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- Accurately monitor PEGylation reactions
- Quality control in metabolomics
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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



RESTEK

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.



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

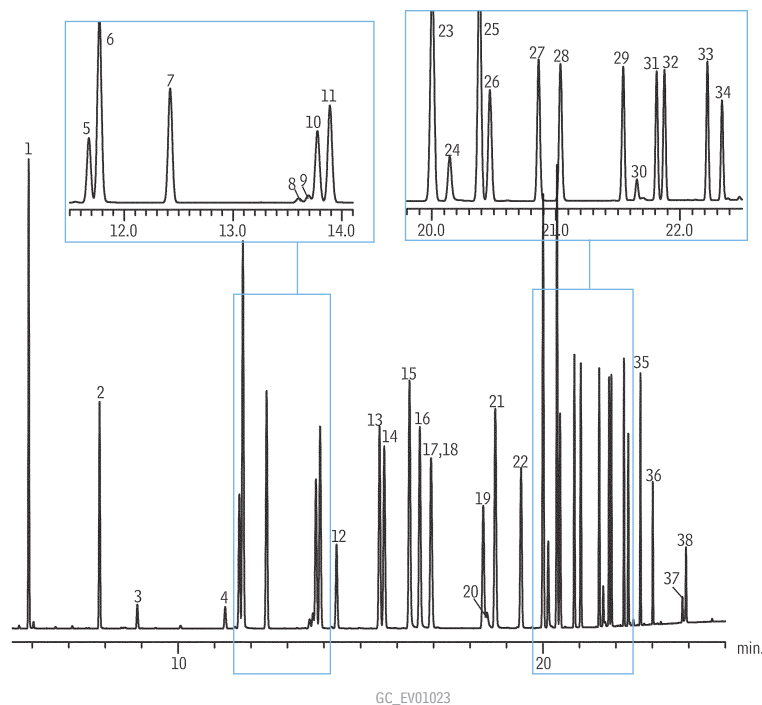
Oven temp.: 80°C (hold 0.5 min.) to 155°C (hold 1 min.) @ 19°C/min. to 210°C @ 4°C/min. to 310°C (hold 0.5 min.) @ 25°C/min.

Detector temp.: ECD @ 325°C

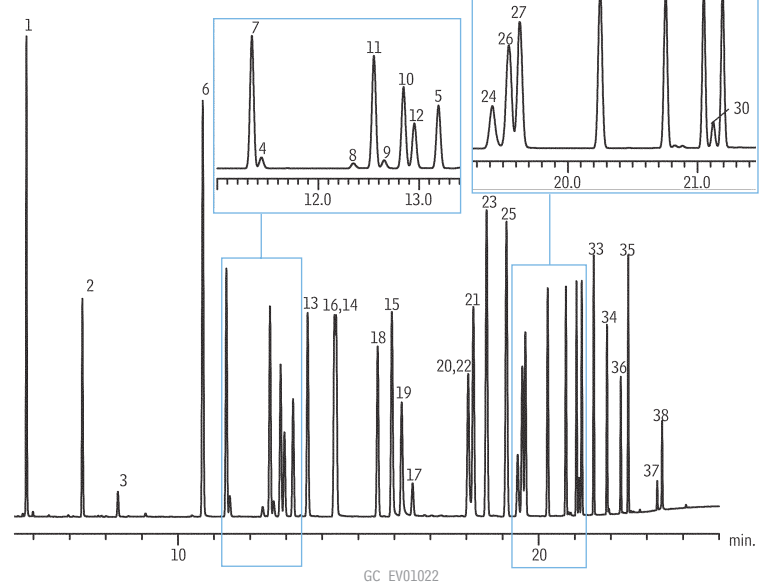
Figure 1 Resolve all critical pairs using Rtx®-CLPesticides and Rtx®-CLPesticides2 columns.

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

Rtx®-CLPesticides2



Rtx®-CLPesticides



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
 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
 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
 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 |



One Stop Shop for EPA Method 535

Reliably Analyze Acetamide Herbicide Degradates by LC/MS/MS

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.

