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THE APPLICATION NOTEBOOK

September 2012

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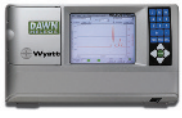

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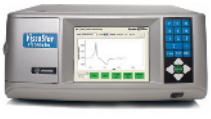

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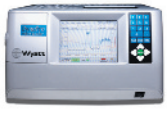

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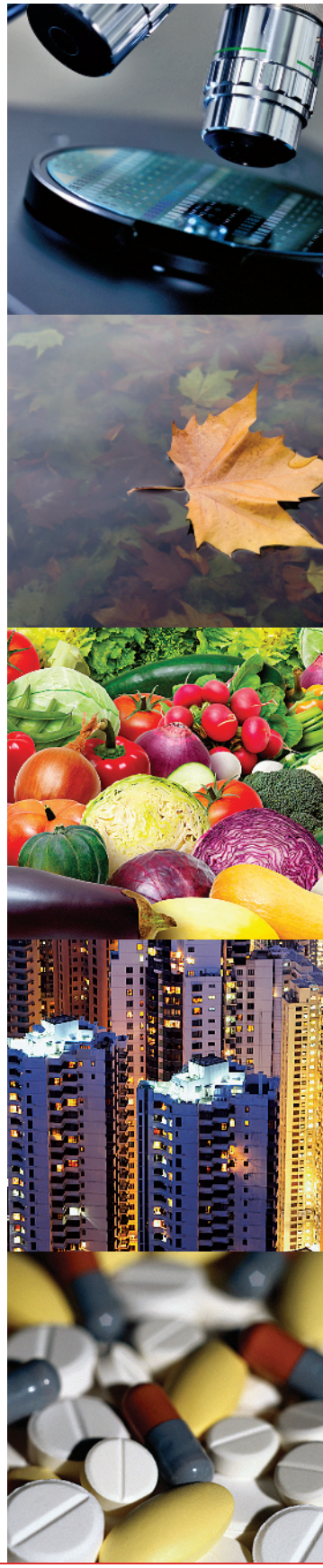

THE APPLICATION NOTEBOOK

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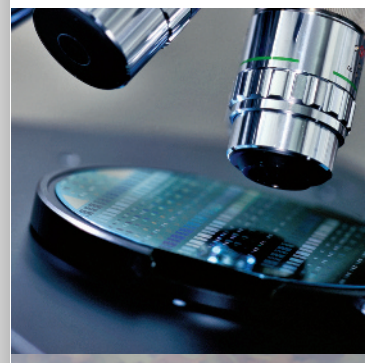

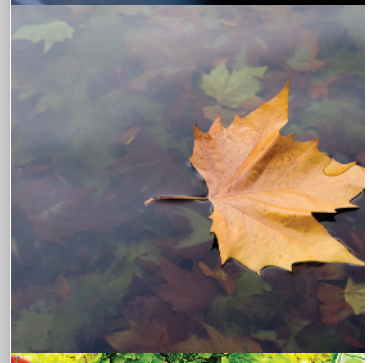

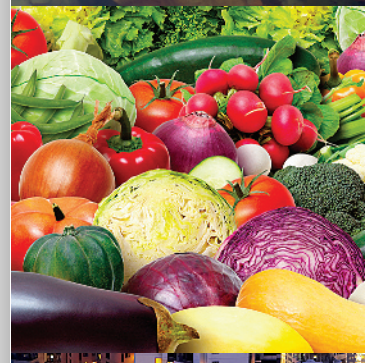

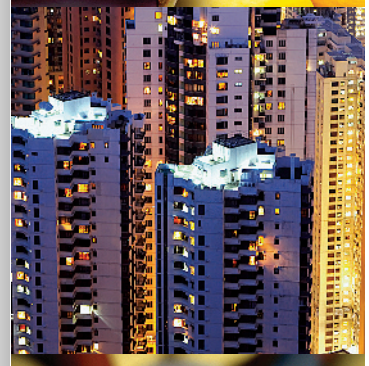

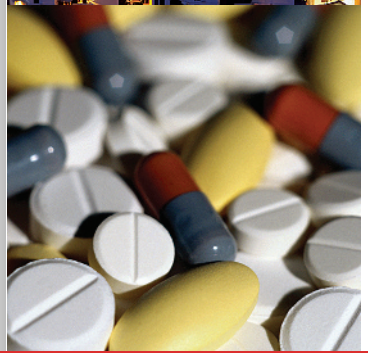

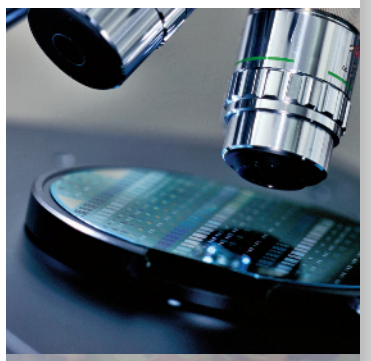

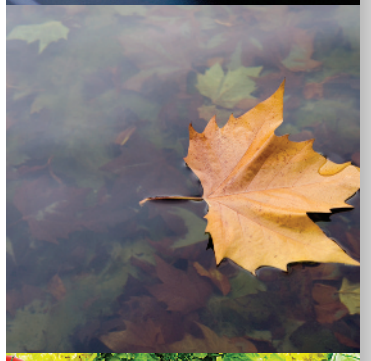

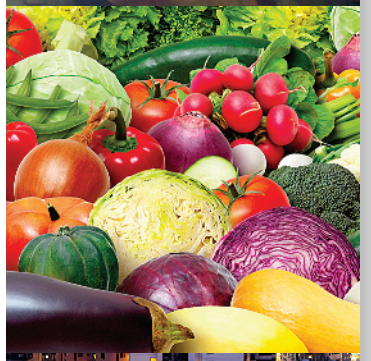

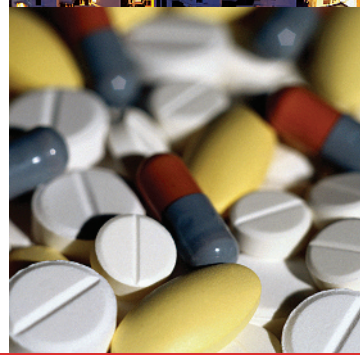

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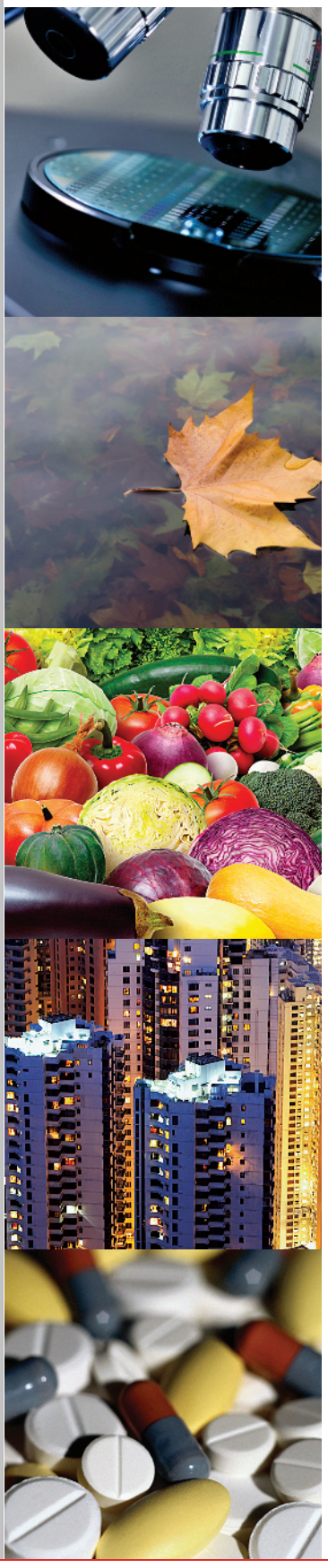

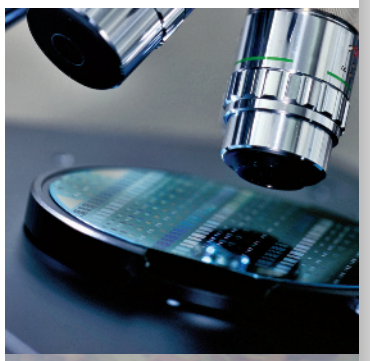

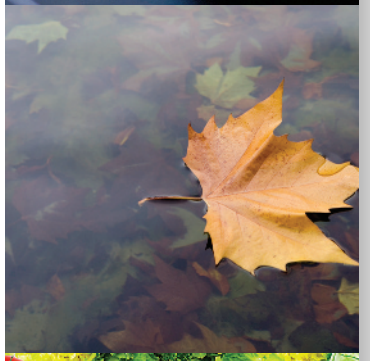

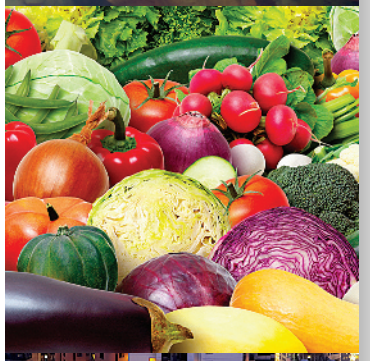

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Analysis of Phosphate Compounds with the Agilent 1260 Infinity Bio-inert Quaternary LC System

Sonja Schneider, Agilent Technologies, Inc.

This application note shows that unspecific reaction of adenosine triphosphate can be completely prevented due to the iron- and steel-free design using the Agilent 1260 Infinity Bio-inert Quaternary LC System.

Severe peak tailing of phosphate compounds is a well described issue in HPLC analysis. Interaction between stainless steel and phosphate groups were described by Liu et al. leading to the formation of phosphopeptide-Fe(III) complexes (1). The 1260 Infinity Bio-inert Quaternary LC System provides a complete metal-free sample flow path throughout the system. With this system, the user can analyze a variety of phosphate compounds without the emersion of peak tailing or other unspecific reaction due to phosphate–iron complexes.

Experimental Conditions

Solvents: 10 mM ammonium acetate with increasing amount of methanol

Sample: Adenosine triphosphate (ATP), solved in H_2Odd (5 mg/mL)

A PEEK restriction capillary was used instead of a stainless steel column.

Results

Significant peak tailing could be observed for ATP analysis in a stainless steel based system, the 1260 Infinity Quaternary LC System. With increasing amount of organic mobile phase, the retention of the phosphate compound was increasing to a huge extent, also resulting in relevant area reduction, see Figure 1.

With the 1260 Infinity Bio-inert Quaternary LC System, the unspecific reaction of the used phosphate sample could be completely prevented, resulting in good peak shapes without substantial peak tailing or area reduction, see Figure 2.

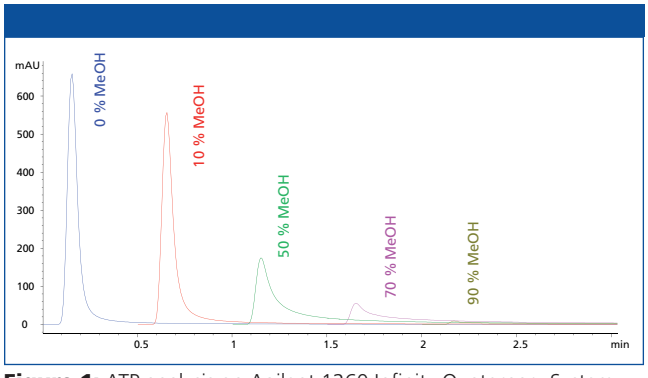

Figure 1: ATP analysis on Agilent 1260 Infinity Quaternary System.

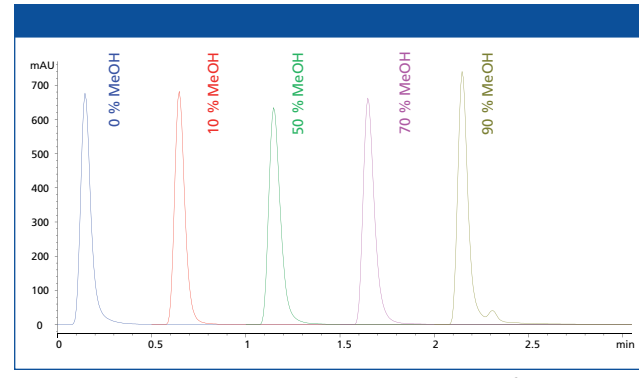

Figure 2: ATP analysis on the Agilent 1260 Infinity Bio-inert Quaternary System.

Conclusions

Unspecific reaction and peak tailing of adenosine triphosphate could be completely prevented when using the Agilent 1260 Infinity Bio-inert Quaternary LC System. Due to the iron- and steel-free design of the 1260 Infinity Bio-inert Quaternary LC system, phosphate compounds can be analyzed without any issues regarding the formation of phosphate–iron complexes as found with stainless steel systems.

Reference

(1) Liu et al., *Rapid Commun. Mass Spectrom.* **19**(19), 2747–2756 (2005).

Ultra-Fast Protein Separations and High Efficiency Peptide Profiling with Agilent LC Columns

James Martosella and Phu Duong, Agilent Technologies, Inc.

Rapid and highly resolved chromatographic separations of proteins are becoming increasingly important to meet the growing demands for faster analyses, greater reliability and overall increased column performance. Additionally, highly selective and efficient separations for peptide and impurity profiling have grown among the biopharmaceutical industry, with an ever increasing need to obtain greater peak capacities.

Agilent Poroshell (superficially porous) and ZORBAX Rapid Resolution High Definition 300 Å (sub-2 µm totally porous) columns can facilitate rapid and high throughput protein analyses and deliver increased peak capacities for high efficiency peptide profiling. For faster analysis, increased flow rates on shorter columns can result in reduced analysis times 5 to 15× faster than traditional run times. Alternatively, these columns can also afford flexibility for delivering ultra high resolution separations during longer run times.

Poroshell 300 5.0 µm — Maintaining Resolution During Rapid Runtimes

Figure 1 compares the speed and the enhanced resolution of Poroshell 300 (Panel B) to another vendor's superficially porous brand (Panel A) using a mixed protein sample. The Poroshell 300 5.0 µm delivers an ultra-fast separation using a shorter column length and higher flow rate (gradient volumes remaining equal). The Poroshell 300 separation completes 12 times faster while maintaining critical resolution, sensitivity, and peak shape. Additionally, Poroshell 300 resolves transferrin (*), which is missing from the separation in Panel A.

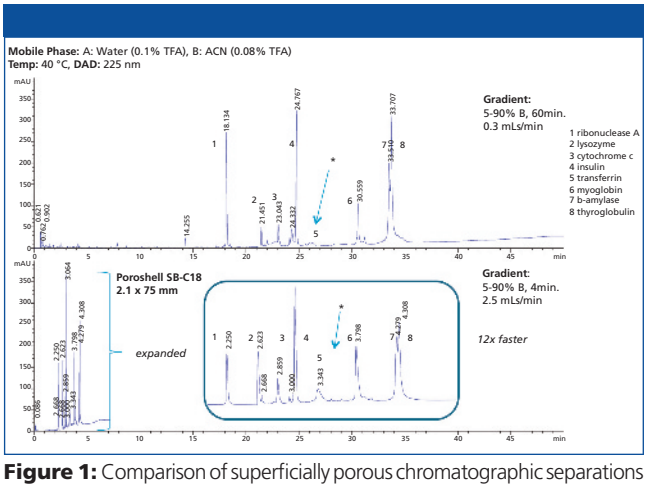

Figure 1: Comparison of superficially porous chromatographic separations using a mixed protein sample. Panel A: 2.1 × 150 mm 3.6 µm superficially porous C18 column, flow rate 0.3 mL/min, mobile phase A = H_2O (0.1% TFA), B = ACN (0.08% TFA), gradient 5 to 90% B over 60 min. Panel B: Agilent Poroshell 300SB-C18, 2.1 × 75 mm, 5.0 µm, flow rate 2.5 mL/min, gradient 5 to 90% B over 4 min. Common conditions; temp. 40 °C, UV 225 nm.

Poroshell 120 EC-C18 2.7 µm and ZORBAX RRHD 300SB-C18 — 1.8 µm High Resolution Peptide Mapping

Comparisons between Agilent Poroshell 120 2.7 µm and ZORBAX RRHD 300SB-C18 1.8 µm columns demonstrate alternate selectivity options for generating high resolution peptide maps. Both peptide separations exhibit excellent separation performance of hydrophobic and hydrophilic peptides and deliver highly resolved peaks and narrow bandwidths. In particular, the ZORBAX RRHD 300 Å column has unique selectivity and retention for hydrophilic peptides (Figure 2, arrows).

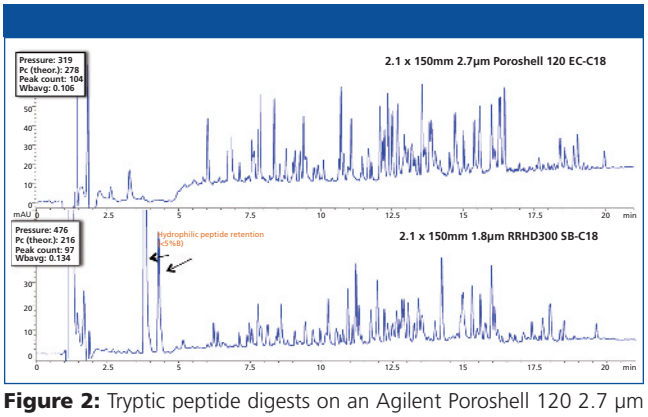

Figure 2: Tryptic peptide digests on an Agilent Poroshell 120 2.7 µm and ZORBAX RRHD 300SB-C18 1.8 µm column, with the latter displaying unique selectivity and retention of hydrophilic peptides (arrows). BSA tryptic digest, 1 pmol/µL (dil. 98/2 A/B), inj. 7 (100 mm) to 10 µL (150 mm), mobile phase A = water (0.1% TFA), B = ACN (0.08% TFA), gradient hold 3% B 3 min, 3 to 65% B 30 min, 6 min post run, flow rate 0.3 mL/min, temp. 40 °C, DAD 215 nm, Agilent 1290 Infinity LC.

Conclusions

As rapid and reliable separation of biomolecules becomes increasingly critical, the speed and resolution benefits offered by Agilent LC columns improve the likelihood of achieving high quality separations. Agilent offers Poroshell and ZORBAX RRHD columns for facilitating a variety of specialized bioseparation needs.

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Rapid Improvements for LC–MS-MS Analysis Using the New Phree™ Phospholipid Removal Plates

Dr. Stuart Kushon, Michael Rummel, and Erica Pike, Phenomenex, Inc.

When performing LC–MS-MS analysis, phospholipids are perhaps one of the most troublesome components of bioanalytical samples. The presence of phospholipids not only reduces the column lifetime and sensitivity but can also cause a phenomenon known as ion suppression.

This work compares the presence of phospholipids in plasma samples after two different sample preparation techniques, protein precipitation and simultaneous protein precipitation and phospholipid removal using a new product, Phree.

Experimental Conditions

Plasma samples from the same lot were prepared (Table I) and were injected on a Kinetex® 2.6 µm C18 core–shell column coupled with an API 3000 MS (AB SCIEX) (Figure 1). A total phospholipid profile was monitored using *m/z* 184–184.

Results and Discussion

When monitoring the total phospholipid profile, the protein precipitated plasma showed a large amount of phospholipids. In comparison, the plasma sample that was prepared using Phree showed virtually no phospholipids (Figure 1).

We also studied analyte response by comparing diclofenac spiked plasma samples. After sample preparation, 20 µL samples were injected on a Kinetex® 2.6 µm C18 core–shell column. The diclofenac signal in the protein precipitated samples was immediately lower than the signal in the Phree extracted samples. The signal in the protein precipitated sample rapidly decreases. The Phree extracted sample shows a steadier signal and is able to withstand > 250 injections (Figure 2). The higher signal strength of the Phree extracted sample is due to the reduction of phospholipid induced ion suppression. The significant reduction in phospholipid build up on the column and MS source also resulted in an increase in column lifetime and a decrease in the amount of MS maintenance required.

Table I: Sample preparation protocols

	Protein Precipitation	Phree Phospholipid Removal
1	Add 300 µL acetonitrile to the wells of a collection plate	Add 300 µL acetonitrile to the wells of the Phree plate
2	Add 100 µL of plasma directly into the acetonitrile	Add 100 µL of plasma directly into the acetonitrile
3	Vortex for 2 min at maximum possible speed	Vortex for 2 min at maximum possible speed
4	Pellet precipitated proteins by centrifuge	Filter using vacuum at 2–7 in. Hg for up to 5 min*
5	Collect supernatant	

*Centrifugation and positive pressure may also be used.

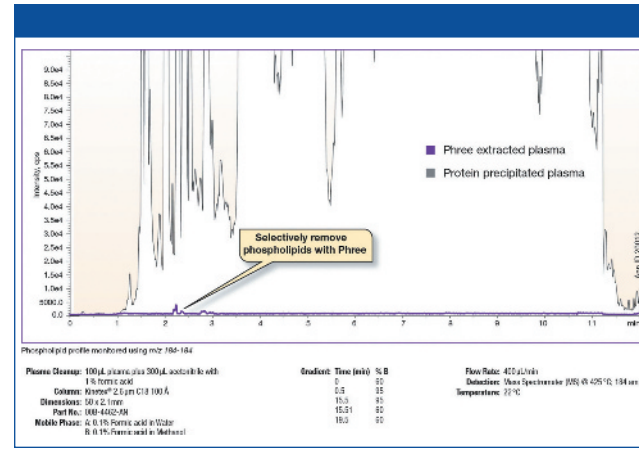

Figure 1: Total phospholipid profile.

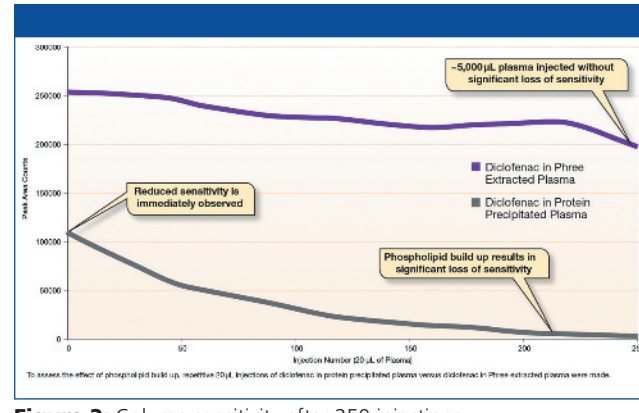

Figure 2: Column sensitivity after 250 injections.

Conclusion

By preparing samples with Phree, analysts can remove proteins and phospholipids in four short steps resulting in immediate improvements to their chromatography work.

For more information about Phree, visit www.phenomenex.com/Phree.

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70-min Amino Acid Analysis of Physiological Samples

Michael V. Pickering, Pickering Laboratories, Inc.

Pickering Laboratories specializes in the manufacturing of cation-exchange columns and eluants for amino acid analysis. Post-column derivatization with Ninhydrin offers unmatched selectivity and reproducibility of the analysis for most challenging matrixes.

Pinnacle PCX post-column derivatization system allows you to combine eluant gradient capabilities of modern HPLC instruments with column temperature gradients. We introduce accelerated amino analysis method for physiological samples that utilizes temperature and eluant gradients.

Method

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column (4.6 × 75 mm), 5 µm
Catalog No. 0354675T
Flow Rate: 0.55 mL/min
Mobile Phase: 1700–1125, Li365, Li375, RG003

Post-column conditions

Post-column system: Pinnacle PCX
Reactor Volume: 0.5 mL
Temperature: 130 °C
Reagent: Trione
Flow Rate: 0.5 mL/min
Detection: UV-vis 570 nm for primary amino acids
440 nm for secondary amino acids
Run Time: 72 min
Equilibration Time: 15 min

Table I: HPLC program

Time	1700–1125%	Li365%	Li375%	RG003%
0	100	0	0	0
10	100	0	0	0
19	40	60	0	0
32	0	100	0	0
43	0	100	0	0
43.1	0	0	100	0
57	0	0	100	0
57.1	0	0	70	30
72	0	0	70	30
72.1	100	0	0	0

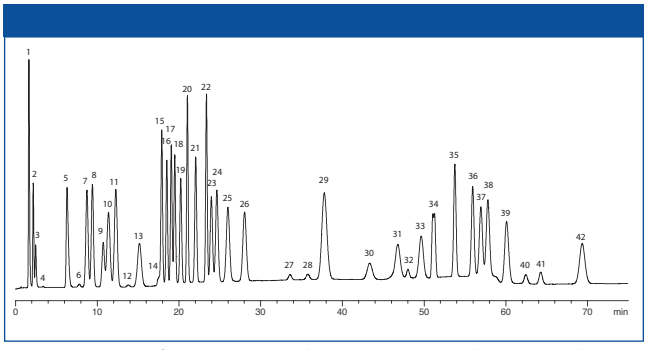


Figure 1: List of amino acids: 1) Phosphoserine, 2) Taurine, 3) Phosphoethanolamine, 4) Urea, 5) Aspartic acid, 6) Hydroxyproline, 7) Threonine, 8) Serine, 9) Asparagine, 10) Glutamic acid, 11)Glutamine, 12) Sarcosine, 13) α-Aminoadipic acid, 14) Proline, 15) Glycine, 16) Alanine, 17) Citrulline, 18) α-Amino-n-butyric acid, 19) Valine, 20) Cystine, 21) Methionine, 22) Cystathionine, 23) Isoleucine, 24) Leucine, 25) Tyrosine, 26) Phenylalanine, 27) β-Alanine 28) β-Amino-i-butyric acid, 29) Homocystine, 30) γ-Aminobutyric acid, 31) Tryptophan, 32) Ethanolamine, 33) Ammonia, 34) Hydroxylysines, 35) Ornitine, 36) Lysine, 37) 1-Methylhistidine, 38) Histidine, 39) 3-Methylhistidine, 40) Anserine, 41) Carnosine, 42) Arginine.

Table II: Column oven program

Time, min	Temperature °C
0	34
6	34
17	65
25	70
70	70
71	34

Analysis of Intact Proteins on a Thermo Scientific Accucore 150 C4 HPLC Column

Joanna Freeke and Valeria Barattini, Thermo Fisher Scientific, Runcorn, Cheshire, UK

This application note demonstrates the analysis of intact proteins using a Thermo Scientific Accucore 150-C4 (150 Å pore diameter) HPLC column. Analysis of six proteins ranging in mass from 6 to 45 kDa is carried out in 15 min with pressures compatible with conventional HPLC instrumentation.

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles have a solid core and a porous outer layer. The optimized phase bonding creates a series of high-coverage, robust phases. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower back pressures than typically seen with sub 2 µm materials. For the analysis of large biomolecules the Accucore pore size has been further optimized and a C4 phase with reduced hydrophobic retention has been prepared. This 150 Å pore size enables the effective analysis of molecules unable to penetrate into smaller diameter pores, whilst the low hydrophobicity C4 phase results in protein separation by hydrophobicity.

Chromatographic separation of proteins at the intact level prior to MS analysis is desirable for reducing sample complexity and maintaining global protein information. In this application note, we demonstrate the excellent performance of an Accucore 150-C4 HPLC column for the chromatographic separation of six intact proteins (6–45 kDa).

