated from the sample matrix and focused on a small, electrically-cooled sorbent trap (Figure 1). This focusing trap is subsequently desorbed by heating it rapidly in a reverse flow of carrier gas causing the VOCs to be injected into the GC/MS system as a narrow band of vapor. Since samples are extracted directly

Thermal desorption is an automatic, high-sensitivity alternative to conventional liquid extraction.

Figure 1 Thermal desorption is compatible with a wide range of sampling strategies.

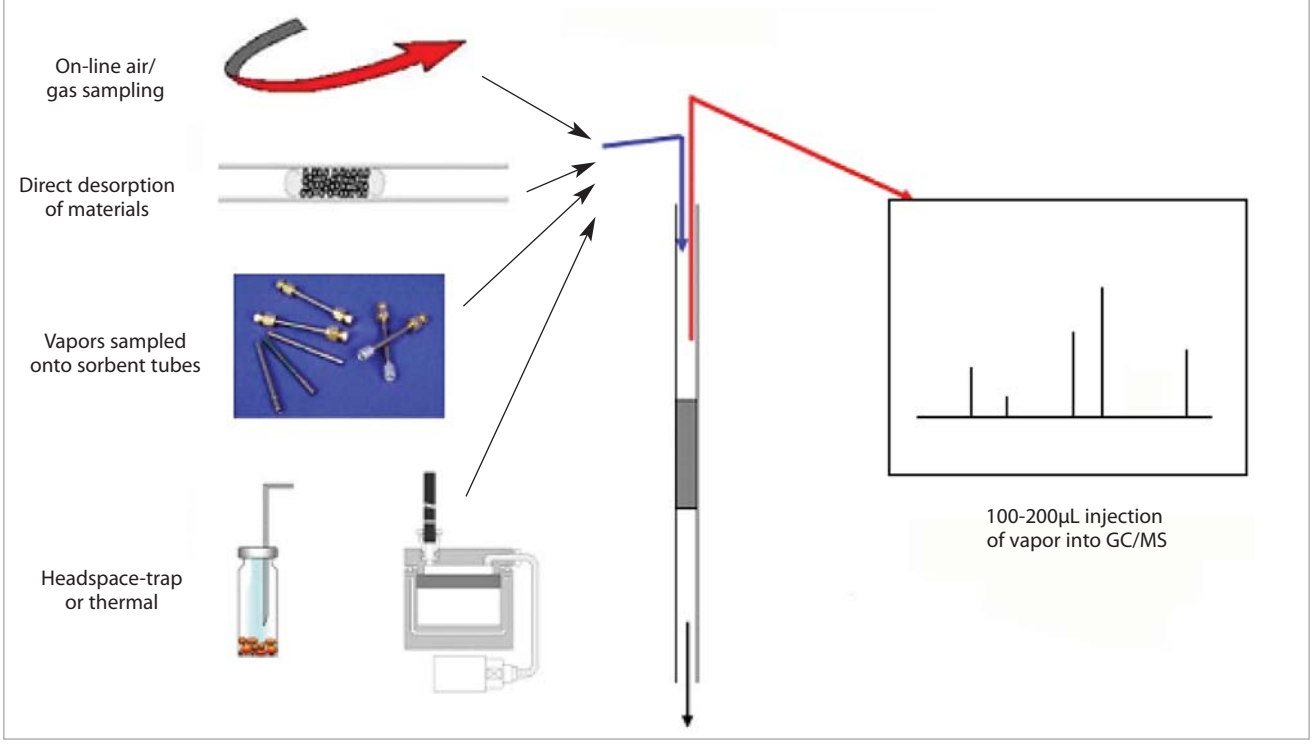

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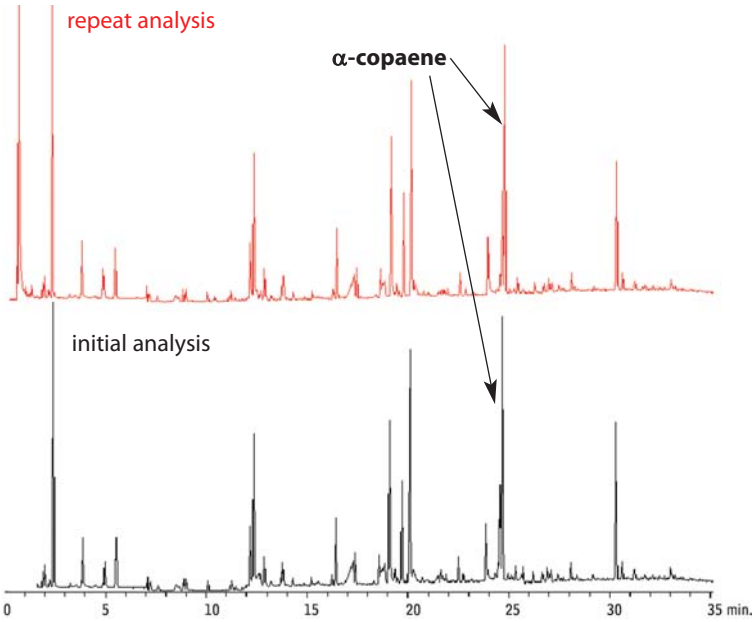
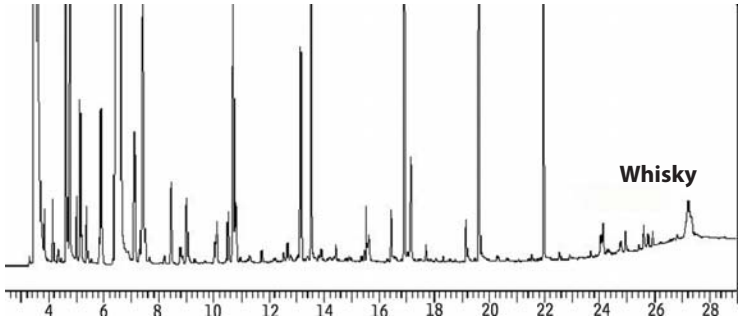
Figure 2 Verify initial results by analyzing re-collected samples.



Analysis of headspace collected above boiling, genetically-modified potatoes. Repeat analysis of the re-collected sample demonstrated excellent recovery of reactive monoterpenes, such as α-copaene.

Figure 3 Thermal desorption allows selective elimination of water and >99% of ethanol vapor, enhancing the determination of key olfactory components.



Analysis of whisky headspace by GC/FID.

Thermal Desorption Unit Tubes, Unconditioned

Fits Markes ULTRA-UNITY, PerkinElmer, and Shimadzu thermal desorbers.

		Unconditioned	
		Stainless Steel	Glass
Description	qty.	cat.#	cat.#
TDU Tubes, Tenax TA	10-pk.	24056	24062