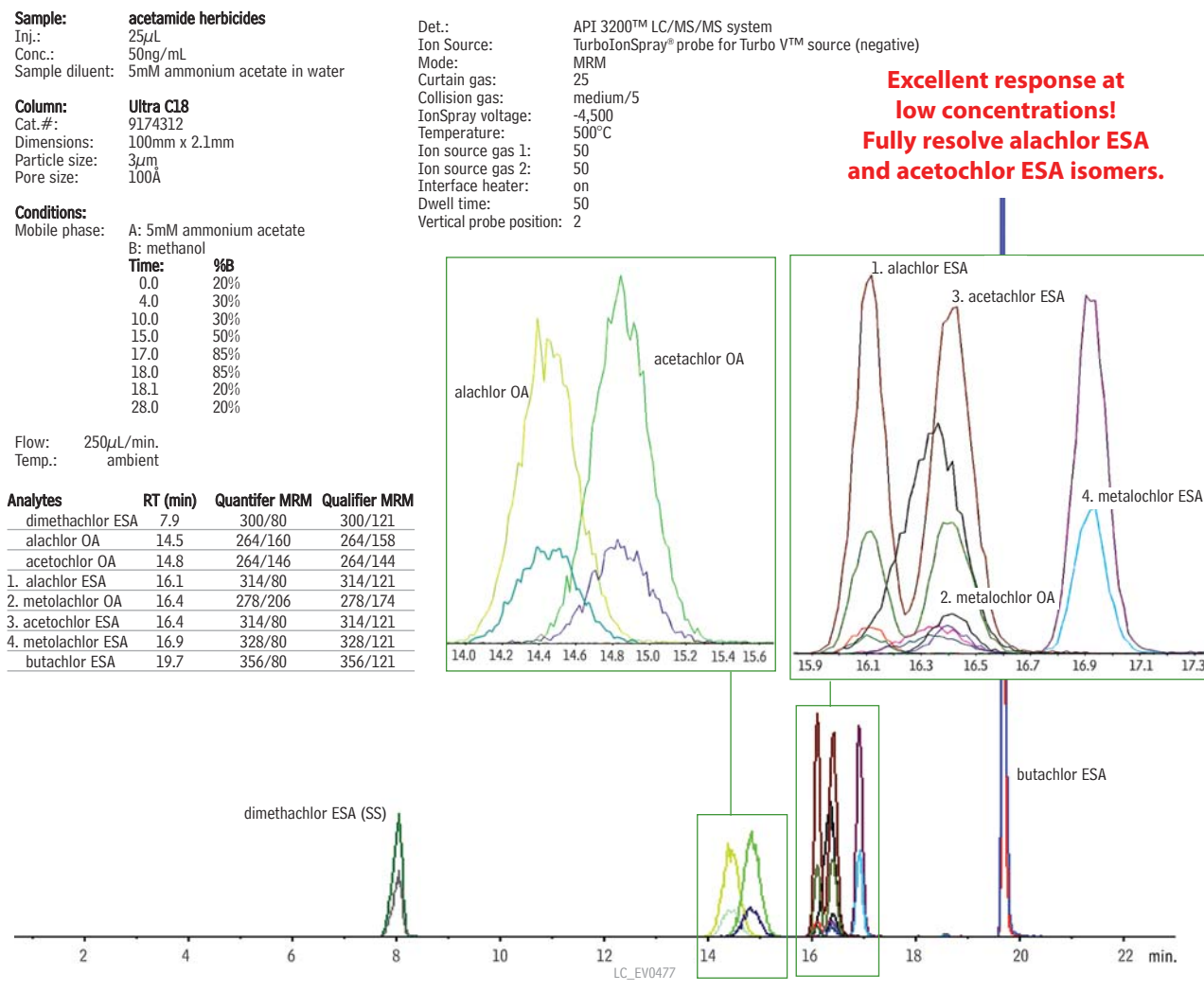


Table I Reliably achieve minimum detection limits of 0.004µg/L or less.

| 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 | endcap: fully endcapped |
| pore size: 100Å | pH range: 2.5 to 7.5 |
| carbon load: 20% | 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.





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!

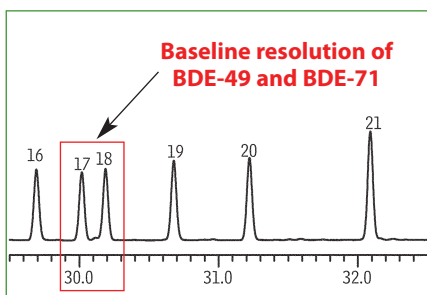
| Column | BDE-209 Average RRF* |
|-----------------|----------------------|
| Rtx®-1614 (15m) | 0.681 |
| Rtx®-1614 (30m) | 0.636 |
| DB-5HT (30m) | 0.502 |

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

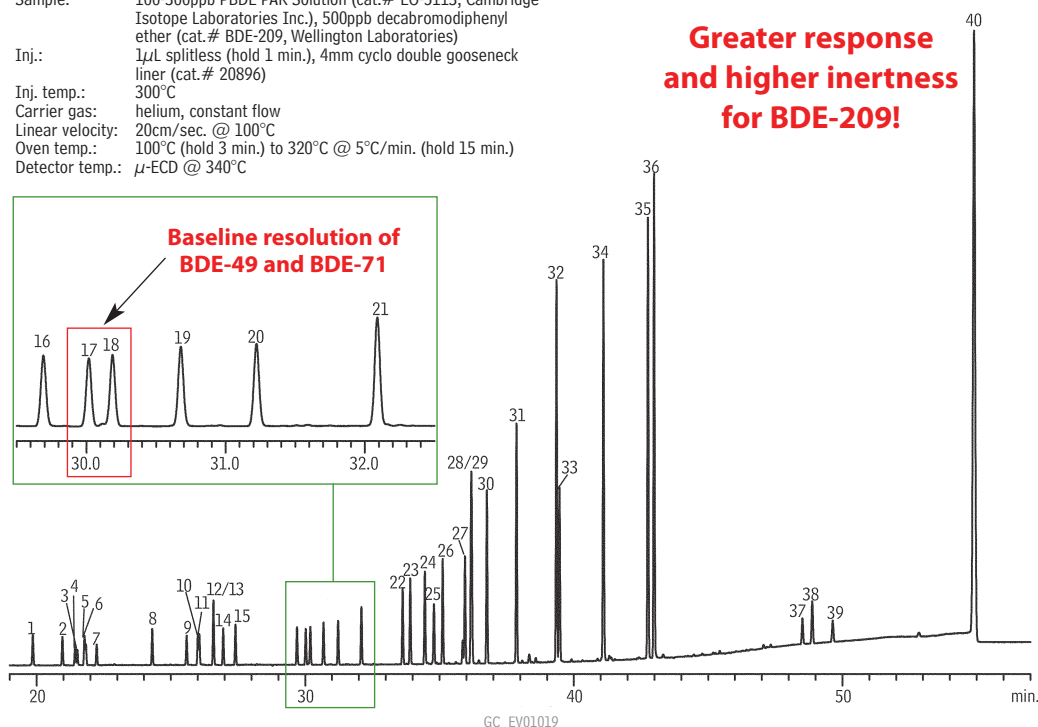
The Rtx®-1614 column meets the method requirements for the resolution of critical pairs, tailing factors, and retention. The data in Figure 1 demonstrate the separation of a large list of PBDEs on the Rtx®-1614 column; note the baseline resolution of congeners 49 and 71, which are required to have a 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