Thermo Scientific Column and Consumables

Accucore 150-C4, 2.6 µm, 100 × 2.1 mm
Vials and closures (P/N MSCERT 4000-34W)

Thermo Scientific Accela HPLC System

Flow rate: 400 µL/min
Run time: 15 min
Column temperature: 40 °C
Injection details: 2 µL (10 pmol/µL solution of each protein)
UV detector wavelength: 214 nm
Backpressure at starting conditions: 185 bar (c.f. 320 bar on sub 2 µm material)

Data Processing

Software: Thermo Scientific Xcalibur 2.0 SR2

Mobile Phase

Mobile phase A: 0.1 % TFA in 30:70 acetonitrile:water
Mobile phase B: 0.1 % TFA in 98:2 acetonitrile:water
Gradient: 0–30% B in 8 min, 30–95% B in 2 min, hold at 95% B for 1 min and re-equibrilate for 4 min

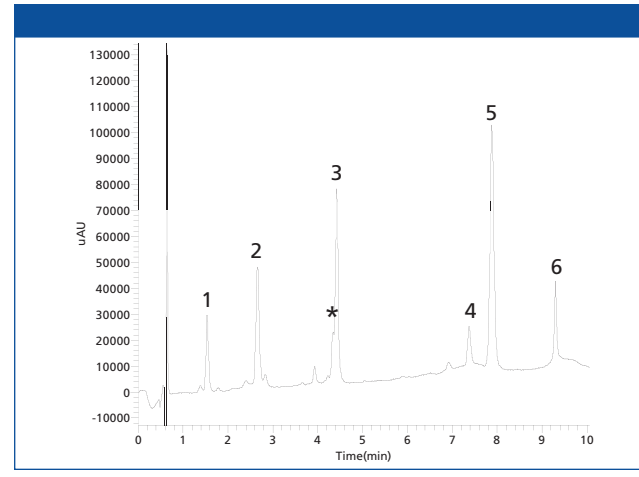


Figure 1: Chromatogram for six proteins separated on an Accucore 150-C4 HPLC column. 1. insulin, 2. cytochrome c, 3. lysozyme, 4. myoblobin, 5. carbonic anhydrase, 6. ovalbumin, * carbonic anhydrase impurity.

Results

Under these conditions, six proteins covering the mass range of 6 to 45 kDa can be separated on an Accucore 150-C4 HPLC column in less than 15 min with back pressures compatible with conventional HPLC equipment. The chromatography is shown in Figure 1 with all of the proteins eluting with sharp symmetrical peaks and being baseline resolved, with the exception of an impurity from carbonic anhydrase which co-elutes with lysozyme.

Conclusion

- Accucore 150-C4 HPLC columns show excellent separation of six test proteins of differing mass (6–45 kDa) within 15 min
- Good peak shape is observed for all proteins
- The back pressure is compatible with use on a conventional HPLC system

Thermo SCIENTIFIC

Thermo Fisher Scientific
Tudor Road, Manor Park, Runcorn, Cheshire WA7 1TA, UK
tel. +44 (0) 1928 534110
Website: www.thermoscientific.com/chromatography

Analysis of Bovine Serum Albumin (BSA) Digest on a Thermo Scientific Accucore 150-C18, NanoLC Column

Valeria Barattini[1], Joanna Freeke[1], Duncan Smith[2], and John Griffiths[2], [1]Thermo Fisher Scientific, Runcorn, UK; [2]The Paterson Institute of Cancer Research, Manchester, UK

This application note demonstrates the analysis of trypsin-digested BSA using a Thermo Scientific Accucore 150-C18 (150 Å pore diameter) nanoLC column.

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. Accucore 150-C18 has been further optimized for the analysis of biomolecules and protein digests by extending the pore size to 150 Å.

The increased pore diameter enables larger peptide fragments to diffuse into the particle and interact with the stationary phase more effectively, resulting in high resolution of these fragments.

Herein, we demonstrate the excellent performance of Accucore 150-C18 nanoLC columns for the separation of digested BSA.

Standard Preparation

A 50 fmol/µL solution of digested BSA was prepared.

Instrumentation, Column, Consumables and Method

Thermo Scientific Dionex UltiMate 3000 RSLCnano LC system, coupled to a Thermo Scientific LTQ-Orbitrap XL mass spectrometer fitted with a Proxeon Nanospray Flex ion source. Accucore 150-C18 2.6 µm, 75 µm i.d. × 150 mm nanoLC column (P/N 16126-157569). Thermo Scientific National Vials and Closures (P/N MSCERT4000-34W).

The sample was loaded directly on the column (1 µL injection volume) through sample loop at gradient start.

Flow rate: 300 nL/min; A: 0.1 % formic acid in water B: 80:20 acetonitrile: water (4–40% B gradient over 30 min; ramp to 95% B over 2 min; hold for 2 min; drop to 4% B over 1 min; hold for 4 min).

Results

Elution of tryptic peptides using the conditions described above was achieved within 36 min (Figure 1). Triplicate analyses showed excellent retention time reproducibility for a set of 12 peptides, with %RSD values below 0.14%. Figure 2 shows the extracted ion chromatograms (EIC) of a subset of the peptides monitored. In all cases the peak shapes were found to be excellent, with minimal peak tailing. A peak capacity value of 200 was obtained (1), showing the high resolution power of Accucore 150-C18 nanoLC columns.

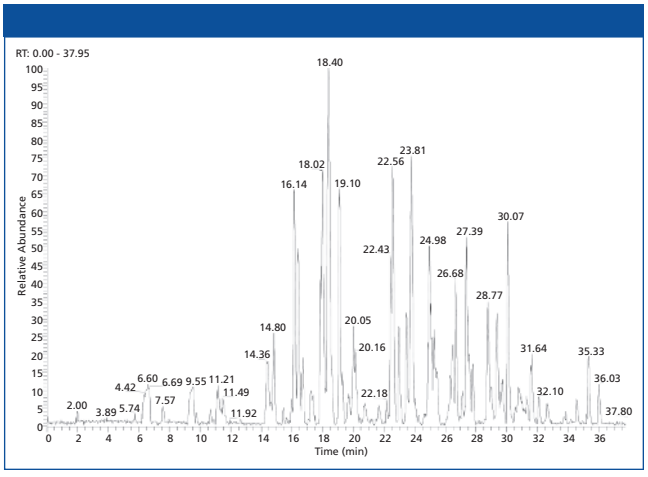


Figure 1: Base peak chromatogram of 50 fmol of digested BSA loaded on an Accucore 150-C18 nanoLC column, 75 µm ID×150 mm.

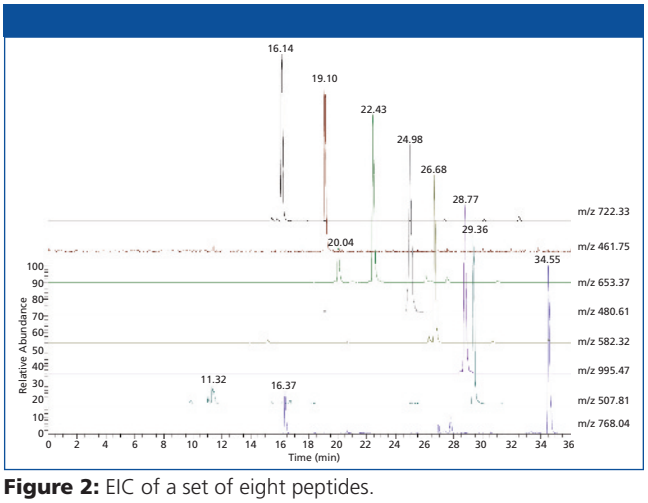


Figure 2: EIC of a set of eight peptides.

Conclusion

Accucore 150-C18 nanoLC columns are an ideal choice for complex proteomic samples, featuring excellent resolution power and run-to-run reproducibility.

References

(1) X. Wang, *Anal. Chem.* **78**(10), 3406–3416 (2006).

Thermo SCIENTIFIC

Thermo Fisher Scientific
Tudor Road, Manor Park, Runcorn, Cheshire WA7 1TA, UK
tel. +44 (0) 1928 534110
Website: www.thermoscientific.com/chromatography

Separation of mAb Isoforms Using Controlled pH Gradients and Ion Exchange Chromatography Columns

Atis Chakrabarti, PhD, Tosoh Bioscience LLC

Monoclonal antibody isoforms differ in modifications of individual amino acid side chains, or the N- or C-terminus. Typical modifications are deamidation, phosphorylation, acetylation, methylation, oxidation, or glycosylation. Fast separation of monoclonal antibody isoforms is important for profiling and mass spectrometric determination. Isoforms may differ in biological activity and stability, making a thorough characterization and quantification of the isoforms necessary to ensure consistent product quality.

Though ion exchange chromatography (IEC) is a useful separation technique for profiling the charge heterogeneity of monoclonal antibodies, these separations are product specific and time consuming to develop. The utilization of controlled pH gradients, however, can provide significant advantages, including improved separation resolution, lower salt concentration in collected fractions, and the ability to correlate the protein isoelectric point (pI) data with elution profiles. Monoclonal antibody isoforms are separated based on differences in their charge states.

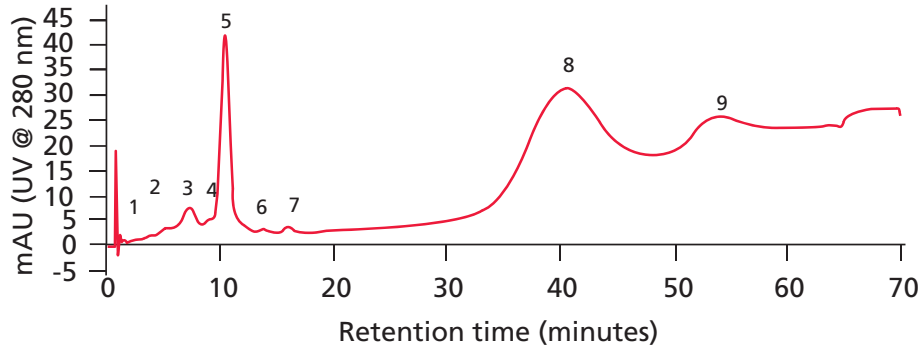
This application note discusses the separation of isoforms of monoclonal antibodies using highly controlled pH gradients on TSKgel® STAT columns packed with 7 and 10 µm hydrophilic nonporous resin particles. The innovative bonding chemistry and relatively large particle size of TSKgel STAT columns result in a respectable loading capacity and a low operating pressure, making these columns suitable for all HPLC and FPLC systems in biomolecule separations.

Experimental Conditions

To create controlled pH gradients a pISep© kit (CryoBioPhysica, Silver Spring, MD), consisting of a software package and two nearly identical buffer compositions, acidic and basic, composed of small zwitterions with overlapping pK_as, was used. pISep buffer composition possesses strong, relatively uniform buffering capacity throughout the pH range 2–12. The pISep software was used to compute column volume or time-based protocols for the development of single or multistep, linear or nonlinear, pH gradients on ion exchange (IEX) columns over any segment of the pH range 2.4–10.8.

Analyses were carried out using an Agilent-1100 HPLC system running Chemstation (ver B.04.02).

Columns:	Strong cation, polymer: TSKgel SP-STAT, 7 µm, 4.6 mm ID ×10 cm (S0004-501N) Weak cation, polymer: TSKgel CM-STAT, 10 µm, 3.0 mm ID × 3.5 cm (N0018-507N) Strong anion, polymer: TSKgel Q-STAT, 7 µm, 4.6 mm ID × 10 cm (R0087-501N)
Flow rate:	TSKgel SP-STAT (1.0 mL/min or 1.66 min/CV); 1CV = 1.66 mL TSKgel CM-STAT (1.0 mL/min or 2.075 min/CV); 1CV = 0.247 mL TSKgel Q-STAT (0.8 mL/min or 0.247 min/CV); 1CV = 1.66 mL



Time (min)	%B	pH
0	0	7.6
15.1	21.4	8.6
23.9	36.9	8.7
44.1	77.3	9.7
50.4	87.6	10.1
57.0	94.9	10.4
63.0	100.0	10.8
70.0	100.0	10.8
70.1	0	7.6

Figure 1: pH gradient-based ion exchange separation of a monoclonal antibody separation using a strong cation exchange TSKgel SP-STAT column.

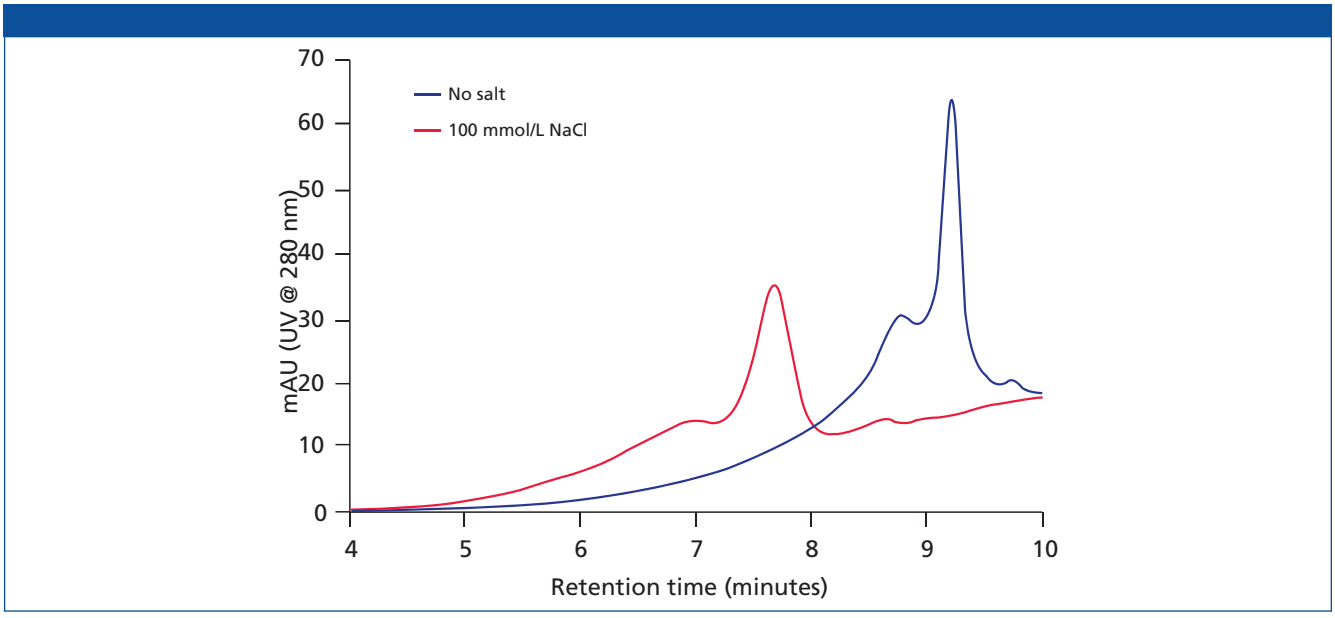


Figure 2: Effect of salt in pH gradient-based weak cation exchange separation of a monoclonal antibody using a TSKgel CM-STAT column.

Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	10 µL
Samples:	monoclonal antibody: BI-mAb-2 from Boehringer-Ingelheim (gift from Tosoh Bioscience GmbH) concentration: 4.5 g/L in glycine/Na phosphate, pH 6.0

Results and Discussion

A size exclusion chromatographic separation using a TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm i.d. × 30 cm column (data not shown here) was used for a quality control study in the purification of a monoclonal antibody and yielded a predominantly pure monomer peak at ambient temperature with a retention time of 7.9 min during isocratic separation using 0.1 mmol/L phosphate buffer at the flow rate of 1 mL/min. Dimer and aggregate peak impurities were resolved from the monomer peak. Linear salt gradient-based separation (not shown here) of a monoclonal antibody using 10 mmol/L phosphate buffer containing 10 mmol/L Na_2SO_4 as a neutral salt (buffer A) and the same with 1 mol/L NaCl (buffer B) on a strong cation exchange TSKgel SP-STAT column was also used to separate the different mAb isoforms and yielded only a few peaks. Controlled pH gradient-based ion exchange separation of a monoclonal antibody using a strong cation exchange TSKgel SP-STAT column under the chromatographic conditions as mentioned above separated nine different isoforms (see Figure 1). The peaks were further resolved by the precise modification of the pH gradient. Large changes in resolution were achieved in controlled pH gradient elution simply by changing the range of elution (not shown here). Figure 2 shows the effect of the addition of 100 mmol/L NaCl salt in improving the peak resolution of the two isoform peaks in the analysis of a mAb using a TSKgel CM-STAT column, a weak cation exchange chromatography column. Similarly an anion exchange chromatography column, TSKgel Q-STAT, was also successfully used for the separation of isoforms (data not shown here).

Conclusions

Controlled pH gradient-based ion exchange chromatography can be an effective method for the separation of protein isoforms. Good resolution was found for pH gradient-based separations using a broad range universal buffer system such as the pISep kit used in this study. TSKgel STAT columns can be used effectively to separate monoclonal antibody isoforms using a controlled pH gradient. Further studies to monitor the effect of different types of salt and organic solvents in improving peak resolution during a controlled pH gradient separation of mAb isoforms is in progress.

TOSOH BIOSCIENCE

TOSOH

Tosoh Bioscience LLC
3604 Horizon Drive, Suite 100, King of Prussia, PA 19406
tel. (484) 805-1219, fax (610) 272-3028
Website: www.tosohbioscience.com

Separation of 20 Amino Acids by Polymer-Based Amino HILIC Column

Kanna Ito, Showa Denko America Inc.

Determination and quantification of amino acids are important in many fields. For the HPLC analysis of amino acids, cation exchange mode method has been used with post-column derivatization. Recently, use of reversed-phase mode method is also increasing. However, because of amino acids' hydrophilic nature, they are usually not well retained and have less selectivity by reversed-phase mode without pre-column derivatization steps. In contrast, HILIC mode is an ideal separation mode for hydrophilic compound analysis. In addition, by employing an ESI-MS detector, target compounds that have similar retention time may also be separated based on their *m/z*. Cation exchange mode generally provides good separation for multiple amino acids, however the eluent used is not desirable for ESI-MS use. A volatile mobile phase used for HILIC mode is advantageous for the ESI-MS analysis. Typically used mobile phase is acetonitrile and buffer, containing ammonium acetate or ammonium formate owing to their high volatilities and sample solubility. In this application, 20 standard amino acids were analyzed simultaneously without additional derivatization steps.

Experimental Conditions

Separation was carried by Shodex NH2P40-2D (2.0 ×150 mm, 4 µm), a polymer-based amino HILIC column. Column temperature was set at 30 °C and flow rate used was 0.2 mL/min. Linear gradient was applied as following: (A) 100 mM ammonium formate, (B) acetonitrile. B% 75% (0 min) → 75% (10 min) → 50 % (11 min) → 50% (35 min). Sample contained 10 µg/mL each of 20 amino acids, diluted with water/acetonitrile = 25/75. Injection volume of 5 µL was used for the experiment. HPLC system was coupled with an ESI-MS and SIM mode was used for detection. Ala and Gly were detected as acetonitrile adducts and Cys was detected as cystine (as a product of ESI).

Results

All 20 standard amino acids were analyzed successfully by the HPLC (HILIC)-ESI-MS method (Figure 1). The method achieved baseline separations of isobaric amino acids such as Leu and Ile, and Lys and Gln providing accurate identification of each.

Effects of ammonium formate concentration were tested during the solvent optimization step. By increasing ammonium formate concentration, tailing which was observed for few amino acids was reduced. The higher ammonium formate concentration also benefits in shortening the analysis time; 53 min (Solvent (A) as 50 mM ammonium formate) down to 33 min (Solvent (A) as 100 mM ammonium formate).

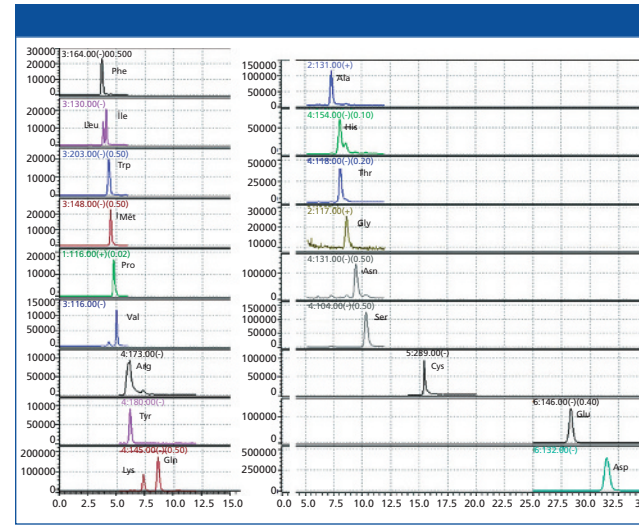


Figure 1: Separation of 20 amino acids by NH2P40-2D. Eluent: Linear gradient (A) 100 mM ammonium formate, (B) acetonitrile. B% 75% (0 min) → 75% (10 min) → 50 % (11 min) → 50% (35 min). Flow rate; 0.2 mL/min, Temp.; 30 °C, Detector; ESP-MS SIM mode.

Conclusions

Shodex polymer-based amino HILIC column, NH2P40-2D, coupled with ESI-MS is feasible for the analysis of 20 standard amino acids within 35 min: NH2P40-2D provides baseline separations of isobaric amino acids such as Leu and Ile, and Lys and Gln. The eluent condition used for HILIC mode is desirable for ESI-MS measurements.

Unique Effects of Two Mobile Phase Alcohols on the RegisPack® CLA-1 Chiral Stationary Phase (CSP)

Ted Szczerba, Regis Technologies, Inc.

Typical chiral stationary phase (CSP) screening paradigms utilize a single alcohol component as co-solvent in its mobile phase. Recent unexpected observations have demonstrated that mixed-alcohol mobile phases can enhance or even introduce peak resolution when none existed in a mono-alcohol system.

The standard protocol for screening with alcohol co-solvents such as ethanol, methanol, and isopropanol is to execute the screen with just a single alcohol. In this particular example, ethanol and methanol were used individually to perform the initial screen. When either alcohol alone was used, there was no resolution suitable for a preparatory scale separation. Select mixtures of the two alcohols showed an unexpected separation of the enantiomers of the racemate sample (compound structure is confidential).

Unlike the unchlorinated RegisPack® amylose-based CSP, this study used the newer chlorinated (tris-(5-chloro-2-methylphenyl) carbamoyl amylose)-based RegisPack® CLA-1. The column dimensions were 25 cm × 4.6 mm using a 5 µm particle size.

Conclusion

Using neat alcohols will typically give acceptable peak resolution of a racemate, provided the CSP is appropriate. This particular compound, however, exhibited no peak resolution when using 100% of a single alcohol as co-solvent. Combinations of the two alcohols gave excellent baseline separations of the example compound. The solvent-CSP interaction, while not obvious, provides an interesting option when trying to develop conditions for difficult separations. Thus, when the two neat alcohols alone don't show promise, one should consider mixtures before ruling a screen unsuccessful.

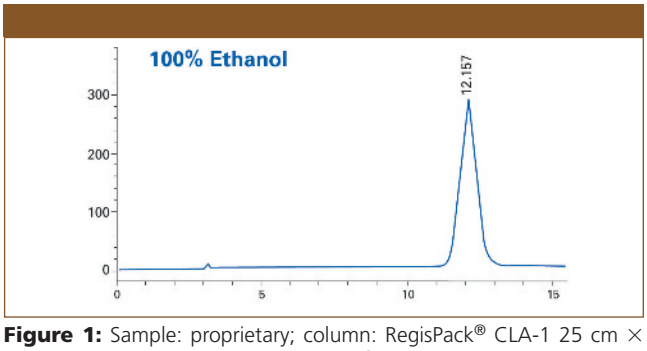


Figure 1: Sample: proprietary; column: RegisPack® CLA-1 25 cm × 4.6 mm; mobile phase: 100% ethanol; flow rate: 1.0 mL/min.

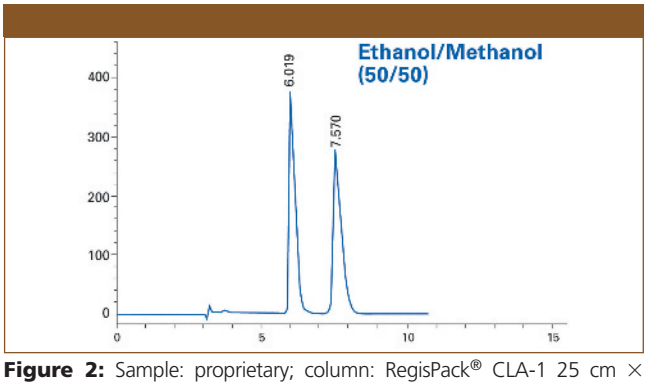


Figure 2: Sample: proprietary; column: RegisPack® CLA-1 25 cm × 4.6 mm; mobile phase: ethanol/methanol (50/50); flow rate: 1.0 mL/min.

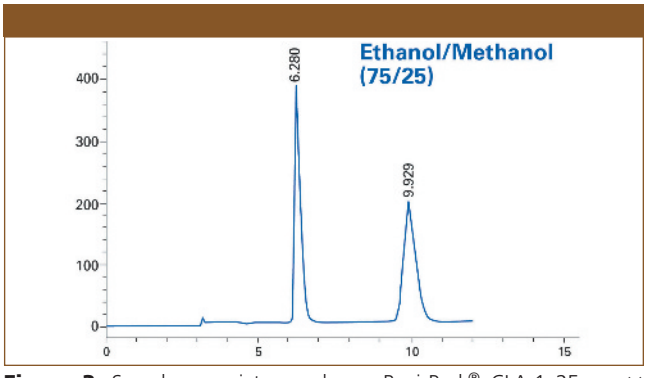


Figure 3: Sample: proprietary; column: RegisPack® CLA-1 25 cm × 4.6 mm; mobile phase: ethanol/methanol (75/25); flow rate: 1.0 mL/min.

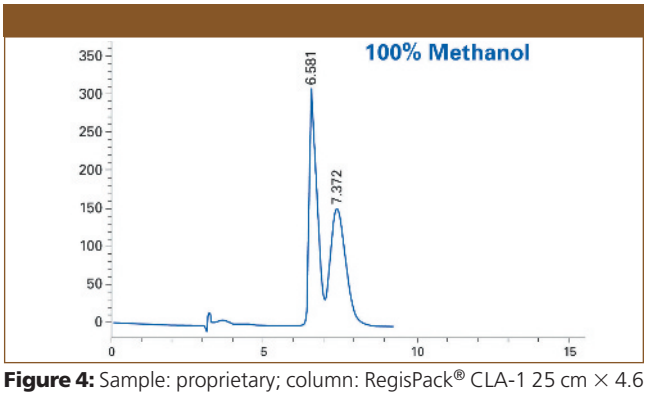


Figure 4: Sample: proprietary; column: RegisPack® CLA-1 25 cm × 4.6 mm; mobile phase: 100% methanol; flow rate: 1.0 mL/min.

Regis Technologies, Inc.
8210 Austin Ave, Morton Grove , IL 60053
tel. (847) 583-7661, fax (847) 967-1214
e-mail: teds@registech.com, Website: www.registech.com/chiral

Developing a UHPLC Method for UV-Based Detection and Quantification of Primary Aromatic Amines in Low Concentrations

Edgar Naegele[1] and Ruediger Helling[2], [1]Agilent Technologies Inc., Waldbronn, Germany, and [2]Saxon State Laboratory for Public and Animal Health, Dresden, Germany.

This application demonstrates that stringent sensitivity requirements for the detection of potentially harmful primary aromatic amines can be fulfilled when using an Agilent 1290 Infinity LC system equipped with the 1290 large volume injection kit.

Primary aromatic amines (PAAs) can originate from printing azo-dyes and azo-pigments. PAAs are potentially harmful and suspected to cause cancer; they have to be detected and determined.

Regulation (EC) 10/2011 sets a limit of 10 ppb for the sum of the released PAAs from plastic food contact materials. In the field of paper and board for food contact a similar limit is mentioned within BfR recommendation XXXVI. Moreover, the upcoming German regulation for printing inks used for food contact materials (Druckfarben V) negates the already introduced limit of 10 ppb for the sum of the released PAAs to all materials. Recently, problems have been reported within industry and enforcement authorities regarding the release of carcinogenic PAAs from heavily printed paper bags and napkins.

Since the legal limits apply to the sum of all PAAs, these compounds have to be detected down to a level of at least 1 ppb (1 ng/mL) for an individual PAA.

Experimental

To meet these requirements, two modifications were made to the standard configuration of the Agilent 1290 Infinity LC System. A 60-mm Max-Light high sensitivity flow cell was used with the 1290 Infinity Diode Array Detector. A 40-µL loop including the 1290 large volume injection kit to inject volumes up to 120 µL was used with the 1290 Infinity Autosampler.