TDU Tubes, Graphitized Carbon	10-pk.	24057	24063
TDU Tubes, Tenax GR/Carbopack B	10-pk.	24058	24064
TDU Tubes, Carbopack B/Carbosieve SIII	10-pk.	24059	24065
TDU Tubes, Tenax TA/Graphitized Carbon/Carboxen 1000	10-pk.	24060	24066
TDU Tubes, Carbopack C/Carbopack B/Carbosieve SIII	10-pk.	24061	24067

into the GC carrier gas stream, no manual sample preparation is required and the problems associated with solvents—masking of peaks of interest, loss of volatiles, and variable extraction efficiency—are eliminated.

Lower Detection Limits and Repeat Analysis

The latest TD systems use thin-walled quartz traps capable of heating at rates over 100°C/sec., maximizing desorption efficiency and lowering detection limits. They also incorporate split re-collection for repeat analysis and simple validation of recovery (Figure 2) through the analytical system. Newer thermal desorption systems are also capable of transferring the vapor profile constituents into the GC capillary column in volumes of carrier gas as low as 100µL. This means that significant concentration enhancement factors can be achieved—typically from 103 to 106—depending on the number of concentration/desorption steps. TD also allows volatile interferences such as water and ethanol to be purged to vent prior to analysis, making it easier to discriminate between samples according to the key olfactory components (Figure 3).