**Greater response
and higher inertness
for BDE-209!**



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

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

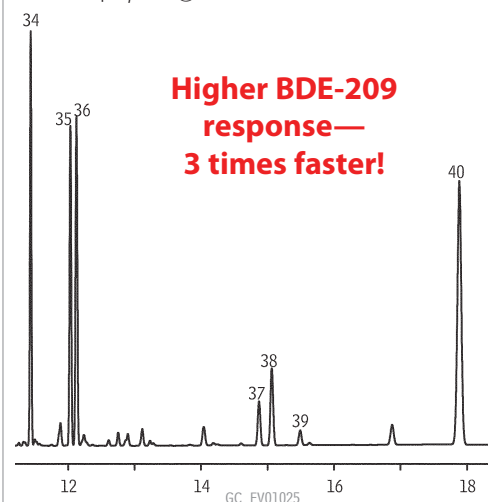
| ID* x OD & Length | qty. | cat.# |
|------------------------------|-------|-------|
| Cyclo Double Gooseneck (4mm) | | |
| 4.0mm x 6.5mm x 78.5mm | 5-pk. | 20896 |

*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

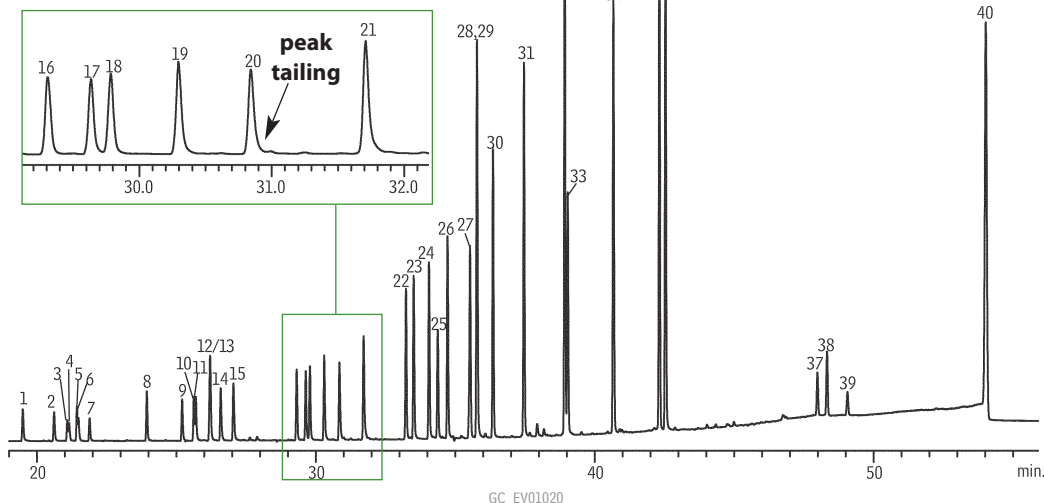


**Higher BDE-209 response—
3 times faster!**

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



**Lower response
than on Rtx®-1614**

See Figure 1 for peak list.



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.

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 (Figure 1). 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.

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

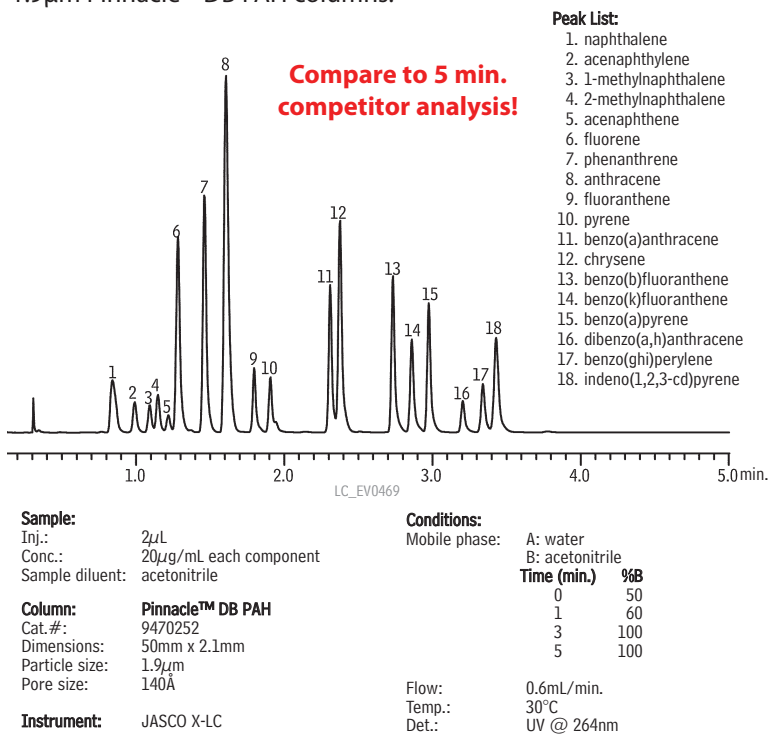
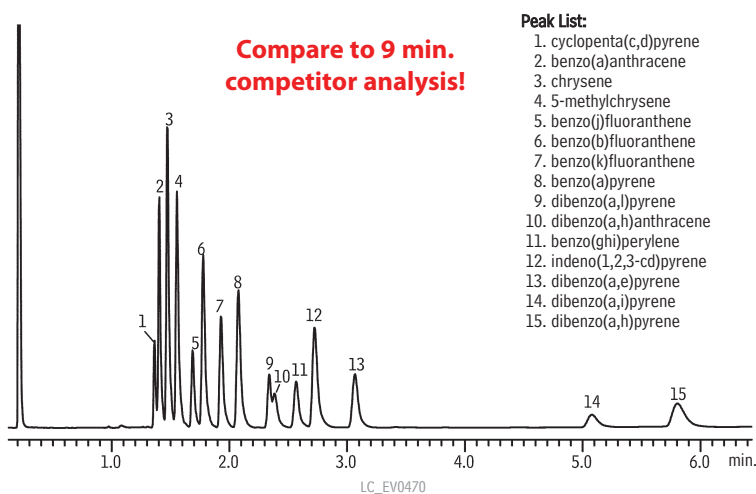


Figure 2 Excellent resolution of EU PAHs on 1.9µm Pinnacle™ DB PAH columns.