The method that was finally applied was developed by using a mixture of six primary aromatic amines at 100 ng/mL: 1) aniline, 2) *o*-anisidine, 3) *o*-toluidine, 4) 2-methyl-5-nitroanilin, 5) 2, 4-dimethylanilin and 6) 2, 4-dichloranilin (Figure 1).

The method starts with an enrichment step for the first minute followed by a steep increase to the starting conditions at 20% methanol. The gradient separation was done in the following 13 min up to 70% methanol on an Agilent ZORBAX Eclipse Plus C18, RRHT, 3.0 × 100 mm, 1.8 µm column.

Results

With the final method, a calibration was done for all PAAs from 100 ng/mL down to a level of 2 ng/mL (2 ppb). All linearity correlation coefficients were above 0.99983. The concentration level at 2 ng/mL was defined as the limit of quantification (LOQ signal-to-noise ratio around 10). The limit of detection (LOD) was 0.5 ng/mL (signal-to-noise ratio around 3).

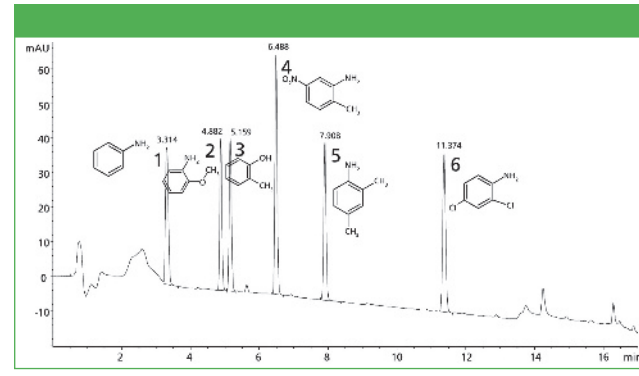


Figure 1: Primary aromatic amines at 100 ng/mL: 1) Aniline: 3.314 min, 2) *o*-Anisidine: 4.882 min, 3) *o*-Toluidine: 5.159 min, 4) 2-Methyl-5-nitroanilin: 6.488 min, 5) 2,4-dimethylanilin: 7.908 min and 6) 2,4-dichloranilin: 11.373 min.

A statistical evaluation was done on the 10 ng/mL level. The relative standard deviation (RSD) of the retention times was between 0.039% and 0.057%, the area RSDs were between 0.5% and 2.7%. Relative standard deviation of the compound response factors were between 0.004 and 0.017%.

To test the method, a blank matrix sample produced from a heavily coloured napkin was spiked with the six PAAs at various concentrations. All six PAAs could be well separated from the matrix compounds and identified and quantified with the developed method and calibration.

Conclusion

This application shows that the Agilent 1290 Infinity LC system can detect low amounts of primary aromatic amines by using the 60-mm Max-Light high sensitivity cell in the diode array detector, and the large volume injection kit in the autosampler.

Agilent Technologies Inc.
5301 Stevens Creek Blvd., Santa Clara, California 95051
tel. (800) 227-9770 (Directory), fax (866) 497-1134
Website: www.agilent.com

Automated Solid Phase Extraction (SPE) of Organochlorine Pesticides in Water

FMS Inc.

Organochlorine pesticides are man-made organic chemicals with a history of wide spread use in both the United States as well as globally. Tending to be very persistent in the environment, they have found their way into sediments and drinking water supplies posing serious health risks. Organochlorines have a wide range of both acute and chronic health effects, including cancer, neurological damage, and birth defects. Many organochlorines are also suspected endocrine disruptors.

In response to growing health concerns, the United States has banned several of these compounds such as DDT, dieldrin and chlordane. Others are still in use including lindane, endosulfan and methoxychlor.

Liquid-liquid extraction via a sep-funnel is the traditional method used for the extraction of organochlorine pesticides. Not only is it time consuming, it also requires a large volume of chlorinated solvent. The result is high cost and low reproducibility. The automated, solid phase extraction method described below allows for rapid, reproducible extractions using a minimal volume of solvent that produce consistent results.

Instrumentation

- FMS, Inc. PowerPrep™ SPE (solid phase extraction) system
- FMS, Inc. SuperVap™ Concentration
- FMS, direct-to-vial concentrator tubes
- 1 g C18 cartridges
- Agilent 7890A GC with uECD

PowerPrep SPE

1. The C18 Cartridge is conditioned with 10 mL methanol
2. The C18 Cartridge is conditioned with 10 mL DI H_2O
3. The sample is loaded onto the C18 Cartridge via vacuum
4. The sample bottle auto rinsed loaded on to the C18 cartridge
5. The C18 cartridge is dried with nitrogen
6. Elute with methylene chloride

SuperVap Concentrator

1. Pre-heat temp: 65 ºC; 2. Pre-heat time: 30 min; 3. Heat in Sensor mode: 65 ºC; 4. Nitrogen Pressure: 15 PSI

Figure 1: PowerPrep SPE and SuperVap Concentrator systems.

Procedure: Sample Prep and Extraction

1. Five, 1 L water samples spiked with 1 mL EPA 8081 surrogate spiking solution (2 analytes)
2. Samples were spiked with EPA 8081 pesticide spiking solution (20 analytes)
3. Samples allowed to equilibrate for 15 min
4. Five samples were loaded onto to corresponding sample ports on FMS PowerPrep SPE system.
5. The program is initiated to run each sample sequentially.
6. The sample is extracted and automatically transferred to the FMS SuperVap Concentrator with direct-to-vial vessels.
7. The sxtracts are concentrated using the SuperVap system to 1 mL exchanged to hexane (15 mLs) and re-evaporated to 1 mL.
8. The extract is removed from the SuperVap system and transferred to Agilent GC for analysis.

Table I: Mean recovery and standard deviation for five replicates

Compound	Spike Conc.	Avg. Rec.	STD Dev.
TCMX	.1 ug/L	70.0%	5.1%
Alpha-BHC	.1 ug/L	81.6%	2.0%
Beta-BHC	.1 ug/L	93.9%	4.7%
Gamma-BHC	.1 ug/L	83.1%	4.7%
Delta-BHC	.1 ug/L	98.9%	5.9%
Heptachlor	.1 ug/L	82.5%	5.0%
Aldrin	.1 ug/L	80.0%	4.5%
Heptachlor Epoxide	.1 ug/L	89.8%	5.2%
Gamma-Chlordane	.1 ug/L	81.0%	4.6%
Endosulfan I	.1 ug L	87.8%	4.7%
Alpha-Chlordane	.1 ug/L	82.9%	4.5%
Dieldrin	.1 ug/L	85.9%	4.7%
4,4"-DDE	.1 ug/L	84.0%	4.7%
Endrin	.1 ug/L	70.6%	5.3%
Endosulfan II	.1 ug/L	90.5%	4.8%
4,4'-DDD	.1 ug/L	81.7%	5.1%
Endrin Aldehyde	.1 ug/L	119.1%	5.9%
Endosulfan Sulfate	.1 ug/L	95.0%	5.1%
4,4'-DDT	.1 ug/L	96.2%	6.4%
Endrin Ketone	.1 ug/L	110.9%	5.8%
Methoxychlor	.1 ug/L	92.5%	6.1%
Deca-PCB	.1 ug/L	77.3%	4.1%

Conclusions

The results of five water samples demonstrate the ability of the FMS PowerPrep SPE system to deliver accurate results with excellent reproducibility. The automated SPE Direct to Vial Concentration method described is superior to traditional, time-consuming, inconsistent and expensive liquid-liquid extractions. The addition of the FMS SuperVap system equipped with direct-to-vial tubes enables the transfer of samples directly from sample bottles to GC vials in a single extraction process without handling the extract allows the extract to go directly to the GC for analysis.

FMS Inc.
580 Pleasant Street, Watertown, MA 02472
tel. (617) 393-2396, fax (617) 393-0194:
Website: fmsenvironmental.com

Simple and Rapid MRM Method Development for Analyzing 288 Pesticides by GC–MS-MS

Keyu Zhou[1], Helen Sun[2], and Kefei Wang[2], [1]Bruker Daltonics, Singapore, [2]Bruker, Division of Chemical & Applied Markets (CAM), Fremont, CA

Gas chromatography coupled with tandem mass spectrometry (GC–MS-MS) operated in MRM mode is a powerful technology for multi-residue analysis in complex food matrices. Good GC separation combined with MS-MS selectivity facilitates monitoring hundreds of MRM transitions within a short cycle time which allows for analysis of large number of compounds in complex food matrices. Nevertheless, MRM method development is time consuming and labor-intensive, which mainly comes from mapping retention time, MRM transition set-up and post-data processing.

In this study, an innovative software feature, compound-based screening (CBS), is introduced on a Bruker SCION™ TQ GC–MS-MS system for method development of 288 pesticide analysis. CBS is implemented with a built-in factory compound library containing optimized MRM transitions along with retention indices for hundreds of pesticides. With retention index (RI), retention time (RT) can be predicted from a small subgroup of pesticides from an initial run within a narrow retention time window, saving time and effort for searching the retention time over the entire run for each pesticide. After obtaining retention time, the scan time (dwell time) for each MRM can be optimized automatically based on the average peak width and desired scan points across each peak. Furthermore, the acquisition and data processing section are integrated within the new platform so that changes on either section can be updated automatically.

Experimental

Sample:

The standard pesticide mix was prepared by spiking 288 standard pesticides in 1:1 hexane/acetone solvent.

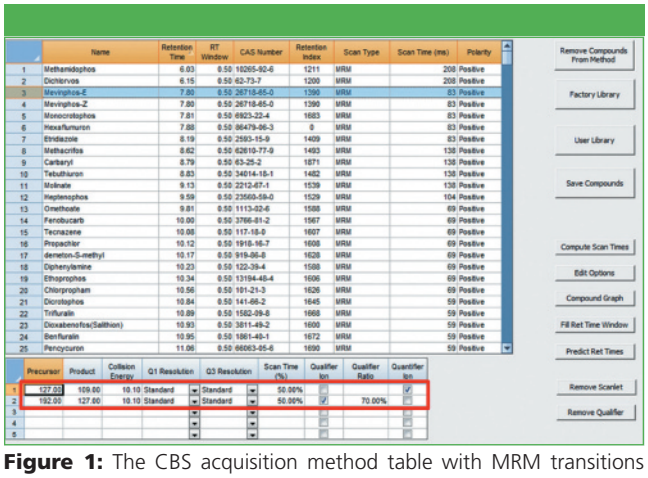


Figure 1: The CBS acquisition method table with MRM transitions linked to each pesticide.

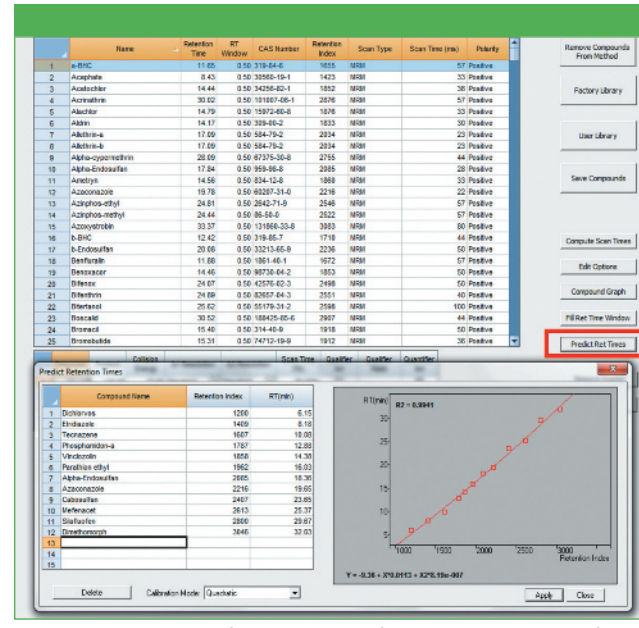


Figure 2: Prediction of retention time for all target pesticides from a subgroup of pesticides.

Instrument:

SCION™ TQ triple quadrupole mass spectrometer coupled to a Bruker 451 GC and CP 8400 Autosampler

GC-MS conditions:

Column: BR-5ms 30 m × 0.25 mm i.d. and 0.25 µm
Carrier gas: He at 1 mL/min
Oven: 80 °C (2 min), at 20 °C/min to 160 °C , at 5 °C /min to 280 °C, at 20 °C/min to 300 °C(5 min), total 36 min
Injection: Splitless, 280 °C, 1 µL
EI source Temp.: 260 °C
Transfer line Temp.: 300 °C
Emission Current: 80 µA
Q2: Ar (1.5 mTorr)

Results and Discussions

A factory-supplied compound library with optimized MRM transitions and retention indices has been implemented in Bruker MSWS 8, the data system for SCION TQ. More than 800 compounds, including more than 400 pesticides, are included in the library. The following illustrates the five-step quick approach for setting up an MRM method for 288 pesticides:

1) Select the 288 target pesticides in the mixture by name or CAS # from factory library to export to method table, the MRM table will be automatically populated (Figure 1).

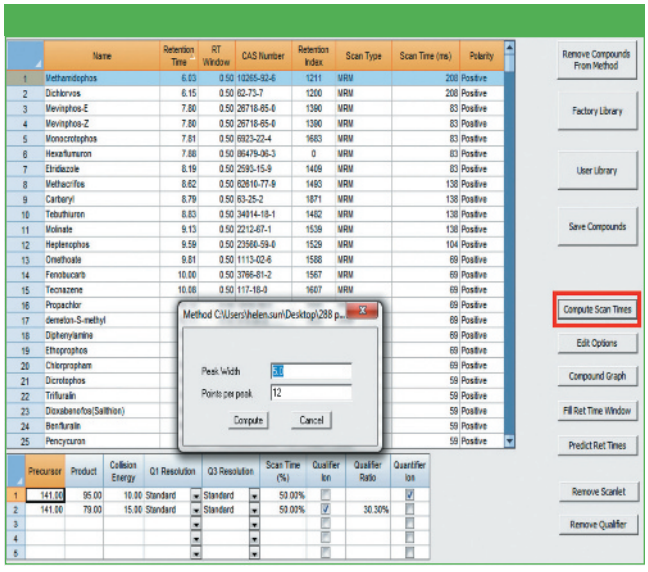


Figure 3: Optimization of dwell time for timed MRM.

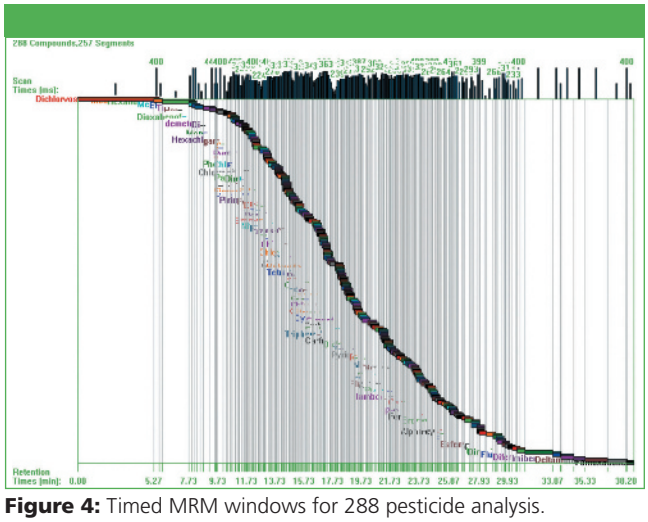


Figure 4: Timed MRM windows for 288 pesticide analysis.

2) Run a full scan of the pesticide mixture standard. Pick up 12 pesticides with identified retention times across the entire run. Click the Predict RT function, select to input this subset of pesticides with RT and RI, plot the RT as a function of RI and to select the best fit, click Apply to input the predicated retention times for all pesticides in the method acquisition table (Figure 2).
3) In the next couple of runs, locate the RT for the rest of pesticides as well as refine those RT that found in above step.
4) Compute the scan (dwell) time for MRM transitions of each individual pesticide by clicking "Compute Scan Time" button, the scan time for each MRM transitions/compound is automatically assigned with the optimized scan (dwell) time to schedule a timed MRM approach (Figure 3).
5) Update the method with the refined RT information. The method is ready for running calibration curves (Figure 4).

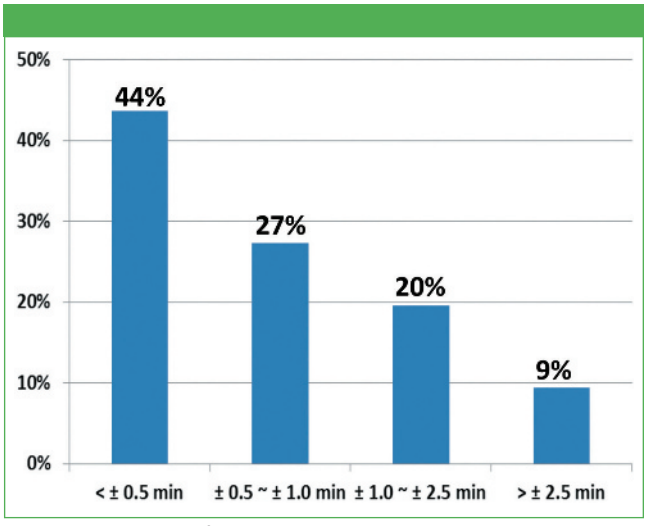


Figure 5: Statistics of predicted retention time vs. real retention time of 288 compounds.

Statistical analysis of predicted RT vs. experimental RT was carried out, and the result listed in Figure 5. As shown, 44% of 288 compounds are within ±0.5 min window from the predicted RT; 27% within a ±0.5–1 min window. A total of 91% pesticides fall within a ±2.5 min window, demonstrating adequate accuracy of the prediction model. The RT prediction function significantly simplifies MRM method development process for multi-residue analysis applications, which ultimately improves the work efficiency in the production lab.

Conclusions

Compound based screening (CBS) along with the factory built-in MRM library, retention time prediction, and dwell time optimization features in Bruker MSWS 8 significantly simplifies MRM method development for multi-residue analysis.

Bruker Daltonics
Billerica, MA
tel. (978) 667-3660, fax (978) 667-5993
Website: ww.bruker.com

The Extraction of Chlorinated Pesticides, Herbicides, and Organohalides for EPA Method 508.1 Using Automated Cartridge SPE

David Gallagher and Michael Ebitson, Horizon Technology, Inc.

This application note will highlight the extraction of EPA Method 508.1 analytes from an aqueous matrix. It will use the SmartPrep Extractor and the DryVap Concentrator system to achieve excellent recoveries while maintaining minimal user-interface.

Instrumentation

- Horizon Technology:
- SmartPrep™ Extractor (6 mL Configuration; 20 mL Tray)
- Bottle Rinse Kit
- DryVap® Concentration System with DryDisk® Membrane
- Phenomenex:
- Strata® C18-E, 6 mL Cartridges
- Zebron™ – MR 1: 30 m × 0.32 mm × 0.50 µm
- Zebron™ - MR 2: 30 m × 0.25 mm × 0.25 µm

Method Summary

1. Prepare 1 L of reagent water using HCl to lower the pH to 2.
2. After mixing, add surrogate solution and spike solution (for blank samples, add only surrogate solution).
3. Attach Sip Tube 1 and Bottle Rinse 7 to the sample container.
4. Place a 20 mL VOA vial in position 1 of the tray.
5. Place a 6 mL C18-E cartridge in position 1 of the carousel.
6. Program and run the EPA 508.1 Method with a N_2P1 pressure of 5 psi and a N_2P2 pressure of 10 psi.
7. Place a 1 mL endpoint Concentration Tube on the DryVap and attach a DryDisk reservoir with a membrane.
8. Transfer the contents of the VOA vial to the DryDisk Reservoir and start the DryVap using a sparge pressure of 20 psi and a vacuum of -10" Hg.
9. Upon completion, bring the volume up to 1 mL using EtOAc, transfer to a vial add internal, and run on a GC-ECD.

Results

A degradation check solution containing DDT and Endrin and was used for every analytical batch.

When performing the analysis, it was discovered that there were multiple sets of co-elutions. On MR-1, a co-elution between Simazine and Atrazine and another between d-BHC and Metribuzin were found. On MR-2, a co-elution between Heptachlor Epoxide B and the surrogate Dibrophenyl was found. In the first case, the co-eluting compounds cannot be reported from the MR-1 column. For MR-2, a different surrogate was selected to clear up the co-elution (Decachlorobiphenyl).

The average recovery for 12 extracts was calculated and is shown in Table I for each column used. With averages of 93% and the highest RSD being 17%, they show excellent results for all the compounds that are able to be reported on each column.

Table I: Average recovery of 12 LCS extracts

		Avg. (%) MR-1	RSD (%) MR-1	Avg. (%) MR-2	RSD (%) MR-2
Etridiazole		115	12.64	84	8.99
Chloroneb		90	5.23	68	6.70
Propachlor		92	9.59	86	8.31
Trifluralin		83	6.11	92	10.06
a-BHC		87	5.67	94	6.89
Lindane (g-BHC)		93	5.37	104	6.91
Simazine	1	N/A	N/A	94	9.60
Atrazine	1	N/A	N/A	95	7.05
b-BHC		94	3.99	103	5.74
d-BHC	2	N/A	N/A	114	7.75
Chlorothalonil		112	8.72	117	10.13
Metribuzin	2	N/A	N/A	83	8.44
Heptachlor		91	13.83	86	13.64
Alachlor		89	11.32	92	10.18
Cyanazine		119	8.60	94	4.48
Metolachlor		100	5.37	N/A	N/A
Dacthal		92	3.75	101	6.13
Heptachlor epoxide B		89	4.22	99	6.66
trans-Chlordane		84	4.93	91	7.37
Butaclor		105	6.81	95	8.19
cis-Chlordane		85	4.62	90	6.66
Endosulfan I		89	4.01	98	6.76
4,4'-DDE		78	7.19	85	9.74
Dieldrin		94	4.54	97	6.26
Endrin		97	6.56	100	7.74
Chlorobenzilate		70	12.60	92	10.99
Endosulfan II		92	4.14	99	8.15
4,4'-DDD		91	5.00	92	8.40
Endrin Aldehyde		90	4.28	92	8.90
4,4'-DDT		107	6.82	94	8.75
Endosulfan Sulfate		96	4.67	96	8.68
Methoxychlor		118	10.22	94	10.99
Permethrin		107	16.09	106	16.99
Decachlorobiphenyl	S	83	6.23	96	10.66
1,2-Co-Elutting compounds on MR-1					

Conclusion

The SmartPrep Extractor Cartridge system was used to extract EPA Method 508.1 compounds from water samples. When coupled with the DryVap Concentration system and DryDisk Separation Membranes, both excellent precision and accuracy was demonstrated. The solutions presented will allow a laboratory to streamline their aqueous extraction procedures and minimize the costs associated with labor and solvent while maintain the level of quality required.

Horizon Technology
45 Northwestern Drive, Salem, NH 03079
tel. (603) 893-3663; fax (603) 893-4994
Website: www.horizontechninc.com

A Broad Spectrum GC×GC–TOFMS Analysis for Endocrine Disruptor Compounds in a Midwestern U.S. Watershed

John Heim, Joe Binkley, and Jeff Patrick, LECO Corporation

Water samples from six urban and rural locations were extracted and analyzed by GC×GC–TOFMS. GC×GC–TOFMS provided an analysis approach for the detection of known EDCs as well as other non-targeted persistent pollutants.

There is increasing worldwide concern among scientists and the public over long-term environmental exposure to endocrine disruptor compounds (EDCs). Serious health effects to humans and global ecological systems have prompted the international community to organize expert groups and establish research in evaluation of EDC-related issues. This application note presents a practical, robust, reliable, and sensitive broad range analysis method for the trace-level detection of EDCs in impacted waterways using solid phase extraction followed by GC×GC–TOFMS.

Experimental Conditions

A solid-phase extraction (SPE) procedure was developed using 1-L aliquots of HPLC water spiked with a prepared 108-component EDC reference standard. The GC×GC–TOFMS method was developed using the reference standard. Thirteen 1-L extractions were conducted from six locations along a Midwestern U.S. watershed. Extractions were conducted using Supel-Select HLB-SPE cartridges (Supelco Analytical, Sigma-Aldrich). Duplicate sample injections by GC×GC–TOFMS analysis were carried out immediately after sample preparation. Data processing was performed with NIST and Wiley library mass spectral search and a "Reference" method developed within LECO's ChromaTOF® software.

GC×GC–TOFMS Analysis Parameters:

Gas Chromatograph: Agilent 7890
Primary Column: 30 m × 0.25 mm i.d. × 0.25 µm film thickness Rxi-5SilMS (Restek Corp.)
Secondary Column: 1.0 m × 0.18 mm i.d. × 0.18 µm film thickness Rxi-17SilMS (Restek Corp.)
Carrier gas: Helium @ 1.5 mL/min
Injection: 1 µL; Splitless Inlet temperature: 250 °C
Primary column temperature program: Initial temperature 75 °C for 1.0 min ramped @ 6.0 °C/min to 300 °C held for 10 min (secondary oven +5 °C)
GC×GC modulator temperature offset: 20 °C
Transfer Line Temperature: 280 °C
TOF Mass range: 35–800 u; Acquisition rate: 200 spectra/s
Source temperature: 230 °C
Total run time: 48.50 min

Results

Duplicate GC×GC–TOFMS analyses of 13 1-L aqueous extractions resulted in the detection of 102 chemicals that would be classified as EDCs. Chemicals found included pharmaceuticals, pesticides, polymer additives, coatings materials, personal care products, flame retardants, industrial by-products, and miscellaneous pollutants. Furthermore, results show that 81% of the 102 detected chemicals were found in five different water samples.

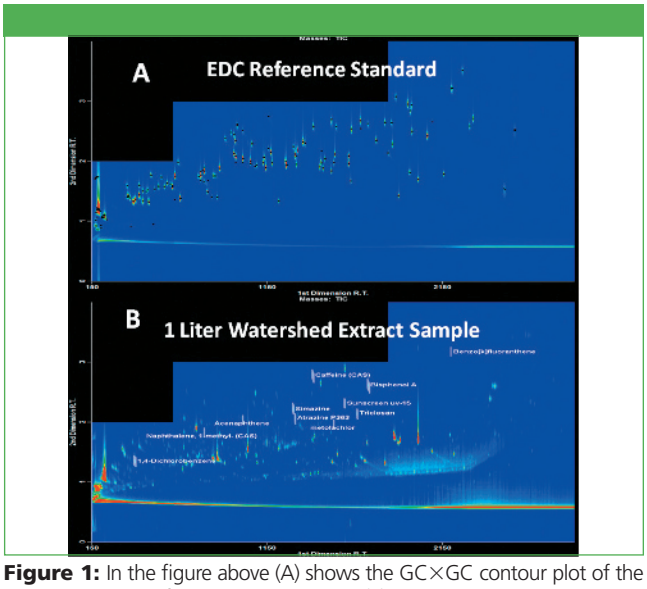


Figure 1: In the figure above (A) shows the GC×GC contour plot of the 108 component reference standard while (B) shows a 1-L extracted sample GC×GC–TOFMS analysis with selected target and untargeted analytes from the EDC study.

Conclusions

GC×GC–TOFMS was utilized in this application to detect 102 endocrine disruptors, and other pollutants in samples obtained from a Midwestern U.S. watershed. GC×GC increases peak capacity and facilitates enhanced detection while TOFMS allows fast non-targeted data acquisition necessary to successfully characterize trace levels of EDCs and other emerging contaminants.

Delivering the Right Results

LECO Corporation
3000 Lakeview Avenue, St. Joseph, MI 49085
tel. (269) 985-5496, fax (269) 982-8977
Website: www.leco.com

Improved Resolution of Triazine Herbicides in Drinking Water and Wastewater

Kory Kelly and Kali Tudela, Phenomenex Inc.

Often used for weed control, triazine herbicides have been found to have detrimental environmental and health effects. Much debate has focused on the level at which these compounds negatively impact health. To monitor and control human exposure to these herbicides, regulatory bodies have established allowable limits of triazines in drinking and wastewater.