Summary

Thermal desorption offers an automatic, high-sensitivity alternative to conventional liquid extraction methods for aroma profiling by GC/MS. It allows vapor profile constituents to be cleanly separated from the sample matrix and facilitates selective purging of volatile interferences in many cases. This helps to ensure that the vapor profile analyzed is most representative of the aroma perceived by consumers and that key olfactory compounds can be identified and measured at the lowest levels possible.

free literature

Thermal Desorption: A Practical Applications Guide
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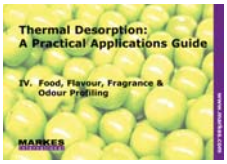
Technical Guide
lit. cat.# FFTG1037



Thermal Desorption Tube Sorbent	Applications
Tenax TA	Vapor phase organics from C6/7 to C26
Graphitized Carbon	Vapor phase organics from C5/6 to C14
Tenax GR/Carbopack B	Vapor phase organics from *n*-C5/6 to *n*-C20 (EPA Methods TO-14/TO-15/TO-17)
Carbopack B/Carbosieve SIII	Vapor phase organics from *n*-C2/3 to *n*-C12/14 (EPA Methods TO-14/TO-15/TO-17)
Tenax TA/Graphitized Carbon/Carboxen 1000	Vapor phase organics from C2/3 to C20
Carbopack C/Carbopack B/Carbosieve SIII	Vapor phase organics from *n*-C2/3 to *n*-C16/20 (EPA Methods TO-14/TO-15/TO-17)

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Tech Tip

Under Pressure?

Reduce System Stress by Backflushing Your HPLC Column

By Tim Herring, Technical Service

Experiencing a higher pump pressure than usual? Or perhaps a complete pressure shut-down of the system has occurred, even after replacing the in-line frit and guard column. High pump pressures can be caused by heavily retained impurities building up within the head of the analytical column. Such contamination can cause poor chromatography, usually in the form of broad, split, or misshapen peaks, and ultimately can compromise results. Backflushing a contaminated analytical column using the following procedure can help restore column performance and reduce pump pressure and system strain.

If back pressure is abnormally high, first take the column out of the equation by disconnecting it from the system altogether. Install a union and run the pumps to verify that the back pressure problem is due to the column, and not to the HPLC system. If the pressure is normal, then the column is most likely the cause of the high back pressure. To address this, reverse the column flow and rinse (backflush) the column to remove the contaminants from the inlet frit and column head. This will move the contaminants down the path of least resistance, instead of forcing them further into the analytical column. Reverse rinse into a waste beaker at low flow (e.g. 0.5mL/min. for a 4.6mm ID column) for 10-15 minutes initially, and then increase the flow to 1.5-2 times the optimal flow (1.5 to 2.0mL/min. for a 4.6mm ID column). Do not reconnect to the detector when backflushing the column. Rather, flush the waste stream into a beaker so that the detector cell is not contaminated by impurities or obstructed by particulate build-up.

Solubility is a key issue when backflushing columns, so remember the old adage, "like dissolves like". For example, if the contaminants are suspected to be oily or hydrophobic in nature, then backflush with a strong, nonpolar solvent such as hexane. If the contamination is polar (a salt for instance), then use a polar solvent, such as water or methanol. Solvent miscibility also needs to be considered, so be sure to use solvents that are miscible with one another. If in doubt, use isopropanol (IPA) as an intermediary solvent between solvent wash steps, as it is miscible with all common solvents. This is particularly true when switching from typical normal phase solvents (such as hexane) to reverse phase solvents (such methanol, acetonitrile, or water) and vice versa. Note that 10 to15 column volumes are generally necessary at each step to remove all traces of immiscible solvents prior to the next step.

If the contaminants are unknown, or vary in chemistry, a series of solvent washes will provide an array of differing chemical interactions and maximize the removal most types of contamination. The solvent order presented in Table I considers miscibility, polarity, and eluotropic strength and is a very effective series for removing most contaminants. Column backflushing, with proper solvent selection, is a simple way to regenerate analytical columns, improving column performance and reducing system stress.

Table I Restore column performance by backflushing with recommended solvent washes.

Reversed phase series:	Normal phase:
A. 1% glacial acetic acid in methanol and water (50:50)	A. isopropanol
B. methanol	
C. chloroform	
D. hexane (or heptane)	
E. methylene chloride (dichloromethane)	
F. methanol	

Contact Restek Technical Service

at support@restek.com or 800-356-1688 with questions on backflushing, or any other technical area. At Restek, we are here to help you!