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

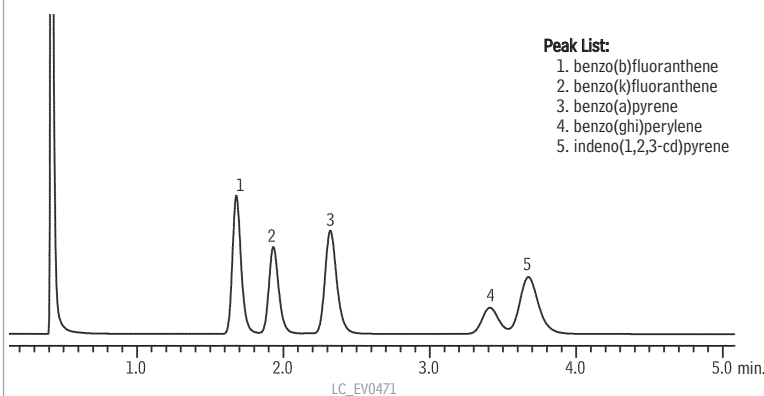
Column: Pinnacle™ DB PAH
Cat.#: 9470252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm
Pore size: 140Å

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 pH range: 2.5 to 10
pore size: 110Å temperature limit: 80°C
endcap: fully endcapped

| 4µm Column, 3.2mm | cat. # |
|-------------------|---------|
| 50mm | 9219453 |

Pinnacle™ DB PAH UHPLC Columns

Physical Characteristics:

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

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

ordering note

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

The Choice Is Yours

Pinnacle™ DB 1.9µm columns offer the widest variety of stationary phases for UHPLC

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Cyano Silica C18 IBD C8 X3 PAH

New phases now available!

Cyano • IBD • C8
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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.

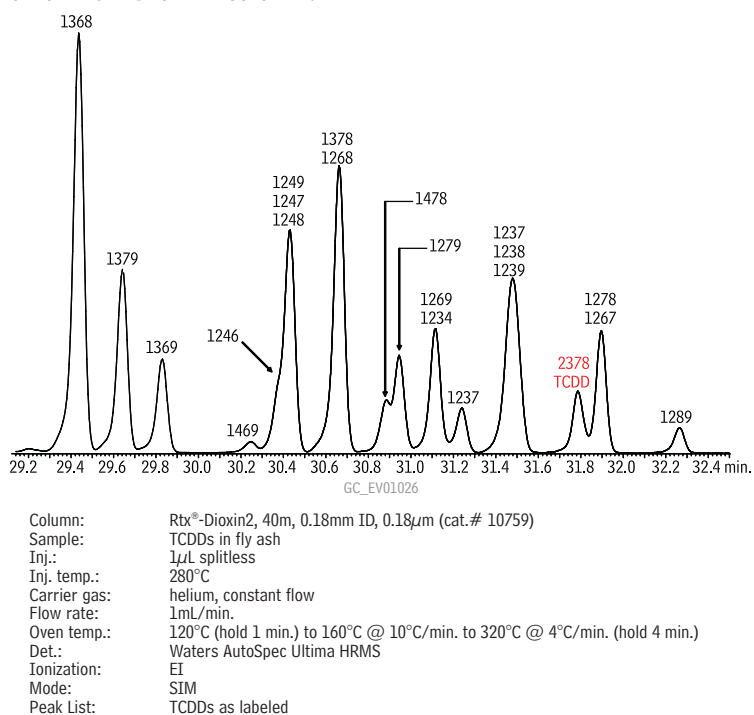
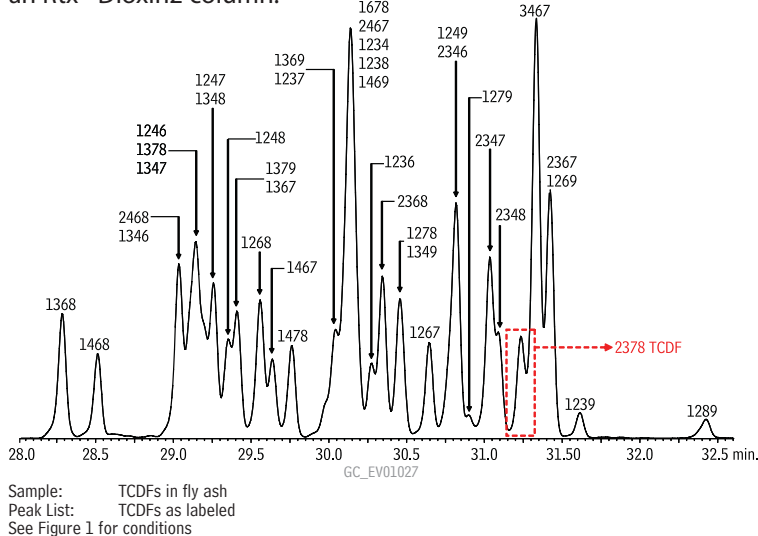


Figure 2 GC/HR-MS analysis of tetrachlorinated furans in fly ash on an Rtx®-Dioxin2 column.



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 coelutions. 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.

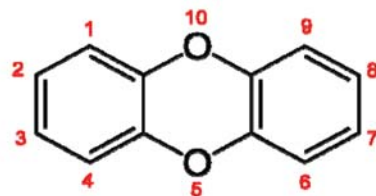
| tetrachlorinated | | | pentachlorinated | | | hexachlorinated | | | heptachlorinated | | | octachlorinated | | |
|------------------|----------|-----------|------------------|----------|-----------|-----------------|----------|-----------|------------------|----------|-----------|-----------------|----------|-----------|
| 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 Dibenzodioxins



RRTs were calculated versus 12378 ¹³C-labeled dioxin.