In this study, two methods for triazine herbicide analysis are presented. They follow the Environmental Protection Agency's Method 536 for drinking water and Method 619 for wastewater, using LC–MS-MS and GC–MS respectively.

Experimental Conditions

For EPA Method 536, an Applied Biosystems API 3000 LC–MS-MS was used. Operating parameters are displayed in Table I. For EPA Method 619, an Agilent 6890/5975 was used. Operating parameters are displayed in Table II.

Results

LC–MS-MS provides the sensitivity needed to accurately quantitate and verify the identity of triazine herbicides in EPA Method 536. The conditions presented provide separation of the herbicides in less than 6 min, as shown in Figure 1. The narrow peaks observed result from the ultra-high efficiency of the Kinetex® 2.6 µm XB-C18 column. The short run time improves laboratory productivity and minimizes sample backlogs.

EPA Method 619 uses gas chromatographic means to detect a longer list of triazine herbicides by GC–MS, as shown in Figure 2. The Zebron™ ZB-50 column provides separation of all compounds, delivering confidence in qualitative and quantitative results.

Table I: LC–MS-MS operating conditions	
LC Column	Kinetex 2.6 µm XB-C18
Column Dimensions	50 x 2.1 mm
Mobile Phase	A: 5 mM Ammonium Acetate; B: MeOH
Flow Rate	0.3 mL/min
Gradient Profile	Please contact Phenomenex for details
Detection	Mass spectrometer (MS)
Note	SecurityGuard™ ULTRA Guard Cartridge system extends column lifetime

Table II: GC–MS operating conditions	
GC Column	Zebron ZB-50
Column Dimensions	30 m × 0.32 mm × 0.50 µm
Injection	Split 40:1, 1 µL @ 250 °C
Oven Program	150 °C to 250 °C at 4 °C/min for 5 min
Carrier Gas	Constant Flow Helium, 1.1 mL/min
Detector	Mass Selective (MSD)

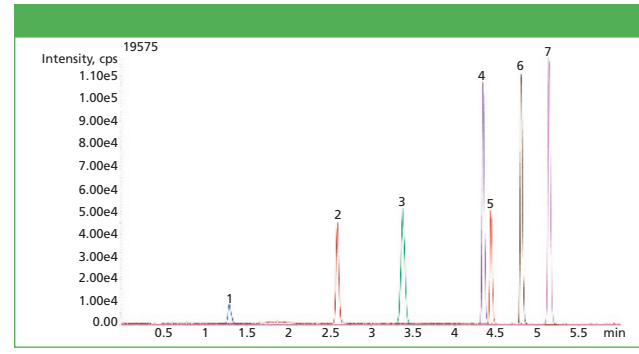


Figure 1: Resultant LC–MS-MS chromatogram of EPA Method 536 using Kinetex 2.6 µm XB-C18.

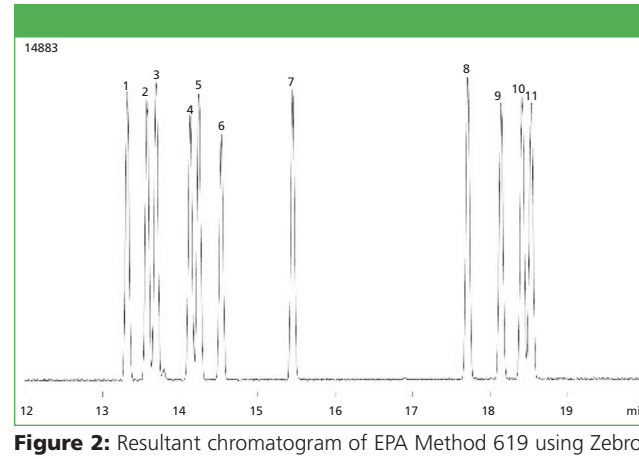


Figure 2: Resultant chromatogram of EPA Method 619 using Zebron ZB-50.

Conclusions

Two successful methods have been presented to monitor triazine herbicides. The narrow peaks achieved with the Kinetex 2.6 µm XB-C18 core-shell technology column increased throughput without sacrificing resolution for LC-based EPA Method 536. The Zebron ZB-50 column provides separation of all 11 triazine herbicides for GC-based EPA Method 619.

Phenomenex
411 Madrid Avenue, Torrance, CA 90501
tel. (310) 212-0555, (310) 328-7768
Website: www.phenomenex.com

Simplified Yet Sensitive Determination of Aniline and Nitroanilines

Xu Qun, Yang Xinlei, Li Lang, and Jeffrey S. Rohrer, Thermo Fisher Scientific Inc.

Aniline is an organic compound widely used in the polymer, rubber, pharmaceutical, and dye industries. Aniline and its derivatives (e.g., nitroanilines) are suspected carcinogens and are highly toxic to aquatic life. Because these compounds are thermolabile and polar, the traditional analytical methods require a derivatization step prior to GC analysis. Most of these procedures are time-consuming and complicated. HPLC analysis is a good alternative because derivatization is not needed. To gain enough sensitivity for UV detection, either liquid-liquid partitioning or off-line SPE must be used prior to HPLC analysis. Here an on-line SPE HPLC system is used to fulfill the simple and sensitive determination of aniline and four nitroanilines (*o*-nitroaniline, *m*-nitroaniline, *p*-nitroaniline, and *o,p*-dinitroaniline) in tap and pond water. Various trap columns and separation columns were tested (best performing are shown here).

Method Conditions and Sample Preparation

The experimental configuration, more experimental results, references, and sample preparation procedures are described in Dionex Application Note 292 (now part of Thermo Fisher Scientific Inc.).

Results

Linearity was tested using standards with concentrations of 1–100 µg/L undergoing on-line SPE under the specified chromatographic conditions (*r* value better 0.9999). Recoveries for each standard in both sample sets ranged from 98 to 108% for the 10 µg/L standard spiked samples, and ranged from 93 to 147% (contaminant excluded after DAD spectra review, pond water only) for the 1 µg/L standard spiked samples. The samples had no detectable aniline or nitroanilines. Method detection limits were 0.1–0.2 µg/L and are therefore 10–100 times more sensitive (analyte dependant) compared to the limits reported in EPA Method 8131. Reproducibility for a 10 µg/L spiked standard was very good with values better than or equal to 0.3% area RSD.

Chromatograms of tap and pond water samples, as well as the same samples spiked with aniline and related standards (1.0 µg/L each and 10 µg/L each, respectively), are shown in Figure 1. A Thermo Scientific Dionex SolEx on-line solid-phase extraction (SPE) HRP cartridge, 12–14 µm, 2.1 × 20 mm, was used for the enrichment. A Thermo Scientific Acclaim 120 C18, 3 µm Analytical, 3 × 150 mm column was used for the separation. Under the optimized chromatographic conditions, the complete analysis required 15 min (30 min including column reconditioning).

Conclusion

This work describes an on-line SPE system using the Dionex SolEx™ HRP cartridge to enrich aniline and nitroanilines, followed by HPLC with UV detection. The enrichment of aniline and nitroanilines in tap and pond water is sufficient, and baseline separation on the Acclaim™ 120 C18 column is achieved. With excellent linearity, sensitivity, and reproducibility, on-line SPE HPLC provides full automation, eliminates operator-related variation, and can help enforce strict process control.

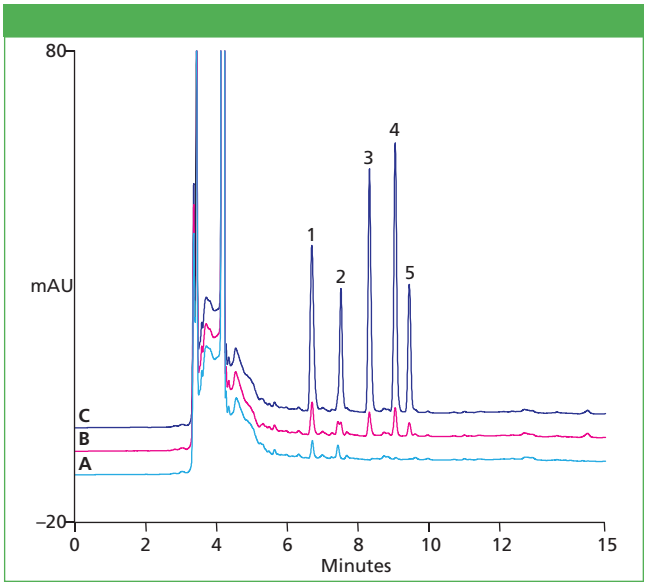


Figure 1: Chromatograms of A) pond water sample, B) A spiked with 1.0 µg/L aniline and nitroanilines standard, and C) A spiked with 10 µg/L. Peaks: 1. Aniline; 2. *p*-Nitroaniline; 3. *m*-Nitroaniline; 4. *o*-Nitroaniline; 5. *o,p*-Dinitroaniline

Receive the complete application note at: www.thermoscientific/AN292

Thermo Fisher Scientific Inc. (formerly Dionex Corp.)
1228 Titan Way, P.O. Box 3603, Sunnyvale, CA 94088-3603
tel. (800) 532-4752
Website: www.thermoscientific.com/dionex

Rapid On-Line-SPE HPLC Determination of Carbofuran and Carbaryl in Tap and Environmental Waters

Xu Qun and Jeffrey S. Rohrer, Thermo Fisher Scientific Inc.

N-Methylcarbamates are widely used agricultural pesticides. For their determination, reversed-phase high-performance liquid chromatography (RP-HPLC) with fluorescence detection following postcolumn derivatization, per U.S. EPA Methods 531.2 and 8318, is typically used. When using HPLC with UV detection, a sample preparation procedure — either liquid–liquid extraction or off-line solid-phase extraction (SPE) — is required to increase detection sensitivity. However, these procedures are time-consuming, require large volumes of organic solvents, and are deficient in terms of process control. This work describes an automated on-line SPE HPLC with UV absorbance method that provides a rapid determination of carbofuran and carbaryl, two of the most frequently used carbamate pesticides, without the need for postcolumn derivatization.

Method Conditions and Sample Preparation

The experimental configuration, sample preparation procedures, more experimental results, and references are described in Thermo Scientific Application Update 186.

Results

Linearity was tested using standards with concentrations of 0.5–100 µg/L undergoing on-line SPE under the specified chromatographic conditions (*r* value better 0.9999). The method showed excellent sensitivity, with detection limits better than those defined in the EPA Method 531.2 (carbofuran 0.062 µg/L, carbaryl 0.036 µg/L), in the standard method enacted by the Ministry of Health, People's Republic of China (7 µg/L for carbofuran), as well as other applicable U.S. and European drinking water regulations. Reproducibility for a 5 µg/L spiked standard was very good, with ca. ±1% area RSD.

Trace B in Figure 1 illustrates good separation and response for carbofuran and carabryl using a 2500 µL injection volume. Here, tap water was spiked with 1 µg/L carbofuran and carbaryl. Injection volumes larger than 2500 µL were not beneficial, as an overloading of the SPE cartridge was observed (Figure 1 Trace A). Spike experiments using tap, pond, surface, and farmland water from the Pudong District of Shanghai, China showed excellent SPE recoveries between 81–120% for a 2500 µL injection volume. Resolution between carbofuran and carbaryl was 3.5, exceeding the value required by the EPA Methods ≤1.0). A Thermo Scientific Dionex SolEx on-line SPE HRP cartridge, 12–14 µm, 2.1 × 20 mm, was used for the enrichment. A Thermo Scientific Acclaim 120 C18, 3 µm Analytical, 3 × 150 mm column was used for the separation. Under the optimized chromatographic conditions, the complete analysis required only 5 min.

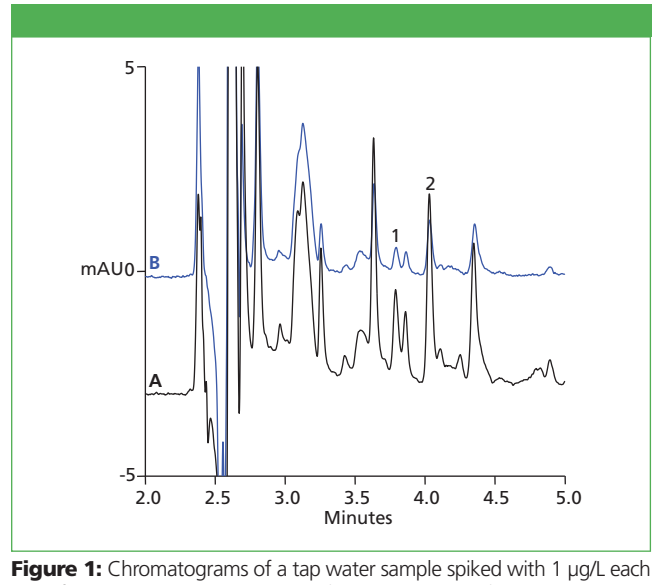


Figure 1: Chromatograms of a tap water sample spiked with 1 µg/L each carbofuran and carbaryl standards: A) 10,000 µL; and B) 2500 µL injection volumes.

Conclusion

Fully automated on-line SPE HPLC as optimized and illustrated here provided good selectivity and suitability for the rapid analysis of carbofuran and carbaryl in tap and environmental water samples. With excellent linearity, sensitivity, and reproducibility, on-line SPE HPLC provides full automation, eliminates operator-related variation, and can help enforce strict process control.

Receive the complete application note at: www.thermoscientific/AU186

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Thermo Fisher Scientific Inc.
1228 Titan Way, P.O. Box 3603, Sunnyvale, CA 94088-3603
tel. (800) 532-4752
Website: www.thermoscientific.com/dionex

High Precision Pesticide Analysis in Produce with GC Triple Quadrupole and U-SRM Mode

Inge de Dobbeleer, Joachim Gummersbach, Hans-Joachim Huebschmann, and Anton Mayer, Thermo Fisher Scientific Inc.

Residuals of pesticides widely used in agriculture are monitored in foods for human consumption through the use of statutory maximum residue levels (MRLs). Regulation EC 396/2005 adopted in the European Union sets MRLs for over 500 pesticides in over 300 food commodities, many at a default value of 0.01 mg/kg (the typical routine analytical method limit of determination). Thus, food safety laboratories must curb costs and turnaround times (often to <48 h) while testing a wide array of foods for a large number of pesticide residues at concentrations levels 10 lower than 0.01 mg/kg. This is most often achieved using multiresidue methods with a combination of LC–MS-MS and/or GC–MS techniques to determine pesticide residues in a single generic solvent extract of the sample.

One such example is the QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure, which is based on acetonitrile extraction and dispersive solid phase extraction. After the QuEChERS extraction, a solvent exchange is made to facilitate the GC injection. Although the QuEChERS extraction technique provides a fast turnaround time for a large number of samples with small sample volumes in the range of 10 g, the heavy matrix load of QuEChERS extraction requires increased robustness of the GC inlet system and increased selectivity from the MS-MS analyzer. Described here is a high-quality, low-level analysis of pesticides in produce samples using the Thermo Scientific TSQ Quantum XLS Ultra GC–MS-MS system.

For most of the pesticide compounds included in this method, the complete list of the compounds with their respective SRM transitions have been downloaded from the Pesticides Method Reference CD (provided with the manual, P/N 120390) into the instrument acquisition method. Each transition has been determined for optimal sensitivity and selectivity, with the complete list documented for TSQ Quantum XLS Ultra™ users.

Over 400 pesticides have been monitored in several matrices, such as wheat, black currants, and cucumber. The results of the most challenging pesticides in terms of activity and response are highlighted, showing calibration curves, repeatability, and ion ratio stabilities.

The TSQ Quantum XLS Ultra is able to perform SRM with a higher mass resolution (0.1 Da) setting to allow for better selectivity. Not all pesticides in all matrices benefit from a higher mass resolution setting, but depending on the matrix and the compound analyzed, there can be a significant improvement on the signal-to-noise ratio. Some examples are shown in the Advanced GC–MS-MS Experiment section of this application note.

Method Conditions and Sample Preparation

The experimental configuration, sample preparation procedures, more experimental results, and references are described in Thermo Scientific Application Note 52279.

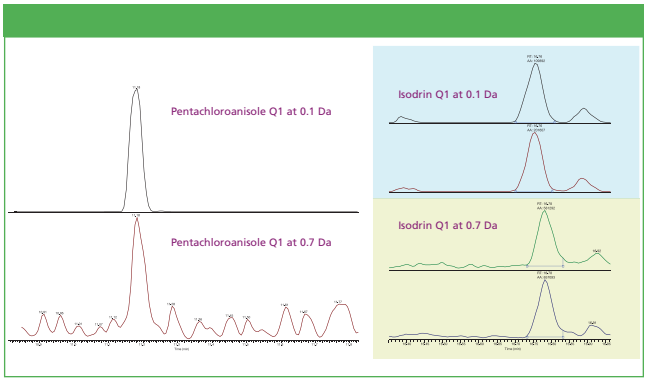

Figure 1: Comparison of U-SRM and standard SRM for pentachloroanisole and isodrin in wheat at 10 ppb level. Top: The chromatogram in U-SRM SRM (Q1 FWHM at 0.1 Da). Bottom: The same sample in standard mode (Q1 FWHM at 0.7 Da).

Conclusions

- Advances in HyperQuad technology offer increased analytical performance for routine applications such as pesticide analysis.
- A true multicompound method was developed for over 400 pesticides using timed SRM, easily transferable from a spreadsheet.
- A high level of accuracy and precision was reached during data evaluation on several cornerstones of analysis, such as repeatability, linearity and ion ratio stability.
- All examples shown are the more challenging pesticides faced analytically in terms of stability, activity, and response.
- This resolution technology development allows for advanced GC–MS-MS operations to be performed, such as U-SRM to further increase selectivity in complex matrices. This not only improves quantitative measurements, but it is also amenable when using a reduced sample cleanup typical for QuEChERS methodologies.

Receive the complete application note at: www.thermoscientific.com/AN52279

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Thermo Fisher Scientific Inc.
2215 Grand Ave. Pkwy, Austin, TX 78728
tel. (800) 532-4752
Website: www.thermoscientific.com/GCMS

Fast Petroleum Hydrocarbons Analysis for Underground Storage Tank Monitoring

Robert Wiedemer, Thermo Fisher Scientific Inc.

Underground storage tanks (USTs) for various petroleum-based substances such as gasoline, diesel fuel, and fuel oil are monitored for leaks as authorized by the Resource Conservation and Recovery Act (RCRA). Leaking USTs (LUSTs) contaminate ground water and soil. Besides the environmental impact, there are significant financial consequences associated with fines and cleanup. UST monitoring is usually done by gas chromatography with flame ionization detection (GC–FID). Because these petrochemical samples often have a wide molecular weight range, a temperature-programmed run is required to get adequate retention of higher volatility components and subsequently elute the high molecular weight components in the same chromatographic run. The chromatographic run time of these types of samples typically takes more than 20 min. Thermo Scientific UltraFast technology makes it possible to reduce the total analysis time to less than 4 min. A Total Recoverable Petroleum Hydrocarbons (TRPH) analytical standard mixture is representative of the molecular weight range typically found in UST samples.

Experimental Conditions

Thermo Scientific UltraFast TRACE GC Ultra Configuration
Column: UltraFast UFC-M1 Phase GC Column,
2.5 m × 0.1 mm × 0.4 µm
Oven: 50 °C (0.5 min hold) -340 °C (2 min hold) at 200 °C/min
Helium carrier gas at 0.5 mL/min

Results

Thermo Scientific UltraFast column technology can be used to achieve fast analysis of a wide molecular weight range of petroleum hydrocarbons such as those found in UST monitoring. Excellent separation of a mixture of even-numbered alkanes from C_8 to C_{40} was accomplished in less than 3.5 min. Analysis of these types of samples on a conventional GC capillary column requires >20 min. Since temperature programming is required for this analysis, the time needed for the GC oven to cool down to the initial temperature also needs to be considered. The time required to cool down the UltraFast from 340 °C to 50 °C for this analysis is approximately 90 s, where the time required to cool down a conventional GC oven for the same temperature range is approximately 4 min, thus further decreasing the overall run-to-run time. Laboratories doing UST analysis can do approximately five times more analyses per day using UltraFast technology versus conventional GC columns.

Conclusion

Analysis of sample containing a wide molecular weight range of hydrocarbons such as those found in underground storage tanks (UST) monitoring can be done in slightly more than 3 min with the use of a Thermo Scientific UltraFast column. This is approximately five times faster than doing the same analysis using conventional GC capillary columns.

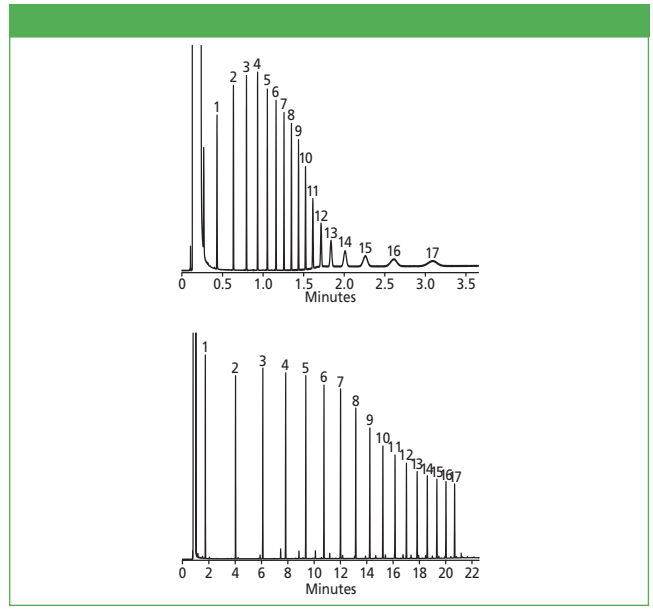

Figure 1: Top: Using UltraFast GC. Bottom: Using conventional GC.

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(2) Leaking Underground Storage Tank Program Implements The Recovery Act, EPA Information Related to the American Recovery and Reinvestment Act of 2009 (Recovery Act), U.S., EPA. www.epa.gov/oust/eparecovery/ (accessed June 12, 2012).

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Receive the complete application note at: www.thermoscientific.com/ANPETRO

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Thermo Fisher Scientific Inc.
320 Rolling Ridge Drive, Bellefonte, PA 16823
tel. (800) 532-4752
Website: www.thermoscientific.com

The Determination of Low Levels of Benzene, Toluene, Ethylbenzene, Xylenes, and Styrene in Olive Oil Using HS-GC–MS

Andrew Tipler, PerkinElmer®, Inc.

Levels of benzene, toluene, ethylbenzene, xylenes, and styrene (BTEXS) are a concern in olive oil. These compounds find their way into olive trees and hence into olives and olive oil mainly as a result of emissions from vehicles, bonfires, and paints into ambient air near orchards. This analysis uses a PerkinElmer® TurboMatrix™ 110 Headspace (HS) sampler with a Clarus® SQ 8 gas chromatograph–mass spectrometer (GC–MS) to achieve detection limits below 5 ng/g and concentrations up to 100 ng/g.

For this method, sample preparation is extremely easy. 10 g of olive oil is weighed into a standard headspace vial, then sealed with a crimped cap. The HS sampler heats the vial at 90 °C for 20 min. During this period the BTEXS components transition from the sample matrix into the vapor phase above. A fixed volume of the HS vapor is extracted from the vial and introduced into a Carbowax-type capillary column for GC separation. A quadrupole mass spectrometer is used to detect and quantify the BTEXS components. By using the MS single-ion monitoring (SIM) mode of operation, the detector sensitivity and selectivity is significantly enhanced and clean chromatography is obtained even at low analyte levels. The Clarifi™ SQ 8 MS detector is able to operate in SIFI mode in which both SIM and full scan data are collected. This enables library searches of MS spectral data to assist with confirmation of the identity of specific components while at the same time benefitting from SIM operation.

The analysis is fully automated and takes just 10.5 min for the chromatography plus 3.5 min for cool-down and equilibration between analyses. Sub-ppb levels are possible using standard headspace sampling of light aromatics in a complex natural oil matrix without the need for vapor pre-concentration (for example with an HS Trap).

The figure below shows typical chromatography obtained from a sample of cleaned olive oil fortified with approximately 17 ng/g of the BTEXS components. Note: the only sample that had low levels of BTEX was in the California sample. It is likely that the olive oil was produced by a solvent extraction method.

Excellent quantitative performance was demonstrated from ten samples of cleaned olive oil fortified with approximately 45 ng/g of the BTEXS components. Each was analyzed using the HS-GC-MS system and an overall quantitative precision of 1.69 to 3.76% relative standard deviation was obtained, which is a very good result for this complex matrix.

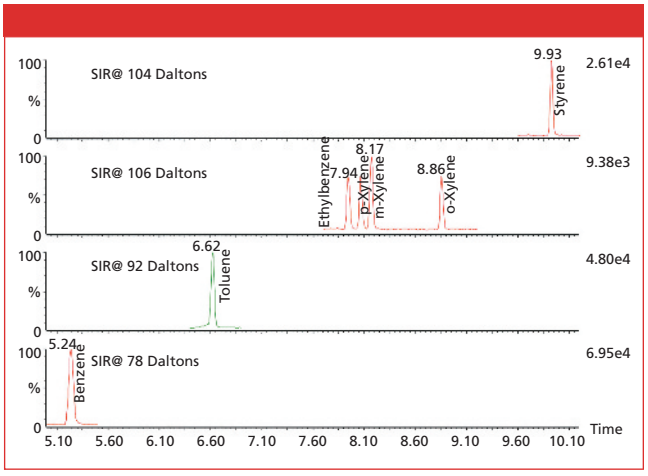

Figure 1: Chromatogram of 17 ng/g BTEXS in 10 g "cleaned" olive oil.

Seven different brands of olive oil were purchased from a local supermarket and analyzed using this method. The results are given in the table below. Alarmingly high levels of BTEXS components were found in many of the supermarket samples. The only sample that had low levels was one from California. Solvents were found in the California sample so it is likely that the olive oil was produced by a solvent extraction method rather than the more traditional olive-pressing method.

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tel. (800) 762-4000, fax (203) 944-4904
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Table I: Results from analysis different brands of supermarket samples

	Concentration in sample (ng/g)						
Sample Source(s)	**Benzene**	**Toluene**	**Ethylbenzene**	***p*-Xylene**	***m*-Xylene**	***o*-Xylene**	**Styrene**
California	0.89	5.86	1.66	1.45	5.24	3.77	3.07
Italy, Greece, Spain, Tunisia	2.86	27.55	6.12	5.86	16.73	8.75	41.34
Italy, Spain, Greece, Tunisia	3.07	24.22	13.47	7.85	23.64	13.97	39.59
Italy, Spain Tunisia, Turkey, Argentina	2.99	17.03	3.74	3.44	9.35	6.14	40.09
Spain, Argentina	2.43	34.99	7.22	7.42	18.97	10.65	126.11
Italy, Spain, Greece, Tunisia, Morocco, Syria, Turkey	4.09	35.71	19.13	17.10	59.31	28.10	61.05
Italy, Greece, Spain, Tunisia	1.25	2.79	ND	1.80	3.74	3.17	7.39

Comprehensive Pesticide Residue Monitoring in Foods Using QuEChERS, LC–MS-MS, and GC×GC-TOFMS

Julie Kowalski[1], Jack Cochran[1], Jason Thomas[1], Michelle Misselwitz[1], Rebecca Wittrig[2]*, and André Schreiber[3],
[1]Restek Corp. [2]AB SCIEX (*Current location: Restek Corporation),[3]AB SCIEX Research and Development, Ontario, Canada

- One fast, simple QuEChERS extraction for a broad range of pesticides.
- Rxi®-5Sil MS and Rtx®-200 column selectivity and orthogonality promote good GC×GC separations.
- Ultra Aqueous C18 LC column retains and gives excellent peak shapes for small polar pesticides.