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Quality Control in Metabolomics

Continued from page 2

process than the hard electron impact ionization in GC/MS. It is insufficient to declare that in LC/MS no major matrix effect is apparent with respect to ion suppression just based on quenching of signal intensity of a single infused compound. This single compound may have characteristics that make it less vulnerable to matrix effects, and thus unsuitable to explore matrix effects. Far better suited are classical approaches, most importantly the use of isotope labeled internal standards. Quality control in metabolomics means that the short-term and long-term influence of matrix effects is carefully evaluated by comparing the metabolite coverage and their relative quantification levels to expected values from background knowledge. Only if quantification of a range of well-known target metabolites validates a specific analytical protocol, can unbiased analysis be furthered to the level of metabolomics and comprise novel metabolite signals. Such integration of classical analytical strategies with modern unbiased data analysis should also include randomized sample sequences, blank controls, and bracketing samples with external calibration standards.

Among the most difficult challenges in metabolomics is the annotation of unknown metabolic signals. The Metabolomics Standards Initiative (MSI) has issued a variety of suggestions for reporting minimal experimental parameters to ensure that metabolomic data can be used and reproduced by other laboratories. Importantly, the identification of metabolites must always be based on at least two orthogonal physicochemical characteristics, such as retention index and mass spectrum. Identifications that are based on authentic chemical standards are generally more trustworthy than annotations based on calculated characteristics. Nevertheless, the metabolome itself is an unrestricted entity that clearly comprises more than the suite of known compounds to be found in classical textbooks or that can be purchased from chemical manufacturers. The metabolome cannot be simply computed from reconstructed biochemical pathways due to enzymatic diversity, substrate ambiguity, and variation in regulatory mechanisms. Hence, the finding of many unknown signals in metabolomic surveys comes as no surprise to biochemists. The sheer complexity of natural products, including isomeric compounds, renders the use of accurate masses and database queries insufficient for annotation of metabolites. Instead, novel algorithms are needed to score metabolic signals based on all available information, from calculated physicochemical characteristics to presence in biochemical databases. Such algorithms might ultimately boost the quality of metabolomic data in a similar way as SEQUEST® did for proteomic analysis. Yet, no software is available to perform this much-needed task.

Dr. Oliver Fiehn is a leading researcher in the field of metabolomics. He is a Professor in the Genome Center at the University of California, Davis. Dr. Fiehn's research focuses on developing and applying analytical and bioinformatic methods, primarily GC/MS and LC/MS, in order to unravel the changes in metabolic networks in sets of biological situations.

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Restek On-the-Road

Tradeshow Schedule

July, 2008

Show: **Florida Pesticide Residue Workshop (FPRW)**
Date: July 20-23
Location: TradeWinds Island Grand, St. Pete Beach, FL

Show: **18th IAFS Triennial Meeting (International Association of Forensic Sciences)**
Date: July 21-26
Location: New Orleans Marriott Hotel, New Orleans, LA

Show: **NSRA -- 39th Street Rod Nationals**
Date: July 31-Aug. 3
Location: Kentucky Expo Center, Louisville, KY

August, 2008

Show: **28th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2008)**
Date: Aug. 17-22
Location: ICC, Birmingham England UK

September, 2008

Show: **122nd AOAC International Annual Meeting & Exposition**
Date: Sep. 21-24
Location: Hyatt Regency Dallas, Dallas, TX

Show: **Northeastern Association of Forensic Scientists (NEAFS)**
Date: Sep. 30-Oct. 4
Location: Renaissance Westchester Hotel, White Plains, NY

Seminar Schedule

Date	Cat. #	City	State
Comprehensive HPLC			
7/22	65733	Linden	NJ
7/23	65734	Melville	NY
7/24	65735	Parsippany	NJ
GC/MS Training Seminar			
7/28	65736	Blue Ash	OH
7/29	65737	Lexington	KY
7/31	65738	Research Triangle Park	NC
Petrochemical Seminar			
9/8	65739	Seattle	WA
9/9	65740	Richmond	CA
9/11	65741	Long Beach	CA
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