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

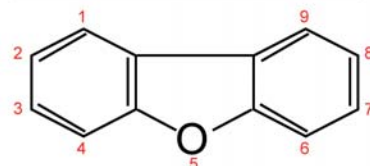
| tetrachlorinated | | | pentachlorinated | | | hexachlorinated | | | heptachlorinated | | | octachlorinated | | |
|------------------|----------|-----------|------------------|----------|-----------|-----------------|----------|-----------|------------------|----------|-----------|-----------------|----------|-----------|
| 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 Dibenzofurans



RRTs were calculated versus 12378 ¹³C-labeled furan.

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



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 Cliquant® 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.

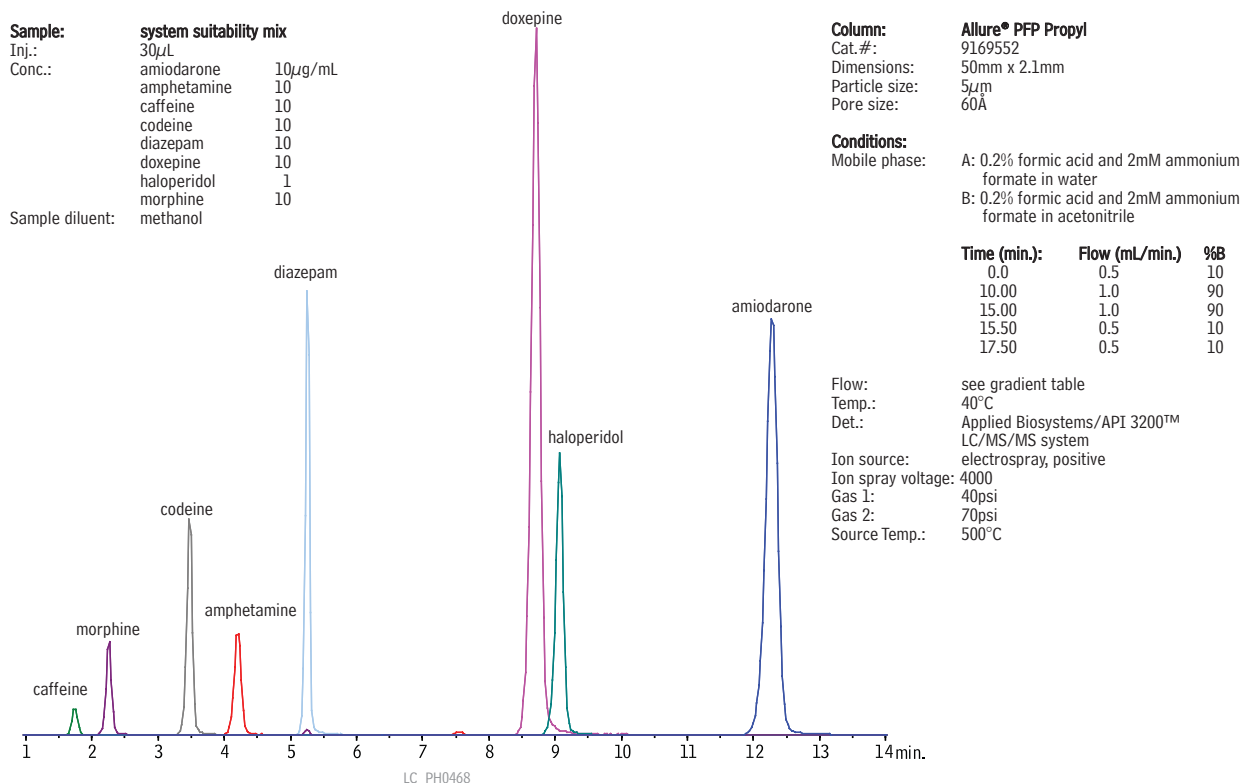
Restek and Applied Biosystems have developed a system suitability mix specifically for drug testing that contains compounds covering a wide range of molecular weights, polarities, and retention times (Table I). This standards mix is designed to verify system performance and identify system problems. 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 Cliquant® 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)



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.

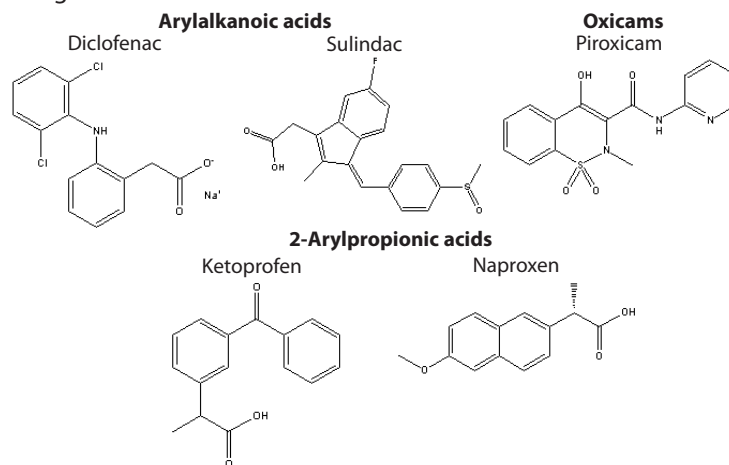
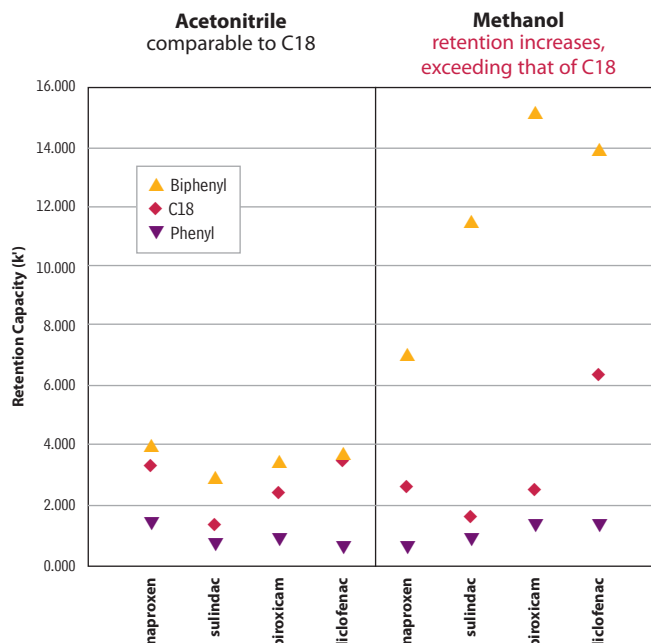