Pesticide residue analysis of food has traditionally been performed using GC, but there is increasing use of LC with tandem mass spectrometry (MS-MS). LC is favored for polar, less thermally stable, less volatile, compounds. GC–MS is preferred for volatile, thermally stable species, and pesticides that do not ionize well in electrospray or atmospheric pressure chemical ionization LC sources. With MS, complete chromatographic resolution of compounds is not always essential, as selected ions or selected reaction monitoring (SRM) transitions are used for pesticide identification and quantification. However, data quality can be improved through better retention and separation of components, especially for structurally similar pesticides and high-level matrix coextractives. In the work summarized here, we employed a comprehensive approach and analyzed QuEChERS extracts of a variety of foods for pesticides by both GC×GC-TOFMS and LC–MS-MS.

Food commodities were fortified with pesticides and processed using Q-sep™ QuEChERS extraction salts and dSPE tubes. QuEChERS (quick–easy–cheap–effective–rugged–safe) is a sample preparation approach developed by Anastassiades et al. (1) as a simple, rapid, effective, yet inexpensive, way to extract pesticide residues from fruits and vegetables, followed by a dispersive solid phase extraction (dSPE) cleanup of the extract. The foods chosen varied in water, fat, and pigment content, so the ruggedness of QuEChERS as well as the performance of GC×GC-TOFMS and LC–MS-MS could be assessed. Commodities tested were red bell pepper, cucumber, black seedless grape, spinach, lemon, raisin, and hazelnut. In this summary, we report data for grape and lemon, the least complex and most complex of the matrices we assessed. Complete results are available at www.restek.com/comp-pest in the full application note.

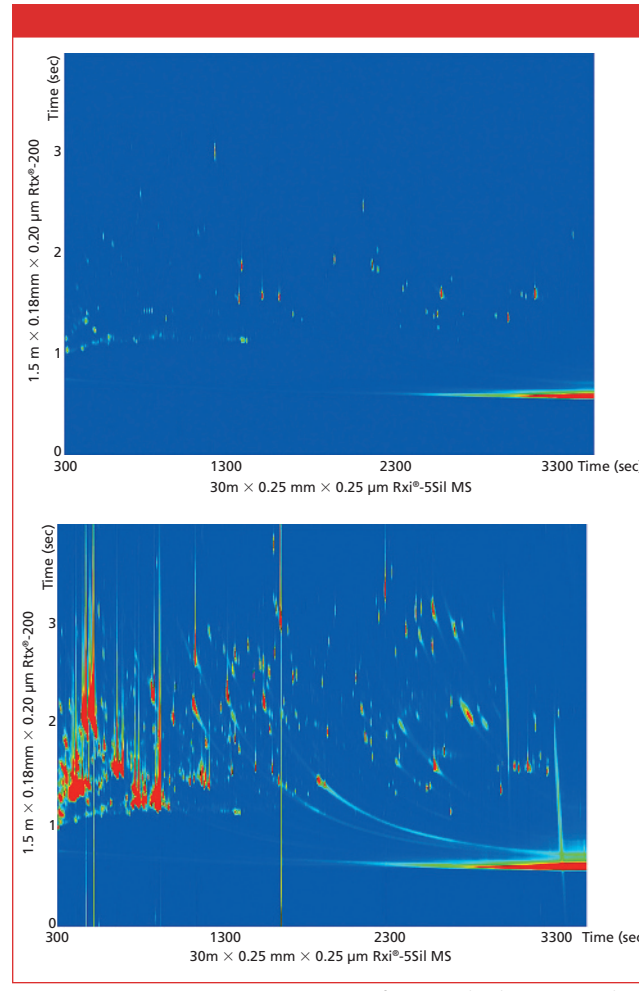

Figure 1: GC×GC-TOFMS contour plots for grape (top) and lemon (bottom) QuEChERS extracts. The lemon extract is much more complex than the grape extract and could not be analyzed by one-dimensional GC.

Column Selectivity and Multidimensional Techniques

We first assessed the complexity of different commodities by examining the total ion chromatogram (TIC) contour plots generated by GC×GC-TOFMS. It is clear from Figure 1 that lemon contains many more coextractives than grape, as demonstrated by the large number of intense (red) signals. While it should be possible to analyze QuEChERS grape extracts for pesticides by one-dimensional GC, multidimensional techniques (e.g., GC×GC–MS, GC–MS-MS, or LC–MS-MS) are necessary for samples as complex as lemon. Column selectivity is an important consideration in multidimensional techniques and the Rxi®-5Sil MS (cat.# 13623) × Rtx®-200 (cat.# 45001) column combination used here provided orthogonal separations that helped isolate target analytes from matrix interferences. Column selectivity is also important in LC–MS-MS methods because coelutions can be problematic if the analytes share MRM transitions. The Ultra Aqueous C18 column (cat.# 9178312) used for this work is both selective for small, polar compounds, showing good retention and peak shape, and has balanced retention for a large number of compounds that vary in physiochemical properties. More balanced retention reduces the number of MRM transitions being monitored at any point in time, and improves data quality by allowing more time to be spent on a smaller number of MRM transitions.

Evaluation of a Comprehensive Approach

Good recoveries were obtained for most pesticides in most commodities as determined by both GC×GC-TOFMS and LC–MS-MS. As shown in Table I, quantitative results for grape were excellent, but lemon proved to be a difficult matrix as demonstrated by the fact that 11 pesticides were not detected by LC–MS-MS and two pesticides had interfering compounds when using the GC×GC-TOFMS method. Given lemon's complexity, ion suppression from coelution with coextractives is likely the cause of the undetected compounds in the LC–MS-MS analysis. Similarly, coextracted matrix compounds likely caused the interference that prevented determination of propoxur and terbacil in fortified samples by GC×GC-TOFMS. While recovery results for most pesticides in most commodities demonstrate successful extract cleanup using dSPE, highly complex matrices will benefit from more exhaustive sample cleanup techniques, such as cartridge SPE (2). Incurred residues were also determined and the number of pesticides detected by each technique was comparable. However, there were some pesticides for which residue concentration could only be reported by either GC×GC-TOFMS or LC–MS-MS.

Table II: Percent recovery values for 10 ng/g fortified samples prepared using QuEChERS and analyzed by GC×GC-TOFMS and LC–MS-MS

Pesticide	Black Grapes		Lemon	
	GC×GC	LC	GC×GC	LC
Propoxur	92	110	INT	75
Methamidophos	170	73	79	66
Acephate	73	NA	88	NA
Propham	100	50	130	ND
1-Naphthol	95	NA	110	NA
o-Phenylphenol	91	NA	100	NA
Tebuthiuron	92	90	110	42
Omethoate	68	98	100	89
Dimethoate	93	91	100	79
Prometon	96	73	110	47
Terbacil	110	NA	INT	NA
Pirimicarb	98	NA	100	NA
Metribuzin	110	76	110	58
Fuberidazole	96	85	98	ND
Carbaryl	120	150	72	14
Metalaxyl	93	81	95	52
Terbutryn	100	79	99	4
Ethofumesate	110	120	81	19
Benthiocarb	85	NA	110	NA
Cyprodinil	99	86	91	ND
Thiabendazole	110	70	83	ND
Furalaxyl	130	85	110	37
Triadimenol	110	NA	100	NA
Doramectin	96	92	109	105
Siduron	98	96	120	35
Imazalil	NA	70	IP	IP
Fludioxonil	120	NA	96	NA
Myclobutanil	130	110	100	13
Buprofezin	IP	IP	94	24
Oxadixyl	120	90	97	40
Mepronil	120	91	100	ND
Carfentrazone ethyl	110	150	110	74
Fenhexamid	120	51	87	ND
Propargite	110	130	100	ND
Piperonyl butoxide	110	95	110	ND
Pyriproxyfen	96	100	99	ND
Fenarimol	89	NA	100	NA
Bitertanol	92	NA	110	NA
Prochloraz	78	80	100	ND
Pyraclostrobin	110	92	61	ND
Azoxystrobin	98	86	110	30
Epinomectin	127	131	92	100
Dimethomorph	90	98	97	25

IP = incurred pesticides NA = not analyzed
ND = not detected INT = affected by interferences

Conclusions

Use of both GC×GC-TOFMS and LC–MS-MS provides more comprehensive results for pesticide residue monitoring in food. The QuEChERS sample preparation approach using Restek Q-sep™ extraction salts and dSPE cleanup tubes worked well for a variety of pesticides and commodities. In general, good recoveries were achieved as determined by both GC×GC-TOFMS and LC–MS-MS. However, more difficult matrices like lemon may benefit from additional cleanup of sample extracts.

Acknowledgments

U.S. Food and Drug Administration/Center for Food Safety and Applied Nutrition; LECO Corporation.

References

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Restek Corporation,
110 Benner Circle, Bellefonte, PA 16823
tel. (800) 356-1688; (814) 353-1300
Website: www.restek.com

Fatty Acid Methyl Ester (FAME) Composition in Fish Oil by Capillary GC

Quadrex Corporation

The health benefits of fish oil in one's diet has been widely established. Common edible fish sources include salmon, trout, tuna, swordfish, and others. The omega-3 fatty acids, EPA and DHA, in dietary supplements (capsules) are primarily derived from smaller oily fish, notably anchovy, horse mackerel, bunker, capelin, and sand eel. These fish offer very little or no demand to normal human consumption. Fish oil from these sources is processed into fish meal, a cooked, dried, and milled material, which is then pressed and refined.

Fish oil capsules have become one of the major cornerstones of the nutritional supplement industry. Health benefits include the regulation of cholesterols (LDL and HDL) and triglycerides, as well as aiding in diabetes, blood pressure, and coronary heart disease. Analytical quality control of fish oil ensures the product reaching the market is unadulterated and has not oxidized.

Method used both as area % method and mg/g method as in European Pharmacopeia 2.4.22 and AOCS CE 1-89 and other pertinent collections.

Discrimination and contamination or breakdown of the long chain polyunsaturated fatty acids is the biggest challenge during the analysis. The official methodology is quite clear on its requirements to document separation and injection effects. What follows is the results of the chromatographic method shown.

Analytical Method

Injector temperature: 250 °C
Splitless time: 0.5 min
Carrier gas: 22 psi Helium
Column: 007-CW (bonded polyethylene glycol, USP G-16)
25 m × 0.25 mm i.d. × 0.20 µm film
(Quadrex Corp. Woodbridge, CT)
Oven program: 90 °C (2 min hold) - 30 °C/min to 150 °C
(3 °C/min) to 225 °C (6 min hold)
Flame Ionization Detector: 270 °C
Standard: Nu Check Prep 68D, (Nu-Check, Elysian, MN)

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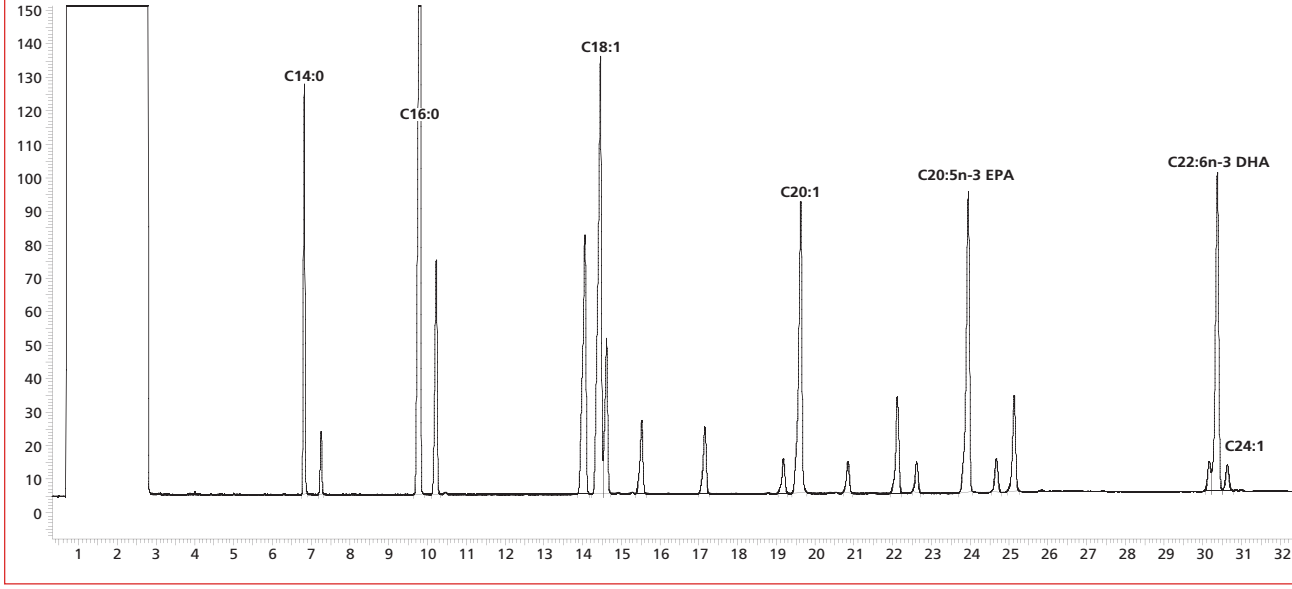
tel. (203) 393-3112, fax (203) 393-0391
Website: www.quadrexcorp.com



Chromatogram courtesy of Terje Aasoldsen, SAMSI AS, Langesund, 3970 Norway.

Acrylamide by QuEChERS Extraction with LC–MS-MS Detection

Brian Kinsella, UCT, LLC

Acrylamide is a neurotoxic compound classified as a probable human carcinogen and genotoxicant (1). In 2002, researchers in Sweden uncovered the presence of acrylamide in certain fried and baked foods at relatively high levels (2). Acrylamide is formed during cooking at high temperatures through the Maillard reaction of asparagine and reducing sugars. This application provides a quick and easy extraction of acrylamide from a variety of food matrices.

Extraction and Clean-up Materials	
ECMSSC50CT-MP	Mylar pouch contains: 4000 mg $MgSO_4$, 1000 mg NaCl
CUMPS15C18CT	Each 2 mL centrifuge tube contains: 150 mg $MgSO_4$, 150 mg PSA and 50 mg endcapped C18

Extraction Procedure

a) Add 5 g of homogenized sample to a 50 mL centrifuge tube
b) Fortify with internal standard
c) Add 10 mL of reagent water, vortex briefly
d) Allow ≥15 min for hydration
e) Add 10 mL of acetonitrile, vortex briefly
f) Add salts from Mylar pouch (ECMSSC50CT-MP)
g) Shake vigorously for 1 min
h) Centrifuge at 5000 rpm for 10 min
i) Supernatant is ready for cleanup

QuEChERS Cleanup

LC: Thermo Accela 1250 pump

- Column: Sepax C18, 150 mm × 2.1 mm, 3 µm
- Guard: Restek C18 2.1 × 20 mm
- Column Temperature: Ambient
- Injection volume: 20 µL
- Mobile Phase: A: water; B: methanol
- Gradient: 0–3 min: 100% A; 5–6 min: 100% B; 7–12 min: 100% A
- Flow Rate: 200 µL/min

MS/MS: Thermo TSQ Vantage

- **Ion source:** positive APCI

Analyte	Parent ion	Product ion
Acrylamide	72.0	55.0
	72.0	27.0
$^{13}C_3$-Acrylamide	75.1	58.03

Matrix (n = 5)	Fortified conc. (ng/mL)	Mean conc. found	RSD (%)	% Rec
Fries	50	53	11.56	106
	250	265	3.71	106
Potato chips	50	56	16.84	111
	250	257	12.54	103
Multigrain cereal	50	49	11.93	98
	250	232	3.97	93

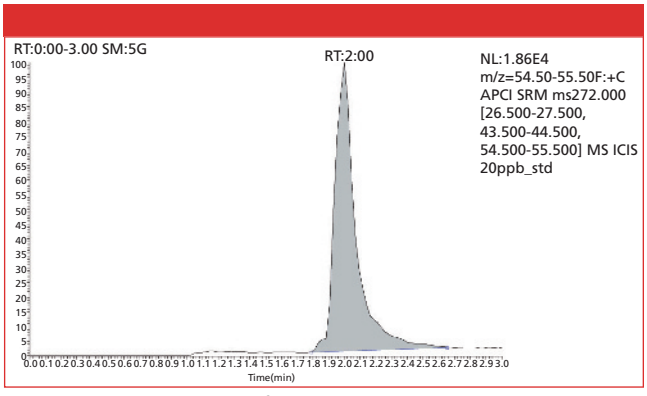


Figure 1: Chromatogram of a 10 ng/mL matrix-matched std.

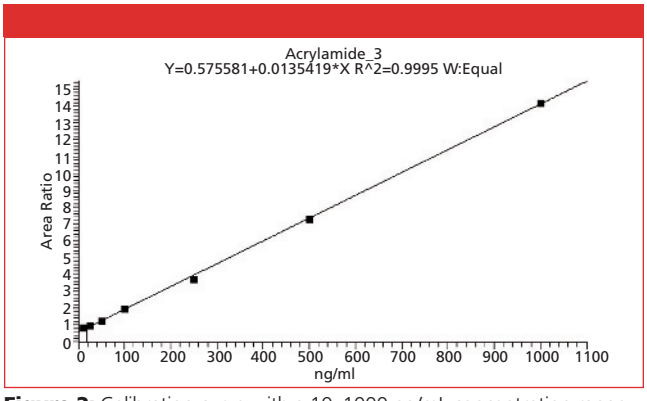


Figure 2: Calibration curve with a 10–1000 ng/mL concentration range.

Conclusions

A QuEChERS method has been successfully developed for the extraction and purification of acrylamide in various food matrices. PSA and C18 in the dSPE step eliminates the need for hexane defatting and results in clean extracts.

References

(1) Zhang et al., New research developments in acrylamide: analytical chemistry, formation mechanism and mitigation recipes, *Chemical Reviews* **109**, 4375–4397 (2009).

(2) Swedish National Food Administration, Information on acrylamide in food. April 24, 2002, www.slv.se.

UCT, LLC
2731 Bartram Road, Bristol, PA 19007
tel: (800) 385-3153; e-mail: methods@unitedchem.com
Website: www.unitedchem.com

QuEChERS Sample Preparation for LC–MS-MS Determination of Avermectins in Meat and Milk

Masayo Yabu, Mia Summers, and Michael S. Young, Waters Corporation

Avermectins are 16-membered ring macrolide drugs that are used as veterinary antihelmintics. The lowest allowable limit for these compounds in food products is set based on worldwide safety evaluations. Regulatory MRLs for avermectins can vary worldwide but are generally in the ppb concentration range. The sensitive analysis of avermectins in food products such as milk and meat can be challenging due to their complex sample matrices.

QuEChERS is a simple and straightforward sample preparation technique that involves a salting-out liquid extraction followed by optional dispersive solid phase extraction (dSPE). Sample preparation using QuEChERS allows for fast throughput and high sensitivity analysis of food products. Although QuEChERS is commonly used for multi-residue pesticide analysis in fruits and vegetables, it is also applicable in the analysis of veterinary drugs in livestock products. In this application note, milk and ground beef are prepared and analyzed for avermectins at the ppb level, using QuEChERS methodology and LC–MS-MS.

Instrumentation & Consumables

Sample Preparation:

Initial Extraction (QuEChERS):

Whole milk or ground beef was placed into a 50 mL centrifuge tube. 10 mL acetonitrile was added and the tube was shaken vigorously for 1 min. The contents of DisQuE pouch salts for CEN QuEChERS (p/n 186006813) was added and the tube was shaken again for 1 min. The sample was centrifuged and a 1 mL aliquot of the supernatant was taken for dSPE cleanup.

dSPE Cleanup

A 1 mL aliquot of supernatant was transferred to a 2 mL dSPE cleanup tube containing magnesium sulfate and C_{18} sorbent and shaken vigorously for 1 min. The sample was centrifuged and an aliquot was transferred to a Maximum Recovery Vial (p/n 600000670 CV) for LC–MS-MS analysis.

UPLC Conditions

System: ACQUITY UPLC

Column: XSelect CSH C_{18}, 2.1 × 100 mm XP 2.5 µm (p/n 186006103)

MS Conditions

System: Xevo TQ-S

Ionization mode: electrospray positive (ESI^+)

Results and Discussion

For the analysis of avermectins in challenging matrices like whole milk and ground beef, good recoveries were obtained using DisQuE pouch sample preparation. The incorporation of a dSPE clean-up step prior to LC–MS-MS analysis aids in the reliable quantitation of avermectins at low ppb concentrations in complex matrices.

Table I: Recoveries of avermectins from ground beef and whole milk samples

	Concentration Range (ppb)		Average % recovery (%RSD) *n*=5			
			ground beef		whole milk	
conc. level	low level	high level	low level	high level	low level	high level
Abamectin	1	10	94(3.6)	88(3.6)	86(14.0)	89(3.7)
Ivermectin	1	10	98(17.7)	85(3.1)	84(5.3)	83(14.8)
Doramectin	10	100	89(4.8)	85(4.2)	101(11.7)	90(5.0)
Epinomectin	10	100	99(2.9)	91(1.5)	94(3.9)	93(3.0)
Moxidectin	10	100	90(4.2)	87(1.8)	100(2.4)	90(5.6)

Table II: Matrix effects of avermectins from ground beef and whole milk samples.
A value > 100% indicates ionization enhancement
A value < 100% indicates ionization suppression

	Concentration Range (ppb)		Matrix effect(%)* *n*=5			
			ground beef		whole milk	
conc. level	low level	high level	low level	high level	low level	high level
Abamectin	1	10	105	96	109	93
Ivermectin	1	10	73	95	83	95
Doramectin	10	100	96	92	109	105
Epinomectin	10	100	127	131	92	100
Moxidectin	10	100	101	94	71	85

Conclusion

- The DisQuE pouch (QuEChERS) protocol is an easy and effective method for the extraction of avermectins from livestock products.
- DisQuE sample preparation provides excellent sample cleanup prior to LC–MS-MS analysis, with high analyte recoveries and minimal matrix effects.
- DisQuE extraction combined with LC–MS-MS analysis using eXtended Performance [XP] 2.5 µm columns provides low ppb quantitation of avermectins in complex matrices, enabling reliable testing.

For full application note, visit www.waters.com/4440.

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Determination of Sulfur in Natural Gas by ASTM Method D 6228-11

Laura Chambers and Gary Engelhart, OI Analytical

Natural gas and other gaseous fuels contain varying amounts and types of sulfur compounds which can be corrosive to equipment and can inhibit or destroy gas processing catalysts. Small amounts of sulfur odorants are added to natural gas and liquefied petroleum gases (LPGs) for safety purposes. Accurate measurement of sulfur species ensures proper process operation and odorant levels for public safety.

This application note describes the use of a pulsed flame photometric detector (PFPD) for determination of sulfur species in natural gas and LPGs by ASTM Method D 6228-11: Standard Test Method for Determination of Sulfur Compounds in Natural Gas and Gaseous Fuels by Gas Chromatography and Flame Photometric Detection.

Experimental Conditions

Instrumentation used for this study was an OI Analytical SPRO-Select GC system equipped with a 5380 Pulsed Flame Photometric Detector. Two capillary PLOT columns were evaluated: the Agilent J&W Select Low Sulfur column and Agilent GS-GasPro column.

Results

Two natural gas samples were blended for this project. Both contained five sulfur compounds at concentrations ranging from 3 to 6 ppmv, and one or more representative hydrocarbons found in different grades of natural gas. The composition of Sample #1 and its repeatability results on two different columns are shown in Table I.

Any capillary column that can provide adequate separation of the target sulfur compounds can be used with the PFPD for ASTM Method D 6228-11. The columns evaluated in this study were chosen because of their superior peak shape, excellent sensitivity for sulfur compounds, and retention time repeatability. Figure 1 illustrates the simultaneous sulfur and hydrocarbon chromatograms obtained from Sample #2 using an OI Analytical SPRO-Select GC system and Agilent Select Low Sulfur column. For complete results of this study, refer to OI Analytical Application Note #3671 (1).

Table I: Blended natural gas sample #1 and repeatability results on two GC columns

Compound	Concentration	Repeatability %RSD (n = 20)	
		GS-GasPro column	Select Low Sulfur column
COS	3.45 ppmv	1.2	2.1
H_2S	4.83 ppmv	2.9	2.5
DMS	4.11 ppmv	2.5	2.0
1-Propanethiol	5.92 ppmv	1.5	1.9
THT	4.46 ppmv	3.3	1.5
Methane UHP	Balance	-	-

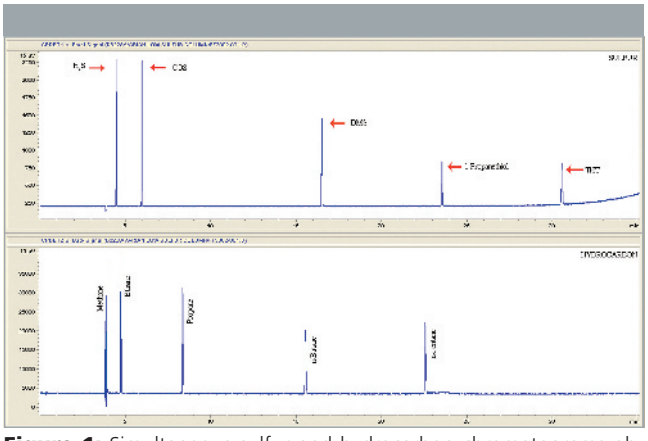

Figure 1: Simultaneous sulfur and hydrocarbon chromatograms obtained from a blended natural gas sample using the SPRO-Select GC system and Agilent J&W Select Low Sulfur column.

Conclusions

The SPRO-Select GC system equipped with a PFPD detects and measures sulfur species in natural gas by ASTM Method D 6228-11 with a high level of precision and accuracy, meeting all method requirements. Both capillary PLOT columns evaluated in this study yielded reproducible chromatograms with symmetric peak shape and chromatographic resolution of the sulfur and hydrocarbon peaks of interest.

References

(1) OI Analytical Application Note #3671, "Determination of Sulfur in Natural Gas by ASTM Method D 6228-11 Using a Pulsed Flame Photometric Detector (PFPD)."

OI Analytical
P.O. Box 9010, College Station, TX 77842
tel. (800) 653-1711, or (979) 690-1711, fax (979) 690-0440
Email oimail@oico.com, Website: www.oico.com

A Single-Column Capillary GC Application for the Determination of Benzene in Reformulated Gasoline

Katherine K. Stenerson, Leonard M. Sidisky, and Michael D. Buchanan, Sigma-Aldrich/Supelco

The amount of benzene in gasoline is a concern because it is a known human carcinogen, and exposure to it has been linked to detrimental health effects. The challenge with the analysis lies in the complex composition of gasoline, which consists of hundreds of different compounds. Reformulated gasoline also contains additives to produce more complete combustion and subsequent lower emissions of harmful compounds. These additives accomplish this by boosting the oxygen content, and are commonly referred to as "oxygenates." Ethanol is a commonly used oxygenate, often added to a 10% level. Therefore, to measure benzene in reformulated gasoline, it must be resolved from the C5-C12 aliphatic portion, other aromatics, and also ethanol. This typically requires the use of a two-column switching procedure (1).

Figure 1 shows the analysis of a reformulated gasoline sample on the extremely polar SLB®-IL111 capillary column, which resulted in the elution of benzene after the aliphatic portion, and also resolution of benzene and ethanol. Additionally, the phase stability of the SLB-IL111 column exhibits a stable baseline when subjected to a temperature ramp. Because this column can be used up to 270 °C, it also allows the timely elution of the heavy aromatic constituents in gasoline. These observations indicate that the SLB-IL111 may be an effective alternative to the two-column switching procedure currently required for the determination of benzene and other aromatics in reformulated gasoline. Complete specifications for this column are listed in Table I. Visit sigma-aldrich.com/il-gc to learn more about Supelco ionic liquid GC columns.

Table I: SLB-IL111 specifications

- Application: This extremely polar ionic liquid column was the world's first commercial column to rate over 100 on our GC column polarity scale. As such, it has the most orthogonal selectivity compared to commonly used non-polar and intermediate polar columns, providing increased selectivity for polar and polarizable analytes. Its temperature limit of 270 °C is very impressive for such an extremely polar column. The 60 m version is excellent at resolving benzene and other aromatics in gasoline samples. The 100 m version is suitable for detailed cis/trans FAME isomer analysis, and is a great complementary column to the SP-2560. Launched in 2010.
- USP Code: None
- Phase: Non-bonded; proprietary
- Temp. Limits: 50 °C to 270 °C (isothermal or programmed)

Reference

(1) ASTM D3606, Benzene and Toluene in Unleaded Gasoline and Aviation Fuel.

Sigma-Aldrich/Supelco
595 North Harrison Road, Bellefonte, PA 16823
tel. (800) 359-3041, (814) 359-3041
Website: www.sigma-aldrich.com/analytical

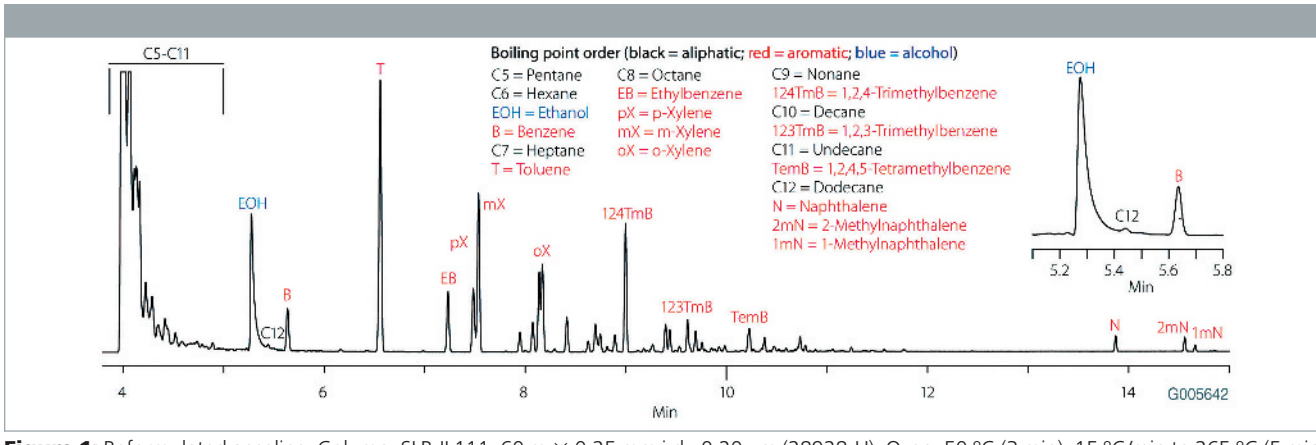

Figure 1: Reformulated gasoline. Column: SLB-IL111, 60 m × 0.25 mm i.d., 0.20 µm (28928-U), Oven: 50 °C (3 min), 15 °C/min to 265 °C (5 min), Inj. Temp.: 250 °C, Detector: FID, 265 °C, Carrier Gas: helium, 30 cm/s, Injection: 0.5 µL, 200:1 split, Liner: 4 mm i.d., split type, single taper wool packed FocusLiner™ design, Sample: reformulated gasoline.

Rapid and Sensitive Determination of Biofuel Sugars

Lipika Basumallick and Jeffrey S. Rohrer, Thermo Fisher Scientific Inc.

Biofuels have emerged as an attractive alternative to fossil fuel. Biofuel refers to any fuel that is derived from biomass (typically, plant and animal waste). A common feedstock for bioethanol production is corn stover (leaves and stalks of maize plants left after harvesting). Corn stover processing involves acid treatment to release a mixture of water-soluble carbohydrates, followed by enzymatic reactions to convert the sugars to ethanol.

This work describes a rapid method based on high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) for determining the sugars in corn stover acid hydrolysate. This method is capable of analyzing high concentration samples encountered in biofuel processing with minimal sample treatment.

Equipment

Biofuel sugars were separated on a Thermo Scientific Dionex ICS-5000 ion chromatography system with a Thermo Scientific Dionex CarboPac SA10 column set and electrolytically generated 1 mM hydroxide at 1.5 mL/min, and were detected by PAD. An injection volume of 0.4 µL (using an internal loop valve) with a 15 mil working electrode gasket is recommended for this method. Samples were prepared according to procedures described in Thermo Scientific Application Note 282 (1).

Results and Discussion

Biomass samples typically have sugar concentration in the range 100–200 mg/mL, and sugars are analyzed after a 100- or 150-fold dilution. Figure 1A shows the resolution of the common biomass sugars in 8 min, and Figure 1B shows the sugars in diluted acid-hydrolyzed corn stover. The high concentration of xylose indicates that corn stover is rich in hemicellulose.

This method exhibited linear peak area response for the biofuel sugars in the range 0.4–2 mg/mL (coefficients of determination were between 0.9984–0.9989). For sugars that are present at low concentrations, an appropriate calibration range should be selected. To achieve a wider linear range, this method can also be used with post-column addition (using a mixing tee) of more concentrated hydroxide (100/200 mM) to the eluent stream. Postcolumn addition can be made using the second pump of the ICS-5000 DP module.

Intra- and between-day precision (RSD) for retention time (RT) ranged from 0.09–1.5%, and peak area RSDs were 0.4–8.0% for sample analysis. Average recovery for the sugars ranged from 69–112%. The aforementioned precisions and recoveries suggest that this method can be used for complex biomass samples.

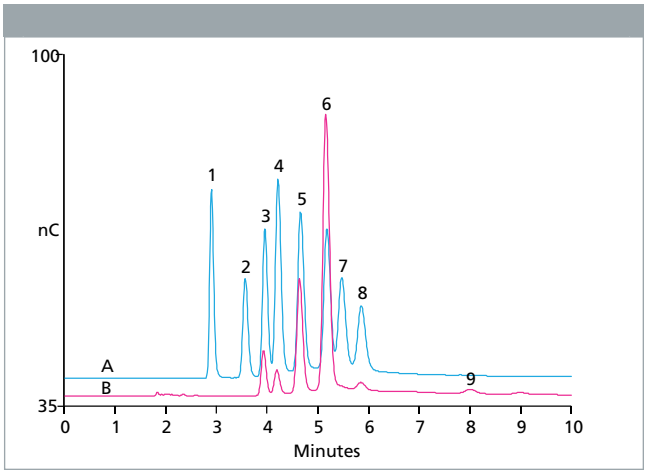

Figure 1: Separation of A) biofuel sugars (0.5 mg/mL) and B) acid-hydrolyzed (diluted 100-fold) corn stover sample. The sugars are: 1) fucose, 2) sucrose, 3) arabinose, 4) galactose, 5) glucose, 6) xylose, 7) mannose, 8) fructose, and one of the later-eluting peaks could be cellobiose.

Reference

(1) L. Basumallick and J.S. Rohrer, Thermo Scientific Application Note 282: Rapid and Sensitive Determination of Biofuel Sugars by Ion Chromatography. Sunnyvale, CA. (Online) www.dionex.com/en-us/webdocs/113489-AN282-IC-Biofuel-Sugars-03May2012-LPN2876.pdf (accessed May 29, 2012).

Receive the complete application note at: www.thermoscientific/AN282

Thermo Fisher Scientific Inc.
1228 Titan Way, P.O. Box 3603 Sunnyvale, CA 94088-3603,
tel: (800) 532-4752
Website: www.thermoscientific.com/dionex

Semi-Quantitative Determination of Volatile Oligomers of Halogenated Compressor Oil in a Manufacturing Process Using a Person Portable GC–MS

Tiffany C. Wirth[1], Tai V. Truong[1], Charles S. Sadowski[1], Douglas W. Later[1], Dan Vassilaros[2], and Jean Baldwin[3],
[1]Torion Technologies Inc.; [2]Vassilaros Consulting; [3]Intertek Chemicals & Pharmaceuticals

During the manufacturing process of industrial gases, volatile oligomers of compressor fluids can enter the product stream. These impurities are typically within specification, but failure of a diaphragm or seal can spike the concentration in a fill batch and the industrial gas no longer meets product specifications. A screening method has been developed to extract, identify and quantitate chlorotrifluoroethylene-based impurities from the compressor fluid using a CUSTODION™ solid phase micro-extraction (SPME) syringe and a TRIDION™-9 person-portable gas chromatograph–toroidal ion trap mass spectrometer (GC-TMS). The compounds were separated and detected rapidly in less than 5 min analysis time.

In this screening method a CUSTODION SPME syringe offers a unique sampling capability where the syringe needle can be exposed to the gas containers via an appropriate interface. The SPME fiber concentrates trace amounts of the volatile components of compressor fluid. Using a TRIDION-9 GC–MS to analyze samples is fast and decision making can take place during the fill process or at least before the product container is sent to a customer. The SPME-GC–MS method can provide contaminant trace analysis at ppb level, which is typically below the lower limit of acceptance for impurities in industrial gases. This analytical method can save time and money by eliminating rework, and can assure the customer that the product meets the specification.

Experimental Conditions

Bromopentafluorobenzene (BPFB) and dibromotetraflurobenzene (DBTFB) were spiked into a Tedlar™ bag at known vapor concentrations, and were used as reference standards. Liquid compressor oil samples were weighed into vials that simulated gas containers. The volatile oligomers were allowed to equilibrate between the liquid and vapor phases (air, at ambient pressure) for 24 h. Both the standards and sample analytes were extracted from the gas phase at ambient temperature for 2 min using a CUSTODION SPME syringe with a 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber.

Following each sample extraction, the SPME syringe was inserted into the TRIDION-9 GC-TMS injection port where the target analytes were desorbed into a split-splitless injector (280 °C) coupled with a low thermal mass metal-clad capillary GC column (MXT-5, 5 m × 0.1 mm, 0.4 µm df). After an initial 10 s hold at 40 °C, the GC temperature was increased at 2 °C/s to 280 °C for a total run time of 2 min and 20 s. The capillary GC is coupled to a TMS detector having a mass range of 45–500 m/z.

Total ion chromatogram area of the samples and standards were used for quantitation. Semi-quantitative results are often applied for in-field measurements. The procedures for in-field calibration are designed to be simple with minimal preparation and generate a level of data quality that provides actionable results.

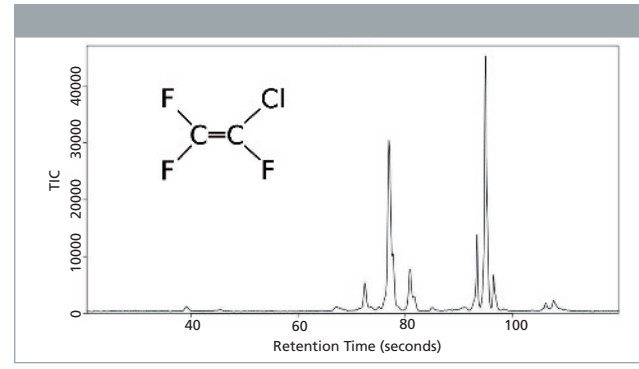

Figure 1: Total ion chromatogram of the volatile chlorotrifluoroethylene oligomers (sample 2).

Results

Figure 1 shows the GC-TMS analysis of the volatile chlorotrifluoroethylene oligomers samples.

Conclusions

The CUSTODION SPME syringe and TRIDION-9 GC-TMS are uniquely suited for rapid quality assurance analysis of product gases for compressor oil oligomers and other organic impurities in the gas phase. This method can be performed at the filling location allowing decisions regarding product purity and filling process integrity be made in real-time. The ~5 min total analysis time makes it possible to collect additional samples within the timeframe of the batch fill.

Acknowledgments

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Torion Technologies Inc.
796 East Utah Valley Drive, Suite 200, American Fork, UT 84003
tel. (801) 705-6600
Email: information@torion.com, Website: www.torion.com

Fast Separation of Eleven Nitroaromatic Compounds on ZirChrom®-CARB

Clayton McNeff, PhD and Kelly Johnson, ZirChrom Separations, Inc.

This technical bulletin details the separation of 11 closely related nitroaromatic compounds, namely RDX, HMX, Nitrobenzene, 2-Nitrotoluene, Tetryl, 2,6-Dinitrotoluene, 4-Nitrotoluene, 1,3-Dinitrobenzene, 2,4-Dinitrotoluene, 2-amino 4,6-dinitrotoluene, 1,3,5-Trinitrobenzene. Our customers have reported that similar separations on silica-based phases produce run times as long as 30 min. Here we report a method on ZirChrom®-CARB at a column temperature of 125 °C in under 4 min.

The rapid and accurate detection of nitroaromatic compounds is difficult due to the structurally similarity of the compounds. The unique surface chemistry of ZirChrom®-CARB enables reversed phase, ion exchange and pi-pi bond interactions. This multi-modal selectivity makes the ZirChrom®-CARB phase an excellent choice for the analysis of structurally similar compounds.

The ZirChrom®-CARB phase is stable up to temperatures of 200 °C. Performing separations at elevated temperatures reduces mobile phase viscosity and improves mass transfer. The resulting decrease in backpressure allows for faster flow rates and translates into significantly shorter run times without the loss of sample resolution.

Experimental

A mixture of nitroaromatics was separated at 125 °C using a ZirChrom®-CARB column (aee Figure 1). The separation conditions were as follows:

Column:	ZirChrom®-CARB, 150 mm × 4.6 mm i.d., (part # ZR01-1546)
Mobile phase:	Isocratic Pre-mixed 35/15/50 Acetonitrile/ Tetrahydrofuran/20 mM ammonium carbonate pH 5.7, 10 mM octylamine
Flow Rate:	2.0 mL/min
Temperature:	125 °C
Detection:	254 nm
Inj. Volume:	1 µL

This separation allows for clear identification of these compounds without the use of expensive MS detection. The separation is also completed using isocratic conditions, thus facilitating a more reproducible transfer from LC to LC.

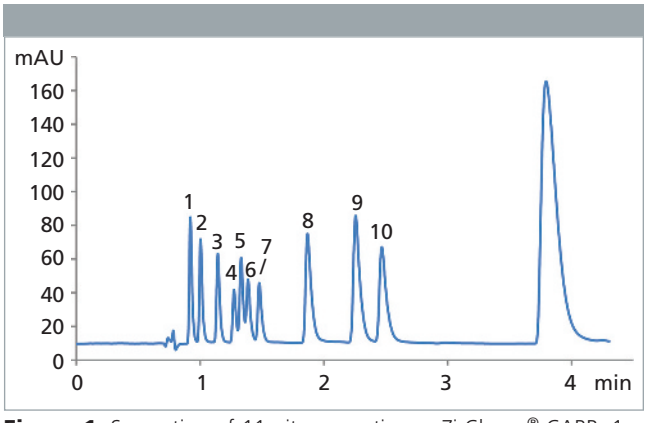

Figure 1: Separation of 11 nitroaromatics on ZirChrom®-CARB. 1 = RDX, 2 = HMX, 3 = Nitrobenzene, 4 = 2-Nitrotoluene, 5 = Tetryl, 6 = 2,6-Dinitrotoluene, 7 = 4-Nitrotoluene, 8 = 1,3-Dinitrobenzene, 9 = 2,4-Dinitrotoluene, 10 = 2-amino 4,6-dinitrotoluene, 11 = 1,3,5-Trinitrobenzene.

Want to learn more about the unique selectivity and stability of ZirChrom phases? ZirChrom is pleased to announce our new, free, on-demand webinar series: ChromU. Webinar topics include the use of elevated temperature and ZirChrom carbon phases. ChromU webinars are, on average, less than 10 min in length and include links to helpful supplemental application notes and newsletters. ChromU is available 24 hours a day, seven days a week on the ZirChrom website www.zirchrom.com. Find your ZirChrom solution today!

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tel.1-866-STABLE-1
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Fast Screening Methods for Steroids by HPLC with Agilent Poroshell 120 Columns

William Long, Agilent Technologies

Selectivity is the most powerful tool to optimize separations in HPLC. This parameter is changed by using different bonded phases or by changing the mobile phase. In this work, Poroshell 120 columns with highly efficient 2.7 µm superficially porous particles and the Agilent 1260 Infinity Series LC Multi-Method Solution were used to quickly evaluate method development choices for the analysis of steroids. Four columns were evaluated, all 2.1 × 100 mm. The short column length and high efficiency provided short analysis times and rapid equilibration leading to fast investigation of selectivity.

Experimental

The Agilent 1260 Infinity Series LC Multi-Method Solution consists of:

- 1260 Infinity Binary pump (G1312B)
- 1290 Infinity Thermostated Column Compartment (G1316C)
- 1260 Infinity High Performance Autosampler (G1367E)
- 1290 Infinity Diode-Array Detector (G4212A), equipped with 10 mm MaxLight cartridge flow cell
- G6140 Single Quadrupole Mass Spectrometer

The system is highly flexible and can be used for up to four (100 mm) columns. The TCC was fitted with a 6-position/14 port column selection valve. Four Poroshell 120 columns were used, all 2.1 × 100 mm: Poroshell 120 EC-C18 (p/n 695775-902); Poroshell 120 SB-C18 (685775-902); Poroshell 120 Bonus-RP column (p/n 685775-901) and Poroshell 120 Phenyl-Hexyl (p/n 695775-912).

Column Choice to Enhance Selectivity

The columns were chosen to improve selectivity in the separation. They included a highly end-capped column recommended as a first-choice in method development (Poroshell 120 EC-C18), and a non-end-capped C18 (Poroshell 120 StableBond SB-C18) that could have interaction with the silanol groups to provide an alternative C18 selectivity using neutral to low pH mobile phases. A polar-embedded amine column (Poroshell 120 Bonus-RP) and a phenyl-hexyl column (Poroshell 120 Phenyl-Hexyl) were also used. Phenyl bonded phases are known for their improved selectivity for aromatic compounds.

A polar-embedded group inserted into the hydrophobic C13 alkyl chain allows the Bonus-RP phase on totally porous Poroshell 120 to minimize interaction of polar samples with silanols, providing symmetrical peaks for a wide variety of applications. This phase is especially useful at neutral pH where amines can interact strongly with ionized

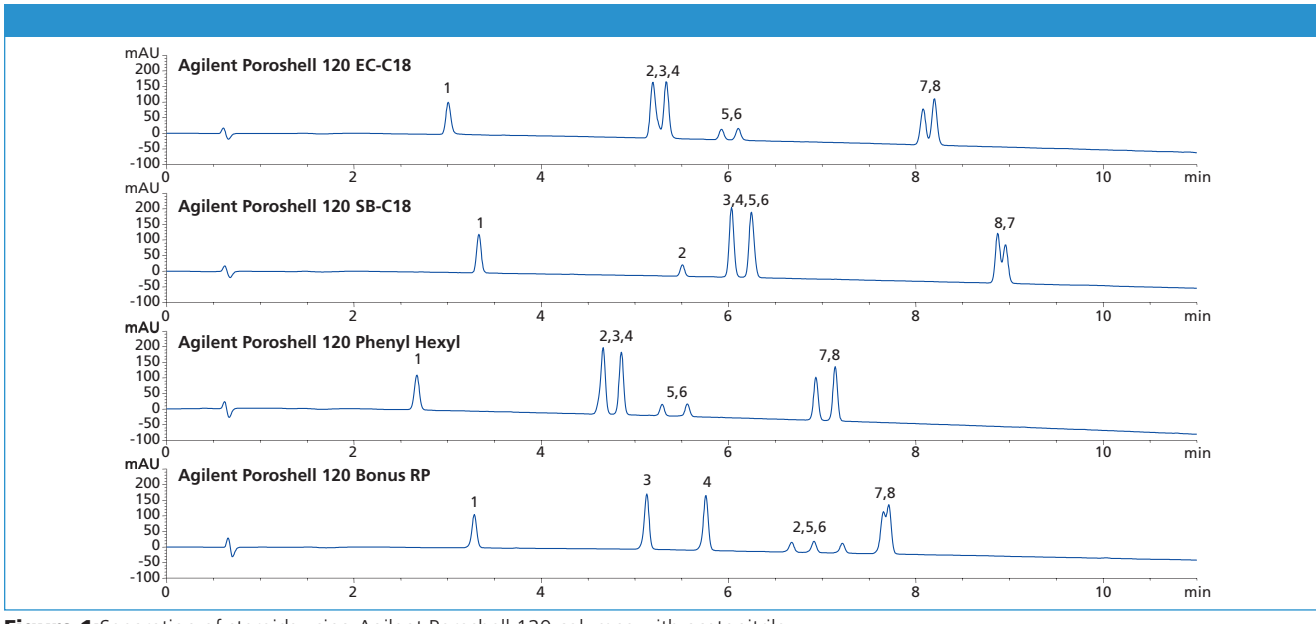

Figure 1: Separation of steroids using Agilent Poroshell 120 columns with acetonitrile.

Peak identification:
1. Hydrocortisone
2. β Estradiol
3. Androstadiene 3,17 dione
4. Testosterone
5. Ethinylestradiol
6. Estrone
7. Norethindrone acetate
8. Progesterone

Conditions:
Columns: Agilent Poroshell 120, 2.1 × 100 mm, 2.7 µm
Flow Rate: 0.4 mL/min
Gradient: 25–80% MeCN/10 min (0.1 % formic acid in water and MeCN)
Temperature: 25 °C
Detection: DAD 260, 80 ref = off

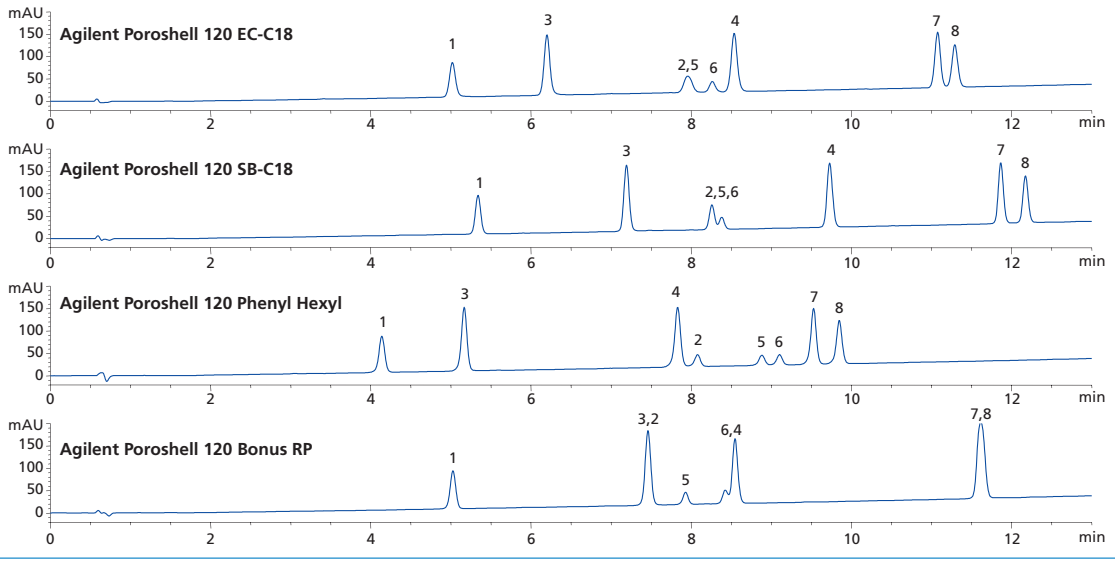

Figure 2: Separation of steroids using Agilent Poroshell 120 columns with methanol.

Conditions:

Columns: Agilent Poroshell 120, 2.1 × 100 mm
Flow Rate: 0.4 mL/min
Gradient:40–80% MeOH/14 min (0.1 % formic acid in water and MeOH)
Temperature: 40 °C
Detection: DAD 260, 80 ref = off

silanols. The polar-embedded group also helps to wet the hydrophobic chains and prevents phase collapse in highly aqueous mobile phases.

Poroshell 120 Bonus-RP can be used for many of the same separations as a C18 column while avoiding some of the disadvantages of C18, such as poor wettability in high aqueous mobile phases. In addition, it is much more retentive for those molecules that can interact by hydrophobic interactions and also by H-bonding with the amide group. Compared to alkyl-only phases, Bonus-RP has enhanced retention and selectivity for phenols, organic acids and other polar solutes due to strong H-bonding between polar group (H-bond acceptor) and H-bond donors, like phenols and acids. Bonus-RP gives slightly less retention than a C18 allows, for easy column comparison without the need to change mobile phase conditions. The Bonus-RP phase gives different selectivity than C18 for polar compounds. It is also compatible with 100% water.

Poroshell 120 Phenyl-Hexyl columns deliver unique selectivity for compounds with aromatic groups, providing superior resolution for these samples. Poroshell 120 Phenyl-Hexyl can also provide optimum separations of moderately polar compounds where typical alkyl phases (C18 and C8) do not provide adequate resolution. Acetonitrile tends to decrease the π-π interactions between aromatic and polarizable analytes and the phenyl-hexyl stationary phases, but methanol enhances those same interactions, giving both increased retention and changes in selectivity. This does not mean that acetonitrile should not be used with a phenyl bonded phase or that it might not provide an acceptable separation, but methanol is more likely to deliver the additional selectivity that is desired from a phenyl phase.

Results and Discussion

As can be seen in Figure 2, the Poroshell 120 EC-C18 and Poroshell 120 Phenyl-Hexyl columns showed very similar profiles, although the elution on the phenyl-hexyl column was faster. This could indicate that the π-π interactions on the phenyl-hexyl column were being reduced by the acetonitrile. The overlap of estradiol and androstadiene was less severe on the phenyl-hexyl column. The Poroshell 120 SB-C18 column delivered a very different separation, resolving estradiol but losing resolution on ethinylestradiol and estrone. This could be due to the exposed silanols on the SB-C18 phase or to some additional shape selectivity derived from the di-isobutyl side changes on the SB-C18 phase. Additional work would be needed to determine this. The Poroshell 120 Bonus-RP phase almost separates all eight compounds, and when using acetonitrile, it would provide the best method development option for further development.

In Figure 2, the separation was carried out using methanol at slightly elevated temperature (40 °C). In this case, the two C18 phases (Poroshell 120 EC-C18 and Poroshell 120 SB-C18) yielded nearly identical profiles. Some additional retention was seen on the SB-C18 phase due to some silanol interaction. The Poroshell 120 Bonus-RP chromatogram had three overlapping peak pairs, which would likely make further method development difficult in methanol. However, the Poroshell 120 Phenyl-Hexyl phase resolved eight compounds at better than baseline resolution.

Agilent Technologies, Inc.
2850 Centerville Road, Wilmington, DE 19808
tel. (877) 424-4536
Website: www.agilent.com/chem/discoverporoshell

The Scherzo Family Columns Effectively Separate Pharmaceutical Compounds

Bhavana Verma and Itaru Yazawa, Imtakt USA (Formerly Silvertone Sciences)

Pharmaceutical compounds are often difficult to analyze on C18 columns alone because they may be charged or have very similar structures. Imtakt Corporation has introduced a family of multi-mode columns that are able to overcome these separation challenges: Scherzo SS-C18, Scherzo SM-C18, and Scherzo SW-C18, containing C18, anion, and cation ligands. The SS-C18 contains a large amount of strong ionic ligands, the SW-C18 contains a small amount of strong ionic ligands, and the SM-C18 contains a medium amount of weak ionic ligands (see Figure 1). These multimode columns can separate the charged pharmaceutical compounds phenylephrine, N-acetylprocainamide, procaine, and naphazoline, as well as the very similar acetaminophen and N-acetyl-4-benzoquinoneimine.

Experimental

All data was generated with a semi-micro HPLC system equipped with UV detection. Separation of phenylephrine, N-acetylprocainamide, procaine, and naphazoline was accomplished with Scherzo SW-C18 of dimensions 150 x 3.0 mm (see Figure 2). This separation was accomplished with three different gradients. All three separations were done at 37 °C and detection occurred at 260 nm.

Separation of acetaminophen and its metabolite NAPQI was accomplished with Unison UK-C18, Cadenza CL-C18, Scherzo SW-C18, Scherzo SM-C18, and Scherzo SS-C18, all of dimensions 150 × 3 mm (see Figure 3). The separation was a gradient elution. Detection occurred at 260 nm and column temperature was 37 °C.