Figure 2 The retention capacity of the Allure® Biphenyl phase far exceeds that of conventional phenyl phases.



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.

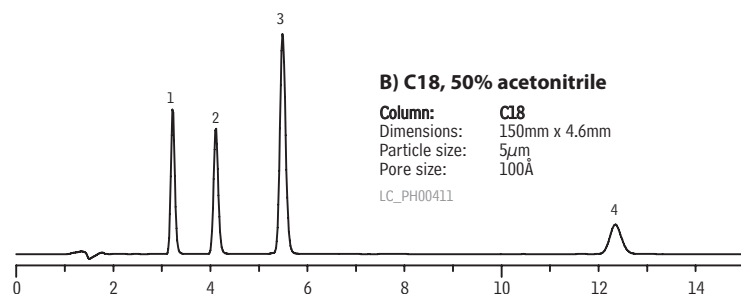
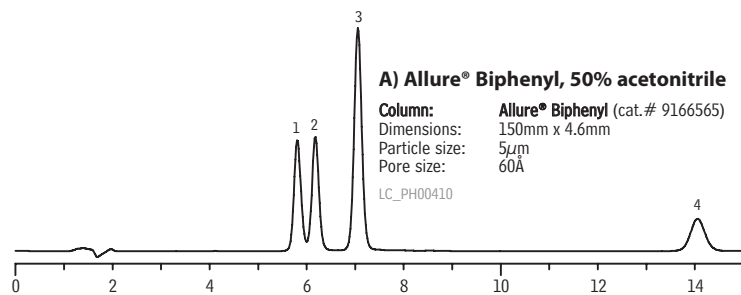
Columns: 5 μ m, 4.6mm x 150mm
Mobile Phase: 10mM potassium phosphate (pH 2.5): acetonitrile or methanol
Det.: UV @ 254nm
Flow: 1.0 mL/min.

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

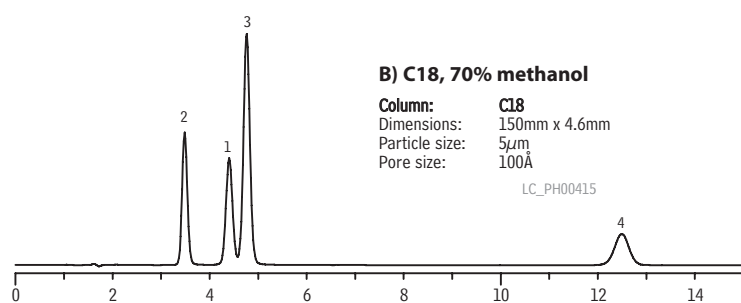
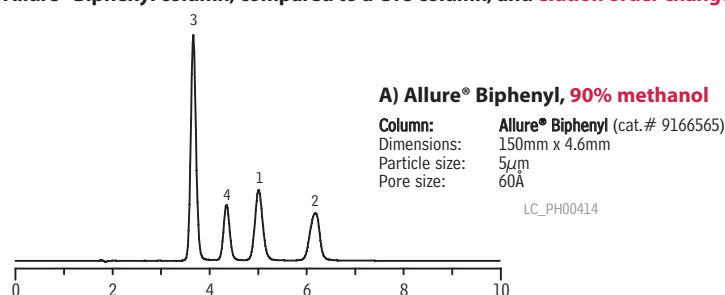
1. sulindac
 2. piroxicam
 3. ketoprofen
 4. diclofenac