Results and Discussion

Separation of acetaminophen and its metabolite NAPQI was accomplished with Unison UK-C18, Cadenza CL-C18, Scherzo SW-C18, Scherzo SM-C18, and Scherzo SS-C18, all of dimensions 150 x 3 mm (see Figure 3). The separation was a gradient elution. Detection occurred at 260 nm and column temperature was 37 °C.. The development of the Scherzo family columns is an important advancement in HPLC technology that can help to improve separations in the pharmaceutical field.

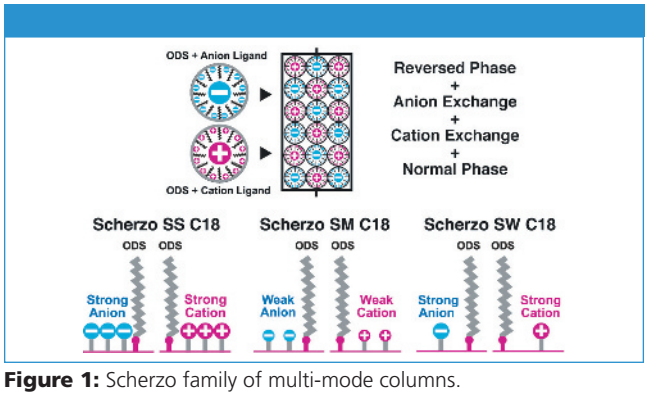

Figure 1: Scherzo family of multi-mode columns.

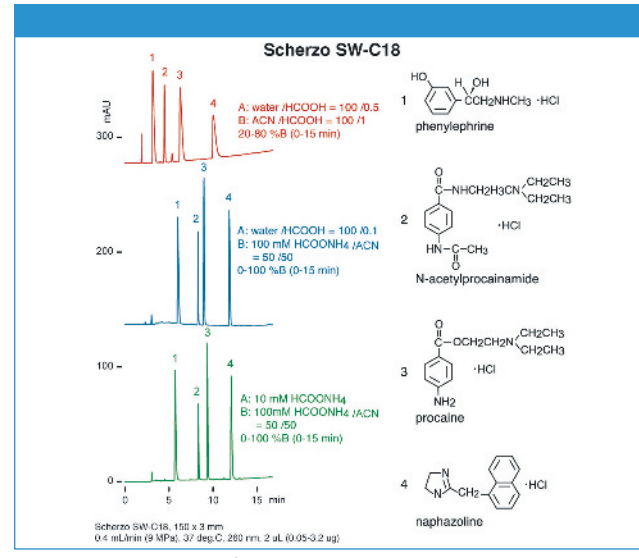

Figure 2: Separation of basic pharmaceutical compounds.

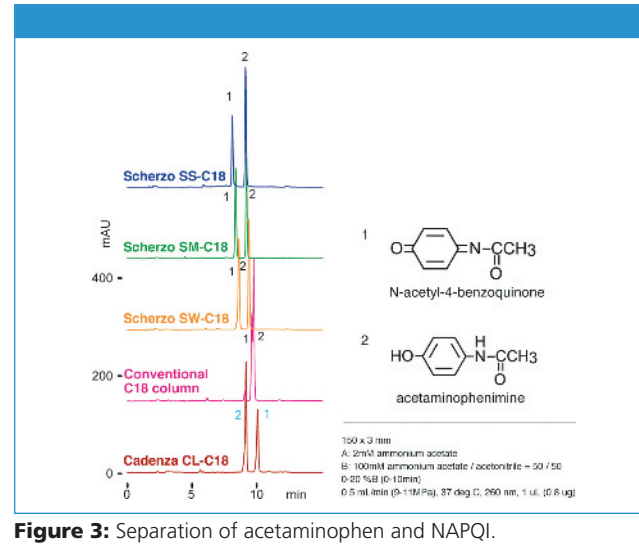

Figure 3: Separation of acetaminophen and NAPQI.

Imtakt USA
6703 Germantown Avenue, Suite 240, Philadelphia, PA 19119
tel. (888) 456-HPLC, (215) 665-8902, fax (501) 646-3497
Website: www.imtaktusa.com; Email: info@imtaktusa.com

Taking Advantage of Sub-2 µm Core–Shell Technology for Ultra-Fast and Ultra-Efficient Urinary Excretion Profiling

Simon Lomas and Jeff Layne, Phenomenex Inc.

Throughout the drug development process, potential new drug candidates (or new chemical entities; NCEs) and their metabolites must be subjected to rigorous and extensive pharmacokinetic evaluations to determine their rates accumulation, metabolism, and excretion from the body. With regards specifically to the excretion, urinary excretion is typically the predominant route for the elimination of drugs and their metabolites, and urinary excretion profiling is an integral portion of the pharmacokinetic characterization of NCEs.

Liquid chromatography tandem mass spectroscopy (LC–MS-MS) allows scientists to rapidly and accurately quantify specific drugs and their metabolites at extremely low levels from various biological matrices, such as urine. On the liquid chromatography side, the ultra-high efficiency delivered by sub-2 µm UHPLC core–shell media provides analysts with the ability to run their samples in extremely short periods of time while maintaining excellent resolution from sample interferences. In this application note, we present an example of the ability of the Kinetex® 1.7 µm core–shell particle to deliver significantly improved performance over larger core–shell particles and conventional fully porous media.

Experimental Conditions

LC–MS-MS conditions

Column:	Kinetex 1.7 µm C18 30 × 2.1 mm Fused-core 2.7 µm C18 50 × 2.1 mm Fully porous 3.5 µm C18 50 × 2.1 mm
Mobile Phase:	A: 10 mM Ammonium formate B: Acetonitrile
Gradient:	(95:5) A/B to (0:100) A/B in 2 min, then re-equilibrate at (95:5) A/B for 1 min
Flow Rate:	700 µL/min
Temperature:	Ambient
Detection:	MS using API 4000 detector
HPLC system:	Agilent 1200 SL
Concentration:	100 ng/mL for active drug and 50 ng/mL for metabolite
Analytes:	Lorazepam-glucuronide spiked into urine at a concentration of 50 ng/mL

Results

In many instances, suitable MRM transitions for an analyte of interest are hidden because of significant matrix interference. Oxazepam-glucuronide did not suffer from urinary interference (Figure 1), however the 497.2 -> 320.9 MRM transition for the glucuronide metabolite of lorazepam does show significant isobaric interference (Figure 2). In cases such as this, the ultra-high efficiency of the core–shell Kinetex 1.7 µm particle can provide significantly greater peak capacity than standard fully-porous media (3.5 µm in this case) and a larger fused-core particle (2.7 µm fused-core).

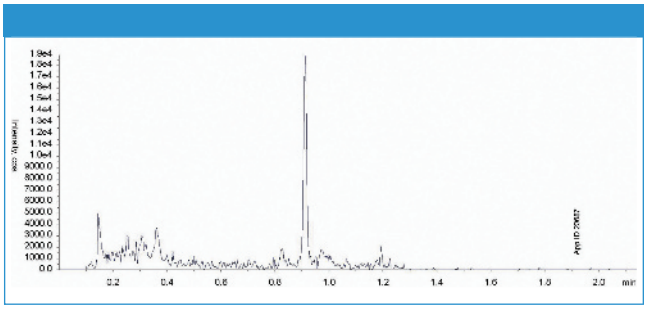

Figure 1: XIC for oxazepam glucuronide (MRM 463.1 -> 287.0) in urine.

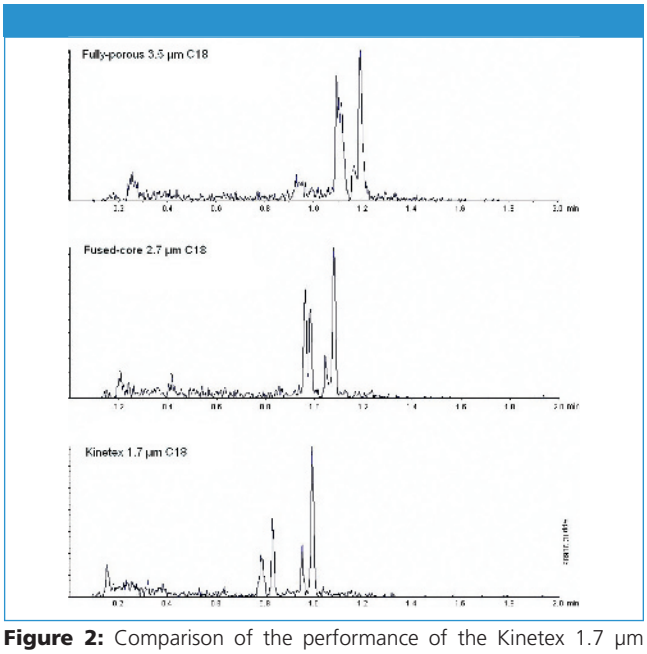

Figure 2: Comparison of the performance of the Kinetex 1.7 µm C18 (30 × 2.1 mm) column versus a fused-core 2.7 µm column (50 × 2.1 mm) and a fully porous 3.5 µm C18 column (50 × 2.1 mm) for the glucuronide metabolite of lorazepam (MRM 497.2 -> 320.9).

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Radio Ion Chromatography

M. Espinosa[1], J. Chesa-Jiménez[2], Beni Galliker[3], Alfred Steinbach[3] and Andrea Wille[3], [1]Gomensoro S.A., Spain, [2]Instituto Tecnológico PET, Spain, [3]Metrohm International Headquarters, Switzerland.

Positron emission tomography (PET) is one of the most powerful non-invasive diagnostic tools for tracing organ functioning. Quality control of the short-lived radiopharmaceuticals is challenging, not least because of the tough time limits, the radiation issue and the near nanomole radiotracer quantities.

This article presents a likewise rugged and versatile multichannel radio IC system that controls the production of the radionuclide [^{18}F]fluoride (precursor) and the two radiotracers synthesized from it, [^{18}F]fluorodeoxyglucose and [^{18}F]fluorocholine, in accordance with pharmacopoeial regulations.

Principles of Positron Emission Tomography (PET)

Radiopharmaceuticals are radioactive substances used in nuclear medicine to diagnose, treat or prevent disease. They contain a radioactive isotope, a so-called radionuclide, attached to a biologically inert or active molecule.

Radionuclides are unstable isotopes that have an excess of either neutrons or protons and, therefore, radioactively decay, resulting in the emission of gamma rays or subatomic particles. In proton-rich nuclides, a proton changes to a neutron, whereby a positron (antiparticle of the electron or an electron with a positive charge, also called ß$^+$ particle) is emitted together with a neutrino (ν) according to

proton (p) → neutron (n) + positron (ß$^+$) + neutrino (ν).

While travelling in the surrounding media, the released positron loses its kinetic energy and then combines with an electron. The encounter annihilates both positron and electron and results in two photons (gamma rays) each with an energy of 0.511 MeV that are emitted in opposite directions (Figure 1).

$m_e^+ + m_e^- \rightarrow 2\gamma$

Sophisticated scanners (detector) can detect such pairs of photons by coincidence detection. From the data collected, three-dimensional images of tissue structures are then calculated. The most commonly used short-lived, cyclotron-produced radionuclides in radiopharmacy are ^{11}C, ^{13}N, ^{15}O and ^{18}F. Their respective half-lives are 20.38, 9.96, 2.03 and 109.7 min.

Radiopharmaceuticals

To administer the radionuclide to a living human or animal, it is either incorporated in a biologically inert molecule (e.g., the blood flow tracers [^{15}O]water or [^{15}O]butanol) or in a biologically active molecule that is absorbed by the organ of interest.

After the radiopharmaceutical is concentrated in the tissue of interest, the patient is placed in the PET scanner. By tracking the photons, computers with sophisticated software generate three-dimensional images of the source of the photons. This allows the study of physiological, biochemical and pharmacological functions at a molecular level. Illnesses such as cancer, cardiovascular disease and even neurological disorders can be detected long before symptoms appear.

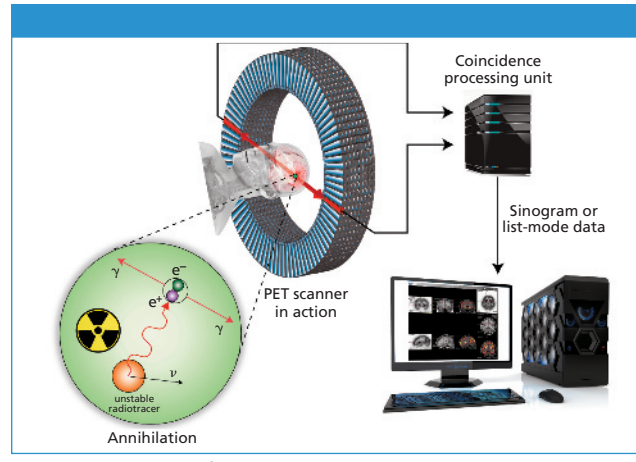

Figure 1: Principle of positron emission tomography.

Production of PET Radiopharmaceuticals

Radionuclides used in PET experiments such as ^{11}C, ^{13}N, ^{15}O and ^{18}F are artificially produced in a cyclotron, where a beam of accelerated charged particles irradiates a prepared target. Subsequently, the resulting radionuclides are isolated and synthetically incorporated into the radiotracer. Since the fluorine atom is similar in size to the hydrogen atom, it acts as a pseudohydrogen and is therefore ideally suited for replacing hydrogen atoms in organic molecules. The positron emitter ^{18}F is thus one of the most important imaging radionuclides in diagnostic nuclear medicine. It is produced by proton bombardment of an ^{18}O-enriched water target. In an ^{18}O(p, n)^{18}F reaction, highly accelerated protons (p) react with the ^{18}O atomic nucleus to emit a neutron (n) and ^{18}F, which immediately decays by positron emission with a half-life of 109.7 min. The product of the ^{18}F decay is the stable isotope ^{18}O. After isolation of ^{18}F from the target water, the radionuclide is incorporated into the chemical compound required. To this end, the preparation of reactive fluorine radionuclides in organic solvents is an important prerequisite for the synthesis of aliphatic carbon-fluorine bonds.

a) [^{18}F]Fluorodeoxyglucose

[^{18}F]Fluorodeoxyglucose, commonly abbreviated [^{18}F]FDG, or simply FDG, is a glucose analogue in which the hydroxyl group at

the 2' position of the glucose molecule is substituted by [^{18}F]fluorine (Figure 2a). It throws light on the use and metabolism of glucose in heart, lungs, and brain. Additionally, it is used in oncology to determine abnormal glucose metabolism to characterize different tumour types. After administering [^{18}F]FDG to the patient, it is incorporated into the cells by the same transport mechanism as the normal glucose, but unlike this, once inside the cell, it is not metabolized and thus remains in the cell allowing PET tomographic imaging. Not least because of its many diagnostic uses, the high number of existing labeling procedures and its advantageous half-life of approximately 2 h, which allows the transport to sites that have no cyclotron, [^{18}F]FDG is actually the most frequently used organic PET radiopharmaceutical.

b) [^{18}F]Fluorocholine

In cells, choline is used as a precursor for the biosynthesis of phospholipids. As the latter are essential cell membrane components and because tumors reveal increased metabolism of cell membrane constituents and increased choline uptake, radiolabeled choline tracers are invaluable diagnostic tools for cancer detection. [^{18}F]Fluorocholine (Figure 2b) is a recently developed PET radiotracer that allows choline metabolism to be imaged *in vivo*. It is based on the tumor-detecting radiotracer [^{11}C]choline. The driving force of the production of the ^{18}F-labeled derivative was the substantially longer half-life, which allows the distribution of this tracer to PET institutions without on-site cyclotron.

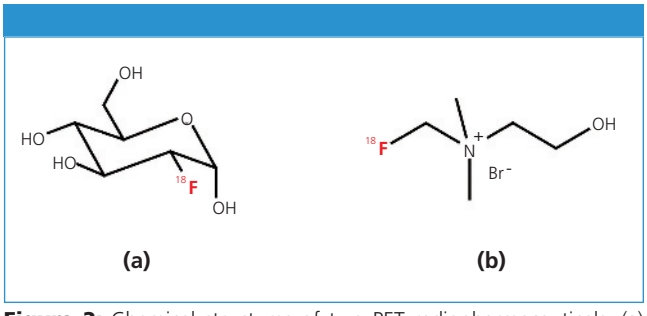

Figure 2: Chemical structures of two PET radiopharmaceuticals. (a) In [^{18}F]FDG, the hydroxyl group at the 2' position of normal glucose is substituted by ^{18}F. (b) In [^{18}F]fluorocholine, a [^{18}F]fluoroalkyl group is attached to the nitrogen atom of N,N-dimethylaminoethanol (DMAE).

Radio Ion Chromatography (IC)

Radio IC is a powerful, fast and sensitive tool for the quality control of a wide range of PET pharmaceuticals. It aims at determining the radiochemical purity of radiopharmaceuticals after radiosynthesis with cyclotron-produced nuclides. To this end, Radio IC uses a flow-through mass and radioactivity detector.

Besides the accuracy and reproducibility of the analytical results, high throughput is a must. One and the same multichannel radio IC takes over the quality control of three production lines. The analytical unit provides the following benefits:

a) Flexibility of the System

The ion chromatography system installed at the ITP (Instituto Tecnológico PET) in Spain combines three quality control systems for PET pharmaceuticals in one. From the very same injection system, the flow can be automatically directed to the three channels. By selecting between an array of different columns, mobile phases and detectors, [^{18}F]fluoride, [^{18}F]FDG and [^{18}F] fluorocholine can be separately determined (Table I). All aspects of system operation and data acquisition are controlled by MagIC Net™ software.

b) Safety

The automated sample injection with Metrohm`s patented Dosino technology allows the aspiration of very low sample volumes with accuracy and precision. By using the MagIC Net™

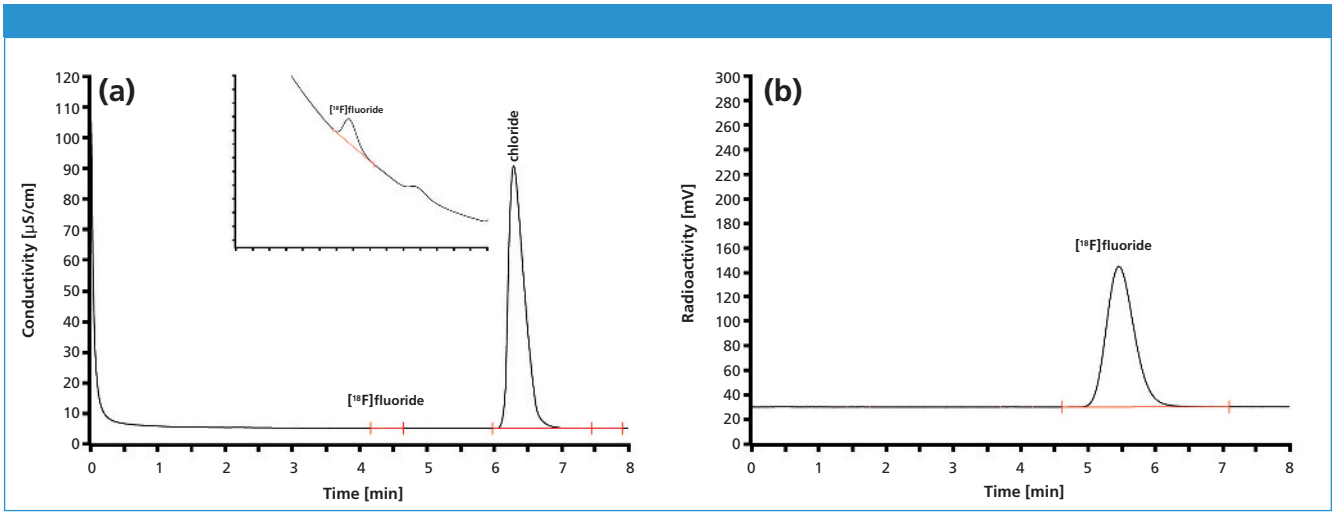

Figure 3: (a) Conductivity and (b) radioactivity chromatogram of cyclotron-produced [^{18}F]fluoride. In the subsequent radiosynthesis (nucleophilic fluorination), tracer (i.e., very low) quantities of [^{18}F]fluoride ions are used to form carbon-fluorine bonds. The IC software converts the radiation units, counts per second (cps), to mV. Chromatographic conditions are shown in Table I.

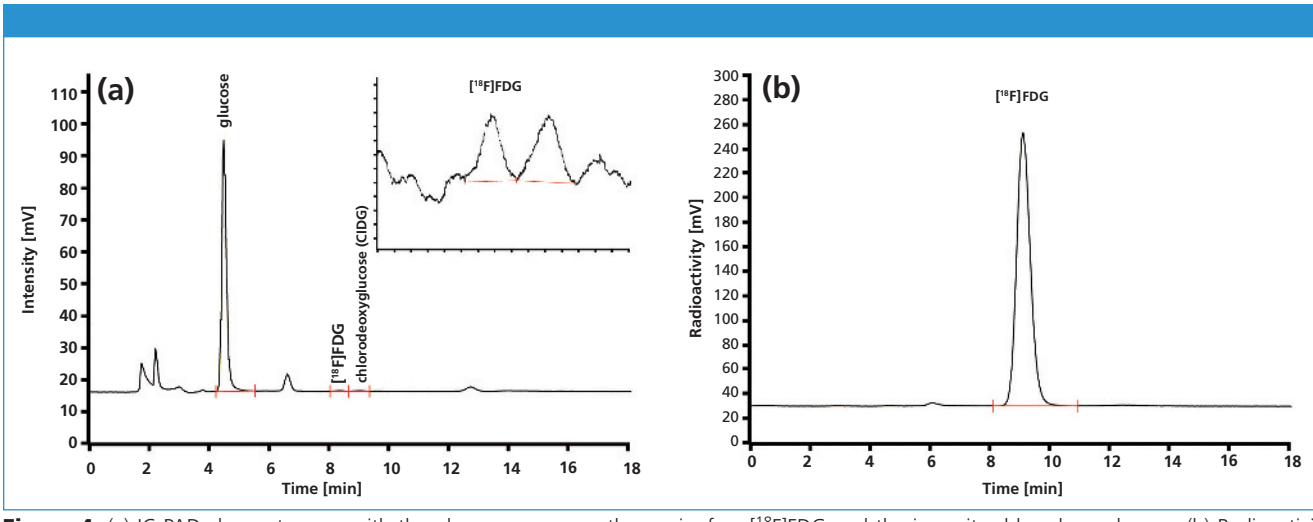

Figure 4: (a) IC-PAD chromatogram with the glucose precursor, the carrier-free [^{18}F]FDG and the impurity chlorodeoxyglucose. (b) Radioactivity chromatogram of the [^{18}F]FDG. The IC software converts the radiation units, counts per second (cps), to mV. Chromatographic conditions are shown in Table I.

Table I: Chromatographic conditions for the quality control of [^{18}F]fluoride, [^{18}F]FDG and [^{18}F]fluorocholine

	[^{18}F]Fluoride	[^{18}F]FDG	[^{18}F]Fluorocholine
Column	Metrohm A Supp 5 - 150/4.0	Metrosep Carb 1 - 150/4.0	Metrosep C 4 - 150/4.0
Column temperature	45 °C	25 °C	40 °C
Sample volume	10 µL	10 µL	10 µL
Eluent	3.2 mmol/L sodium carbonate 1.0 mmol/L sodium hydrogen carbonate	0.1 mol/L sodium hydroxide	1.7 mmol/L nitric acid 0.7 mmol/L dipicolinic acid
Flow-rate	0.7 mL/min	1.0 mL/min	1.5 mL/min
Detection	Conductivity detection after chemical suppression	Pulsed amperometric detection (PAD)	Conductivity detection
Analysis time	8 min	18 min	14 min

software, liquid handling as well as dosing and rinsing tasks are completely automated without any carryover. The system's modular design supports the installation of the required lead shielding and thus guarantees user safety. The injection valve is placed inside a 5 cm thick tailor-made lead housing, while radiation from the radiotracers in the separation columns is attenuated to a safe level by a sufficiently thick lead shielding. In addition, a lead sample holder avoids user exposure to gamma radiation.

c) Comprehensive Quality Control Using Multichannel Radio IC

Radiopharmaceuticals have unique characteristics and require special tests described in numerous pharmacopoeias. The quality control includes testing for both chemical and radiochemical purity before the radiotracer is administered to the patient. The radiochemical purity of a radiotracer — the ratio of the radionuclide in bound form (e.g., [^{18}F]FDG) to the radionuclide in unbound form (e.g., [^{18}F]fluoride) — guarantees the image quality of the PET scan and protects the patient from unnecessary radiation.

In the first instance, the concentration of the cyclotron-produced radionuclide, the ^{18}F, which is used as a precursor in the subsequent radiosynthesis, has to be determined (Figure 3).

Subsequently, the appropriate chemical purity — as for any other pharmaceutical preparation — and the concentration of the synthesized radiopharmaceutical, the ^{18}F, which mostly is in the nanomolar range, have to be determined. In doing so, excessive precursors and radiolabeling-derived impurities have to be quantified also. Figure 4 shows the chromatogram with the glucose precursor, the carrier-free [^{18}F]FDG and the chlorodeoxyglucose impurity. The analysis is completed in less than 10 min.

Figure 5 shows the chromatogram of the reaction mixture of [^{18}F] fluorocholine synthesis. Besides nanomole quantities of the [^{18}F] fluorocholine radiotracer, considerable amounts of the reactant N,N-dimethylaminoethanol and trace-levels of calcium impurities were detected in the reaction mixture.

d) Analysis Time

As most positron-emitting radiopharmaceuticals are characterized by short half-lives, there is a strong drive to reduce the time spent

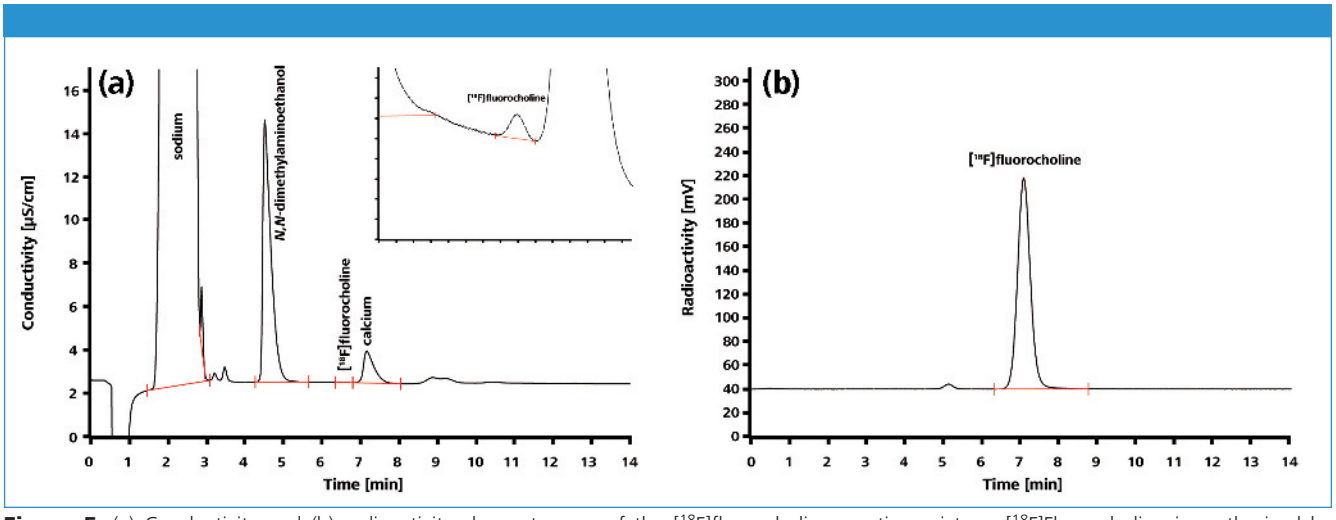

Figure 5: (a) Conductivity and (b) radioactivity chromatogram of the [^{18}F]fluorocholine reaction mixture. [^{18}F]Fluorocholine is synthesized by ^{18}F-fluoroalkylation of N,N-dimethylaminoethanol (DMAE) using gaseous ^{18}F-fluorobromomethane. This labeling reaction results in high levels of residual DMAE. Other potential by-products such as bromocholine (not detected) can additionally be determined. The IC software converts the radiation units, counts per second (cps), to mV. Chromatographic conditions are shown in Table I.

on quality control. Fast and precise analyses are guaranteed by optimally harmonized and computer-controlled determination and rinsing sequences for detector pathways and the sample injection circuit.