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

150mm cat. # 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 |





Easily Resolve Oxytocin PEGylation Reaction Products

Using Viva Wide Pore HPLC Columns

Julie Kowalski, Ph.D., Bioanalytical Innovations Chemist

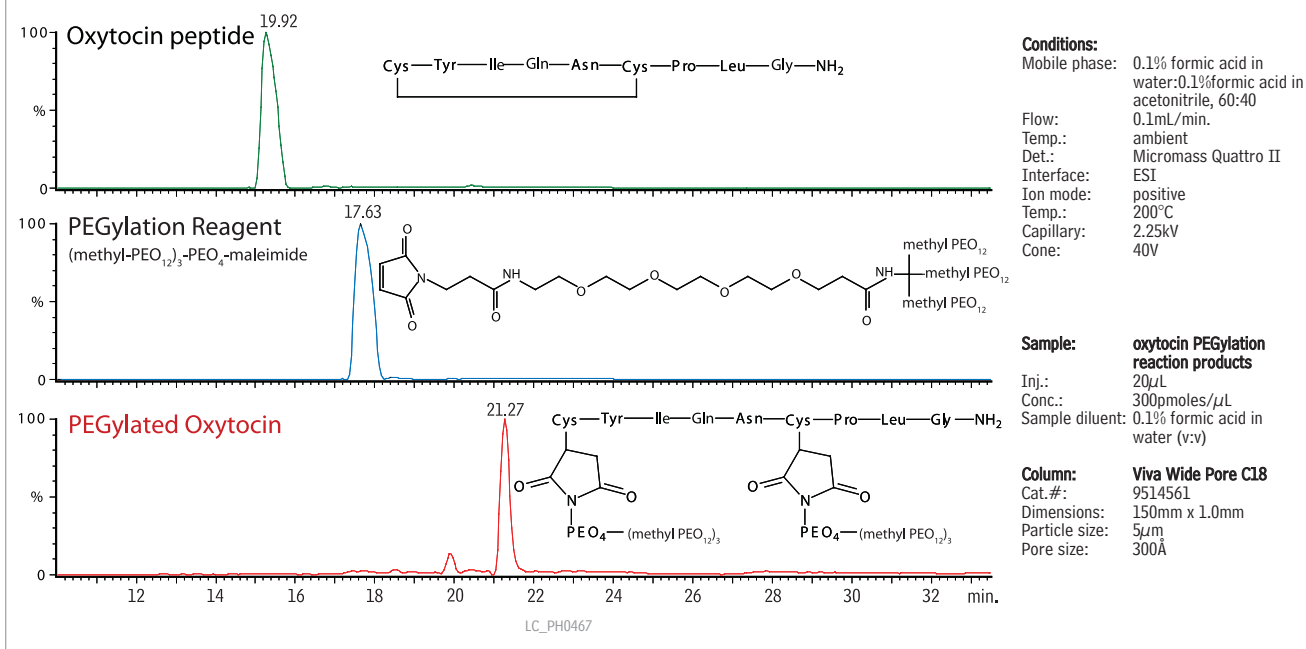
- Ideal for PEGylation reaction monitoring.
- Easy isocratic method saves time, eliminating column equilibration time between injections.
- Largest available surface area in 250-350Å pores; engineered for proteins, peptides, and other large biomolecules.

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-PEO₁₂)₃-PEO₄-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-PEO₁₂)₃-PEO₄-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.



Viva C18 Columns (USP L1)

5µm Column, 1.0mm
150mm

cat. #
9514561

ordering note

For other dimensions and guard cartridges for these columns, visit our website at www.restek.com.



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)

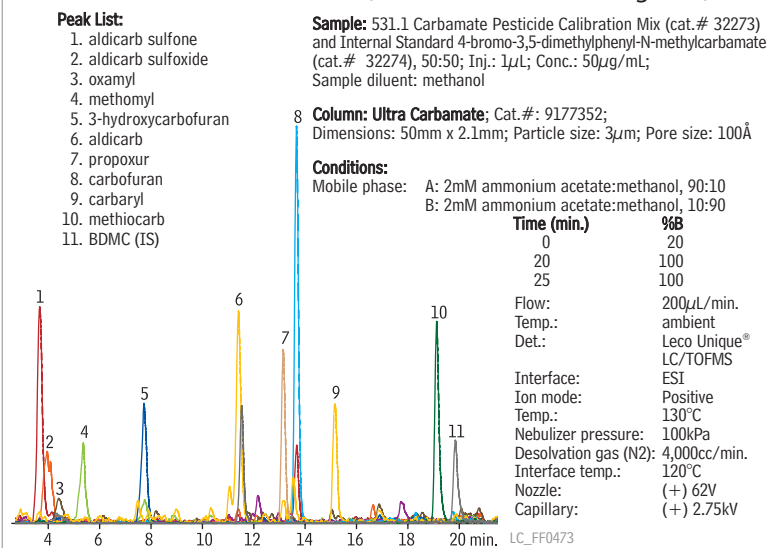


Figure 2 Positive identification of carbamates in orange oil injected with no sample preparation. (extracted ion chromatograms)

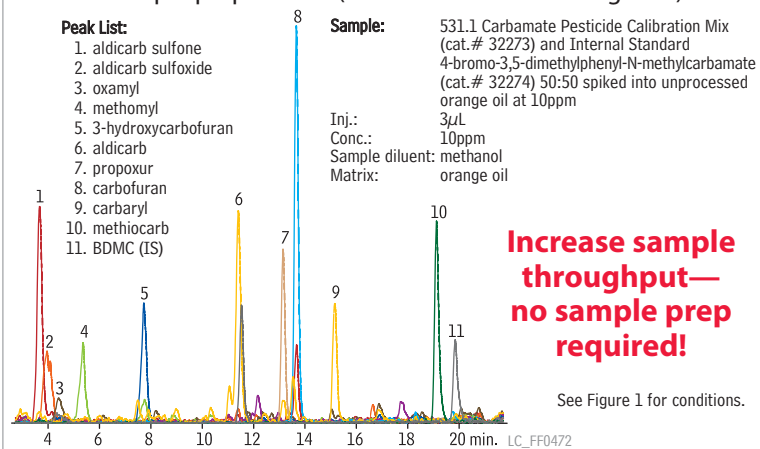


Table I Carbamates were positively identified in matrix using both retention time and mass.

| | | 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 | 11.92 | 116.052* | 11.53 |
| | | | 116.0751* | | | |
| 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



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.

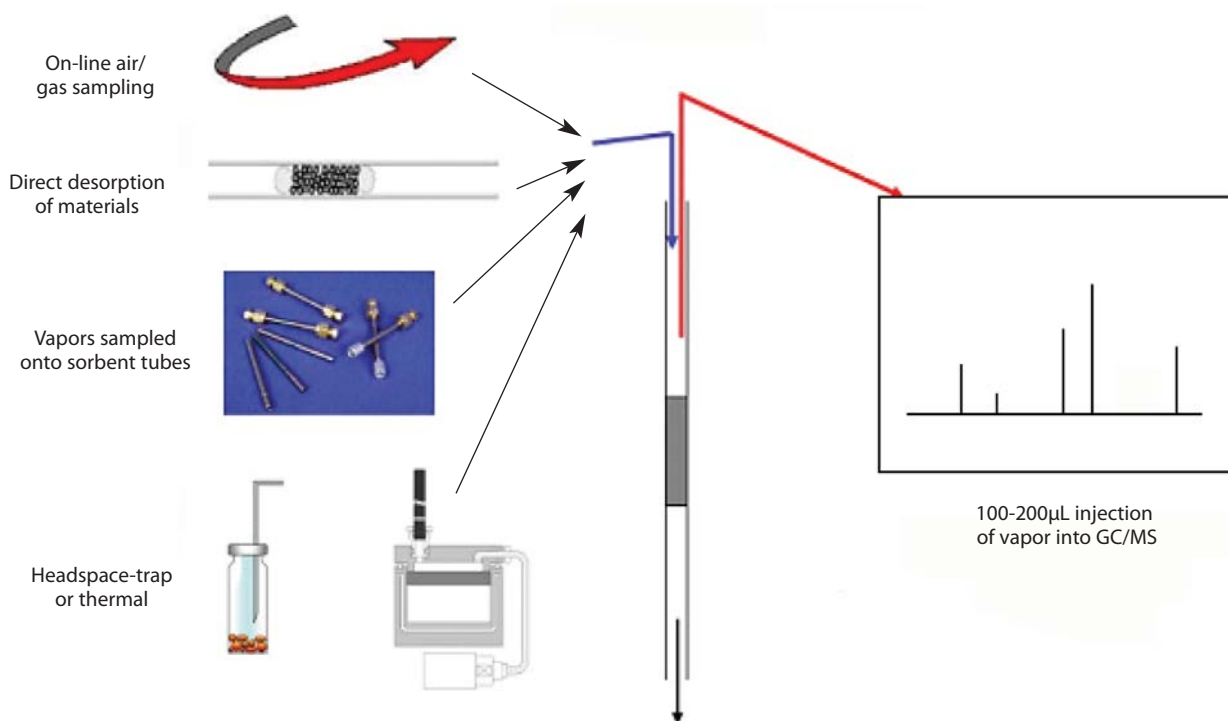


Figure 2 Verify initial results by analyzing re-collected samples.

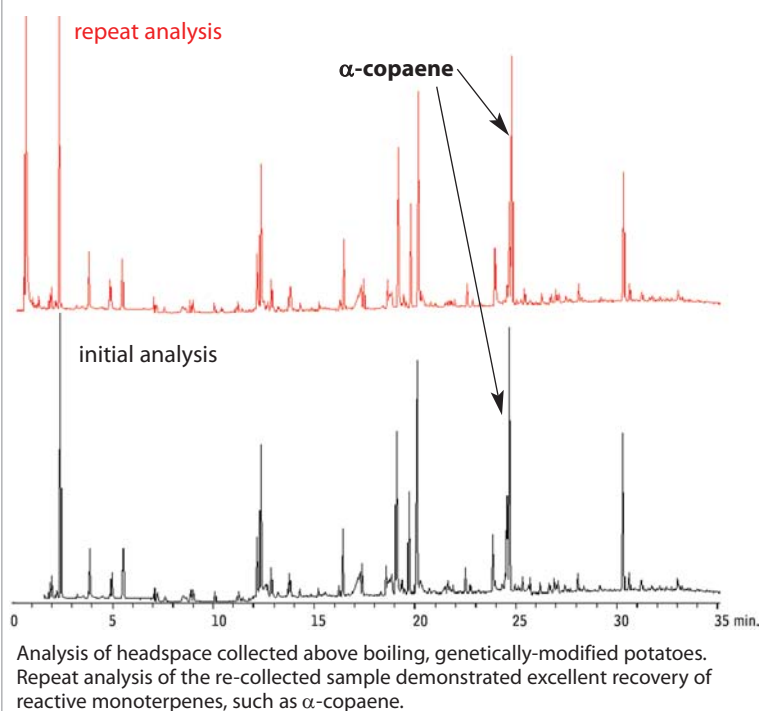
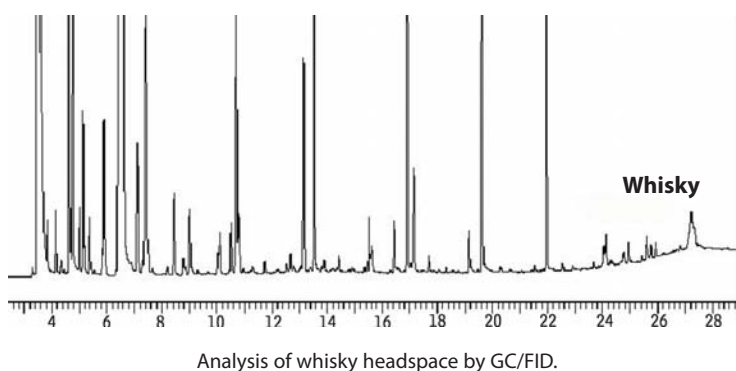


Figure 3 Thermal desorption allows selective elimination of water and >99% of ethanol vapor, enhancing the determination of key olfactory components.



Thermal Desorption Unit Tubes, Unconditioned

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

| Description | qty. | Unconditioned | |
|--|--------|-----------------|--------|
| | | Stainless Steel | Glass |
| | | 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 100 μ L. 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 <i>n</i> -C5/6 to <i>n</i> -C20 (EPA Methods TO-14/TO-15/TO-17) |
| Carbopack B/Carbosieve SIII | Vapor phase organics from <i>n</i> -C2/3 to <i>n</i> -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 <i>n</i> -C2/3 to <i>n</i> -C16/20 (EPA Methods TO-14/TO-15/TO-17) |



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 as methanol, acetonitrile, or water) and vice versa. Note that 10 to 15 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 of 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:

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

Normal phase:

- A. isopropanol

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

Tradeshows 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 |
| 9/12 | 65742 | Salt Lake City | UT |
| 9/30 | 65743 | Edison | NJ |

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