Conclusion

Metrohm's highly customizable chromatography system copes with the tough requirements of the radiopharmaceutical industry and pharmacopoeial regulations. One single multichannel radio IC meets the quality control requirements of various production lines. Besides the high quality, the Metrohm IC presented ensures user safety, low maintenance costs and outstanding ruggedness.

Acknowledgments

The authors thank the entire group of the Ion Chromatography Department of Gomensoro for their outstanding support and fruitful discussions during this project.

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Metrohm International Headquarters
Ionenstrasse, 9101 Herisau, Switzerland
tel. +41 71 353 85 04
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Residual Solvents Testing of Packaging Materials per ASTM F1884-04 with the HT3 Automated Headspace Sampler

Roger Bardsley, Teledyne Tekmar

Printing inks used on packing materials of consumer products contain colorants and pigments, binder systems, solvents, and additives. ASTM F 1884-04 (1), Standard Test Methods for Determining Residual Solvents in Packaging Materials determines residual solvents in packaging materials from printing processes utilizing solvent-based printing inks.

Test method A recommends an autosampler to perform the headspace-gas chromatography examination of residual solvents in packaging materials. The Teledyne Tekmar HT3 automated headspace sampler will be used to meet the rigorous demands of ASTM F 1884-04. This method prepares a standard curve in the package sample matrix.

Standard Preparation

A 20 µL/L 4-heptanone solution was used as an internal standard. A five-point calibration curve was prepared by diluting a commercially available residual solvent standard mix with the internal standard solution. A calibration curve was prepared by adding 2 mL of the standard or internal standard solutions into 22 mL headspace vials.

The samples were automatically injected onto a GC/FID system by the Tekmar HT3 automated headspace sampler.

Results

The relative standard deviation for the average of the response factors (%RSD) and correlation coefficients (r^2) were calculated by the internal standard method for the five-point calibration curve. An example of this calibration data (labeled Peak #9) is presented in Figure 1. Figure 2 shows a comparison of a calibration standard to a film blank standard.

Conclusions

The Teledyne Tekmar HT3 automated headspace sampler provides excellent linearity for residual solvent standards used in the analysis of residual solvents in packaging material as defined by ASTM method F1884-04, Standard Test Methods for Determining Residual Solvents in Packaging Materials.

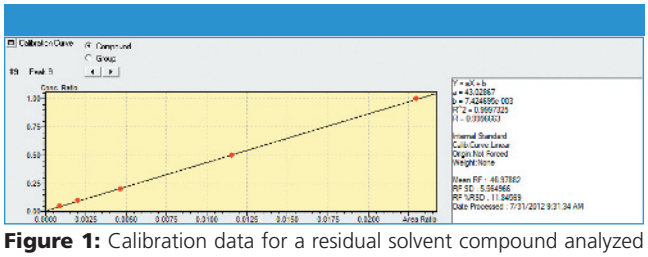

Figure 1: Calibration data for a residual solvent compound analyzed by ASTM F1884-04.

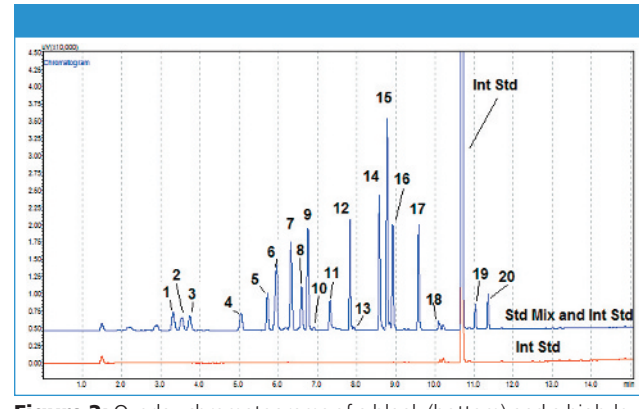

Figure 2: Overlay chromatograms of a blank (bottom) and a high-level standard (top).

Additional data for the HT3 and the new Versa automated headspace sampler using ASTM Method F1884-04 will be presented at the 2012 Eastern Analytical Symposium and Exhibition.

References

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Teledyne Tekmar
4736 Socialville Foster Rd., Mason, OH 45040
tel. (800) 874-2004
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Accurate Determination of Tartrate in Drug Products in Just 10 Minutes

Suparerk Tukkeeree, Chanita Chantarasukon, and Jeffrey S. Rohrer, Thermo Fisher Scientific Inc.

Tolterodine, a quaternary ammonium compound, is used to treat urinary incontinence. To create the drug substance, tolterodine is paired with tartrate to form tolterodine tartrate. This drug substance can then be used to create drug products. One way to confirm the amount of a drug substance in a drug product is to assay for its counterion. This is especially true for counterions such as tartrate that are unlikely to originate from any other sample components. Ion chromatography (IC) with suppressed conductivity detection is a common technique for counterion determinations. Here we report an IC method to determine tartrate in a tolterodine tartrate drug product that is faster, requires less labor, and eliminates analyst exposure to strong acid relative to a recently published IC method (1).

Equipment

A Thermo Scientific Dionex ICS-5000 IC System configured with an EG Eluent Generator module, DC Detector/Chromatography Compartment, and CD Conductivity Detector was used. Samples were introduced by an AS-AP Autosampler. All data collection and processing was controlled by Thermo Scientific Dionex Chromeleon Chromatography Data System software. Complete experimental details are found in reference 2.

Results

Figure 1 Trace A shows the determination of tartrate in a capsule drug product containing tolterodine tartrate. Tartrate is resolved from other anions in the sample using a Thermo Scientific Dionex IonPac AS20 Hydroxide-Selective Anion-Exchange Column set with 20 mM potassium hydroxide. The eluent is produced by the eluent generator, and therefore the labor and possible error associated with eluent preparation are eliminated. Tartrate is detected by suppressed conductivity in the recycle mode, indicating that the analyst does not have to prepare a strong acid solution for suppressed conductivity detection. Figure 1 Trace B shows the same sample to which an additional 4 mg/L tartrate was added. For a set of five samples there was 100% recovery of the added tartrate. The same unfortified five samples had 98.4% of the labeled amount of drug substance as measured by tartrate concentration. Both results indicate good method accuracy. Overall this is an accurate IC method for determining tartrate that does not require the analyst to prepare eluent or strong acid regenerant.

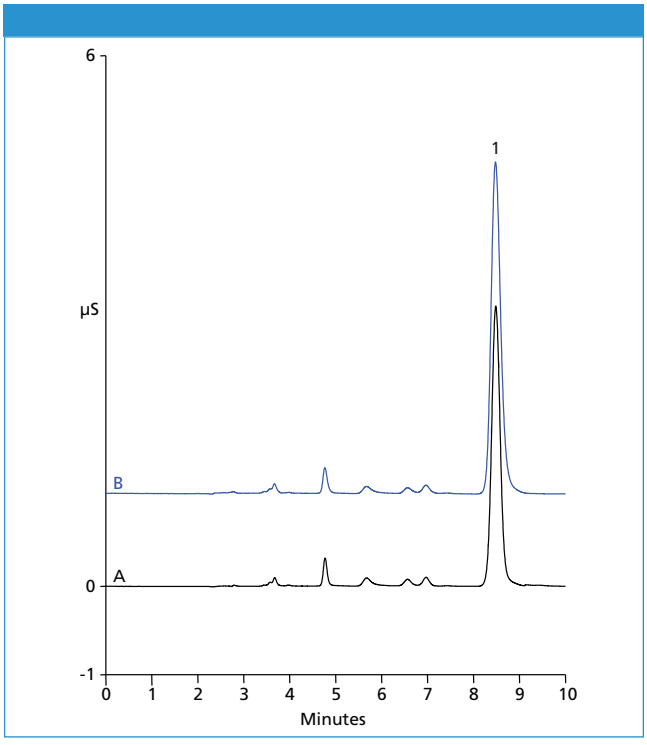

Figure 1: Determination of tartrate in a drug capsule: Trace A shows the sample and Trace B is the sample to which 4 mg/L tartrate was added before sample preparation.

References

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Challenges in Developing an Ultra Sensitive Bioanalytical Method for Ethinylestradiol in Human Plasma

Introduction

Ultra sensitive LC–MS-MS quantification of steroids is particularly difficult due to the presence of countless other steroid interferences, many isobaric, in human plasma. In particular, for compounds such as ethinylestradiol (EE), where the required detection limit (LOD) is 1 pg/mL, this challenge is even greater. Not only must one use the highest sensitivity MS system, but both LC and sample preparation must also be carefully optimized for selectivity.

Instrumentation & Consumables

LC Conditions

Instrument: ACQUITY UPLC®
Column: ACQUITY UPLC HSS C_{18} SB, 2.1 × 100 mm, 1.8 µm
Part number: 186004119
Mobile phase A: 0.1% HCOOH in H_2O
Mobile phase B: 0.1% HCOOH in 80/20 ACN/IPA (v/v)

Initial mobile phase conditions were 60% mobile phase B (MPB). After a 1 min hold, the percentage of MPB was increased to 90% over 4 min. The flow rate was 0.4 mL/min. The injection volume was 35 µL.

Sample Preparation Conditions

Human plasma samples were extracted with 75/25 hexane/ethyl acetate (v/v), derivatized with dansyl chloride, and then the derivatives cleaned up with Oasis® MCX in µElution format (part number 186001830BA.)

MS Conditions

Instrument: Xevo® TQ-S
Ion Source: ESI^+

Results/Discussion

Figure 1 demonstrates the importance of a multi-step clean-up process for high sensitivity analysis of EE. Following liquid-liquid-extraction (LLE) alone, the MS scan in red clearly highlights the overwhelming level of interferences remaining. This interference peak not only co-elutes with EE, but also saturates the detector. These interferences prohibit one from reaching the desired LOD. The interferences present after LLE alone contain a high concentration of phospholipids amongst other things. When LLE is followed by mixed-mode cation exchange (Oasis MCX – green trace), the interferences are practically eliminated, allowing one to reach the required detection limits.

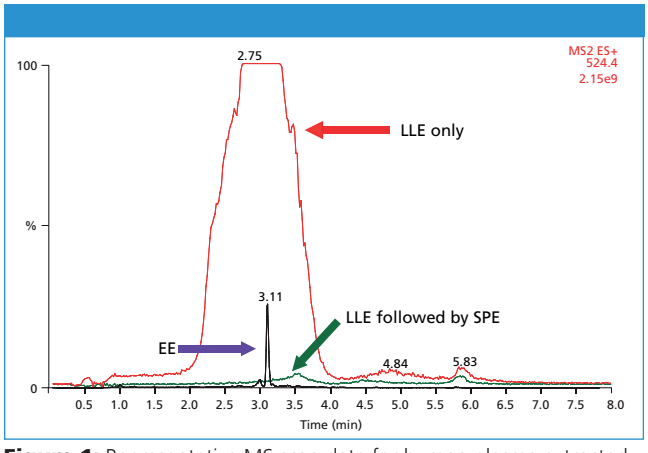

Figure 1: Representative MS scan data for human plasma extracted using liquid-liquid-extraction (LLE) alone (red) and LLE followed by mixed-mode SPE (green). The retention time for EE is shown in purple.

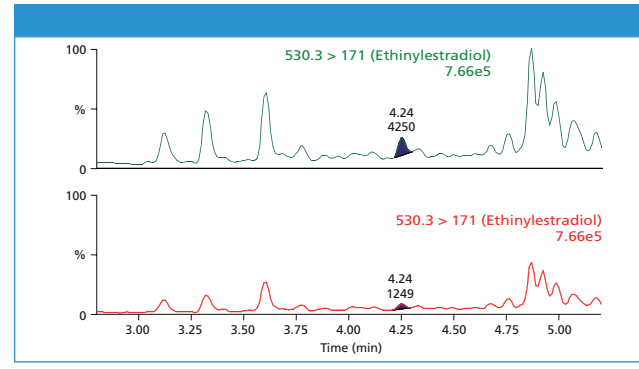

Figure 2: Panel A is a representative chromatogram of EE at 1 pg/mL in extracted human plasma compared to Panel B, which is the blank extracted plasma.

The chromatograms in Figure 2 demonstrate that one can easily meet the detection limit of 1 pg/mL in human plasma, as the level in panel A is > 3× the level in the blank extracted plasma sample (panel B). To assess accuracy and precision, standard curves were prepared from 0.001–1 ng/mL. Quality control (QC) samples were prepared at low, medium and high concentrations: 0.003, 0.075 and 0.75 ng/mL, respectively. Regression analysis of the data produced standard curves with an r^2 value of 0.999 using a $1/x$ weighting. The average % accuracy for the points on the standard curves is 99%. The average % accuracy for the QC samples is 96%.

Conclusions

The proper combination of optimized sample prep and chromatography coupled to high sensitivity MS produces a bioanalytical method for EE which reaches an LOD of 1 pg/mL in human plasma.

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Molecular Weight Determination of LMWH SEC–MALS vs. SEC–UV-RI

Wyatt Technology Corporation

Low-molecular-weight heparins (LMWHs) are obtained by fractionation or depolymerization of natural heparins. They are defined as having a mass-average molecular weight of less than 8000 and for which at least 60% of the total weight has a molecular mass less than 8000.

Size-exclusion chromatography (SEC) has been the most common way of measuring the molecular weight and molecular weight distributions of LMWHs by using the two most common detection technologies: ultraviolet (UV) coupled with refractive index (RI) detection. However, these detectors embody a relative method in order to determine molecular weights, requiring calibration standards. A newer, absolute method involves the use of multi-angle light scattering (MALS), which does not require any standards. The European Pharmacopeia (EP) monograph for LMWH specifies the use of the UV-RI detection method and provides a known calibration standard. Many laboratories around the world have adopted this method.

We previously developed an SEC–MALS method and found it to be very suitable for the analysis of LMWHs. We have recently adopted the UV-RI method described in the EP monograph and compared the molecular weight results generated for LMWH using each detection type. The adopted method uses an Agilent LC- 1200 series HPLC, 0.2 M Sodium Sulfate pH 5.0 mobile phase, Tosoh TSK-gel G2000 SWxl column with Tosoh TSK-gel Guard SWxl, Waters 2487 dual wavelength UV detector, and Wyatt Optilab rEX refractive index detector. For MALS analysis the UV detector was replaced with a Wyatt miniDAWN TREOS detector; all other methods aspects remained the same.

The results indicated that both detection types are suitable and acceptable for the analysis of LMWHs. The molecular weight and distribution results generated using each detection type are comparable. This indicates that a SEC–MALS method could be adopted in place of the SEC–UV-RI method currently required by the EP monograph, and that it would result in less time because it obviates the need for calibration standards.

This note was graciously submitted by Lin Rao and John Beirne, Scientific Protein Laboratories LLC.

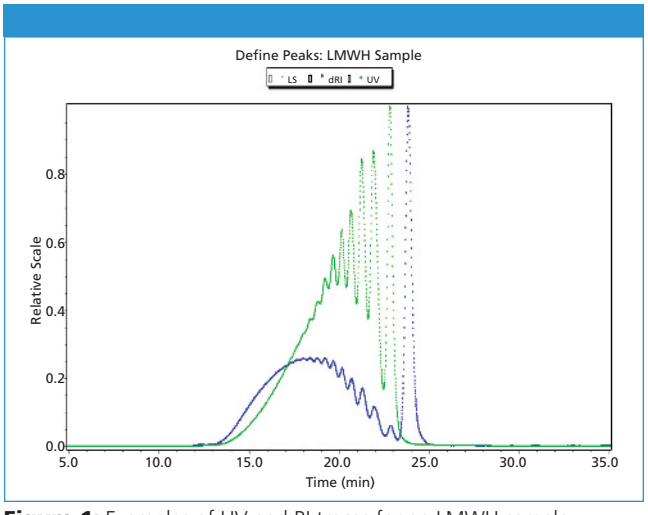

Figure 1: Examples of UV and RI traces for an LMWH sample.

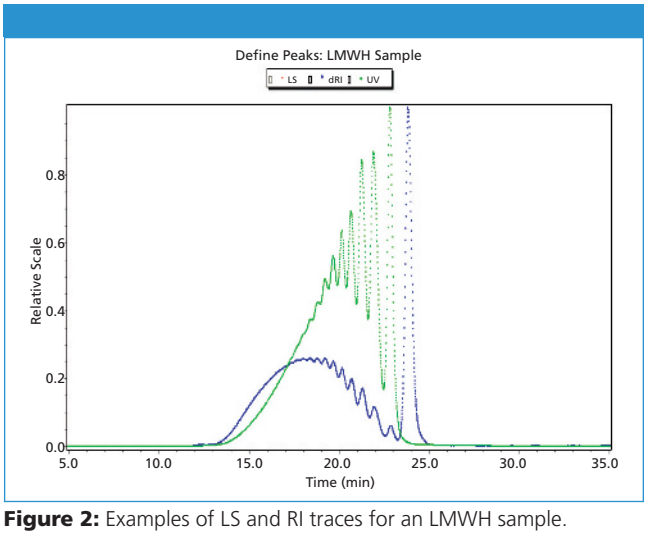

Figure 2: Examples of LS and RI traces for an LMWH sample.

Wyatt Technology Corporation
6300 Hollister Avenue, Santa Barbara, CA 93117
tel. (805) 681-9009, fax (805) 681-0123
Website: www.wyatt.com

Characterization of PLGA Using SEC–MALS-VIS

Wyatt Technology Corporation

Poly(lactic-*co*-glycolic acid) (PLGA) is a copolymer based on glycolic acid and lactic acid. The two monomer units are linked together by ester linkages and form linear polyester chains. The obtained product is biodegradable and biocompatible, and it is approved by the Food and Drug Administration (FDA) for production of various therapeutic devices as well as for drug delivery applications. The properties of PLGA can be tuned by the ratio of the two monomers and by its molar mass distribution.

The characterization of PLGA by means of conventional size-exclusion chromatography (SEC) is problematic because of the lack of suitable calibration standards. In addition, the linear polyester structure can be modified by the addition of small amounts of polyfunctional monomer to obtain branched chains of differing degrees of branching. The degree of branching becomes an additional parameter that can be used to adjust PLGA properties — all of which renders conventional column calibration an inadequate analytical technique.

In this application note, two commercially available samples were analyzed by SEC coupled to a multi-angle light scattering (MALS) detector (HELEOS), a refractive index detector (Optilab rEX), and a viscosity (VIS) detector (ViscoStar). The ViscoStar was used in order to discover additional information about the molecular structure of the analyzed polymers. In addition to molar mass distributions, the SEC–MALS-VIS system yields the relationship between intrinsic viscosity and molar mass (Mark-Houwink plot) that can provide deep insight into the molecular structure of the polymers being analyzed.

In Figure 1, the molar mass distributions are given as differential distribution plots. As seen from the plots, the two samples span markedly different molar mass ranges. The Mark-Houwink plots of the two samples are shown in Figure 2 together with the plot of linear polystyrene that is shown simply for the sake of comparison. The slope of the Mark-Houwink plot of the linear polystyrene is 0.71, a typical value for linear random coils in thermodynamically good solvents. The slope of the red sample roughly corresponds to a linear structure as well. However, there is a slight indication of deviation from linearity at the region of high molar masses that may indicate the presence of branched molecules. The Mark-Houwink plot of the blue sample is curved. Curvature of the Mark-Houwink plot generally reveals branching. In addition, the slope of the higher molar mass portion of the Mark-Houwink plot of 0.48 suggests significant branching.

SEC-MALS-VIS is an excellent method for the characterization of PLGA polyesters as it has the ability to determine not only the molar mass distribution, but to reveal subtle differences in PLGAs molecular structure.

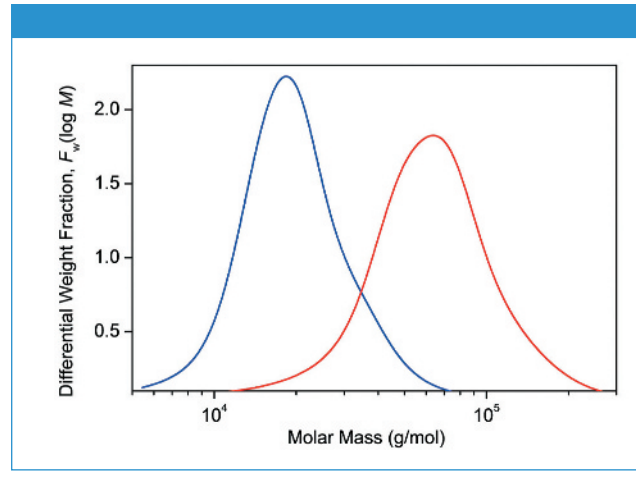

Figure 1: Differential molar mass distribution curves of two PLGA samples.

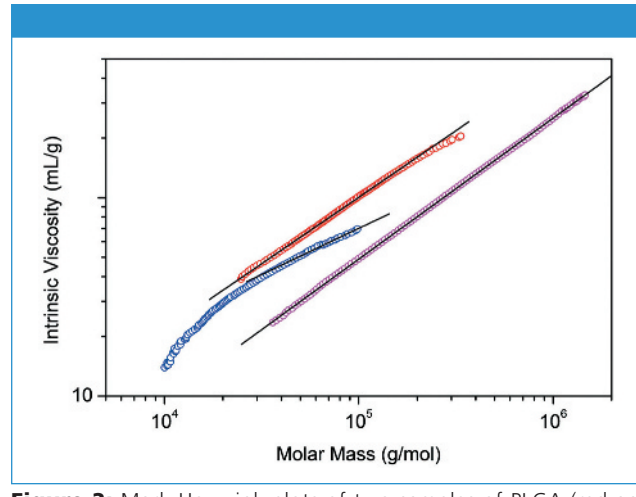

Figure 2: Mark-Houwink plots of two samples of PLGA (red and blue) and linear polystyrene (magenta). The lines are linear extrapolations of the data.

Wyatt Technology Corporation
6300 Hollister Avenue, Santa Barbara, CA 93117
tel. (805) 681-9009, fax (805) 681-0123
Website: www.wyatt.com

2D Analysis of Thermoplastic Elastomers TPE

Peter Kilz, PSS Polymer Standards Service GmbH

Introduction

Blockcopolymers such as SBS (styrene-butadiene-styrene) are an important product class and a typical example of complex polymers. In addition to the molar mass distribution, a chemical composition distribution may also be present in copolymers. While GPC–SEC is the established method for the determination of molar mass averages and distribution, gradient HPLC can be applied to separate based on chemical composition.

Gradient HPLC can be hyphenated with GPC–SEC in a fully automated setup to measure both distribution simultaneously with a high peak capacity and to detect differences in batches (cf. Figure 1).

Experimental

All experiments were performed on PSS SECcurity equipment using the following conditions:

Eluent 1st dim.:	n-Hexane/THF p.a. gradient
Column 1st dim.:	PSS Si-60 5 µm
Flow-rate 1st dim.:	0.1 mL/min
Injection volume:	20 µL
Transfer:	PSS 2D tandem transfer valve with two 100 µL loops
Eluent 2nd dim.:	THF p.a.
Column 2nd dim.:	PSS HighSpeed SDV 5 µm, 10000 Å
Flow-rate 2nd dim.:	6.25 mL/min
Detection:	SECcurity VWD 1260 UV at 254 nm
Calibration:	PSS Polystyrene ReadyCal Standards, PSS Polybutadiene standards
Data system:	PSS WinGPC Unity 7.5

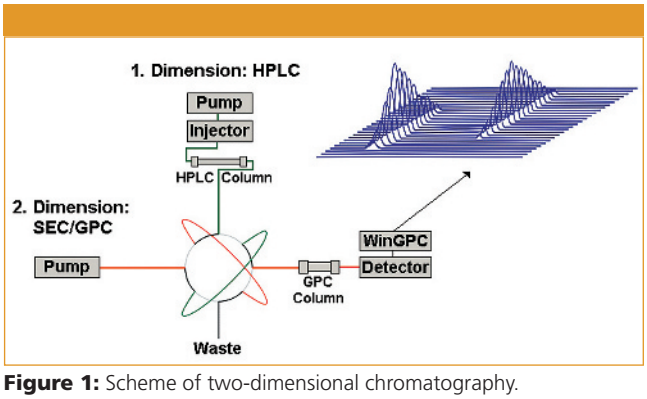

Figure 1: Scheme of two-dimensional chromatography.

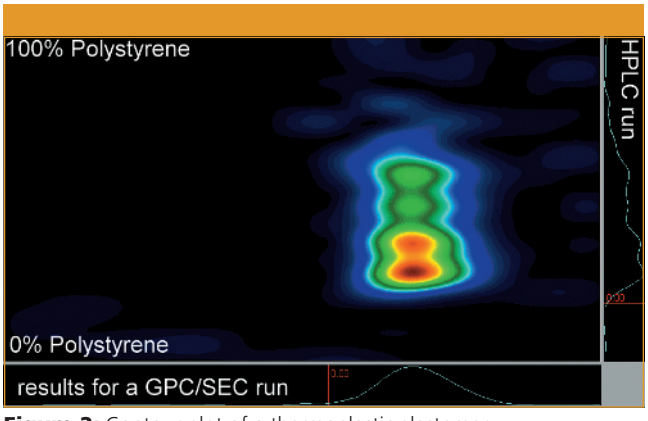

Figure 2: Contour plot of a thermoplastic elastomer.

Results

The 2D approach is the only way to determine two property distributions independently and unambiguously. The online combination of gradient HPLC and GPC–SEC increases the peak capacity of the separations and those peaks which cannot be separated by either method alone to be examined more closely. The HPLC conditions are selected to separate according to polybutadiene content.

Figure 2 shows the contour plot for a thermoplastic elastomer that shows one narrow main peak in GPC–SEC. However, 2D separation reveals that four different compositions are present that co-elute in the GPC–SEC experiment. The species differ in composition and polybutadiene content. The color code indicates the concentration of each peak. Simultaneous molar mass results and composition results can be measured using the calibrated GPC–SEC and HPLC axis.

PSS Polymer Standards Service GmbH
In der Dalheimer Wiese 5, D-55120 Mainz, Germany
tel.+49 6131 96239 0, fax +49 6131 96239 11
E-mail: Pkilz@polymer.de
Website: www.pss-polymer.com

High Temperature Gel Permeation Chromatograph (GPC–SEC) with Integrated IR5 MCT Detector for Polyolefin Analysis: A Breakthrough in Sensitivity and Automation

B. Monrabal, J. Sancho-Tello, J. Montesinos, R. Tarín, A. Ortín, P. del Hierro and M. Bas, Polymer Char

Introduction

Gel permeation chromatography (GPC), also referred to as size exclusion chromatography (SEC), when applied to polyolefins analysis (Polyethylene, PE and Polypropylene, PP) has been considered a demanding task due to the high temperature required for operation and the use of hazardous chlorinated solvents for resin dissolution.

In the last 20 years the sample preparation process, of major importance in the GPC–SEC analysis of polyolefins, has not advanced significantly; vials are being filled with solvent manually and in most cases external filtration after dissolution is carried out with subsequent manual vial transfer before injection. Sample degradation, especially in the case of polypropylene, has not been fully eliminated and samples still remain at a high temperature longer than required for proper dissolution.

It has been common practice to place the GPC–SEC columns, injector valves, and detectors in the same oven compartment and when loop size or filter needs to be changed or the detector maintained the whole system needs to be cooled down to prevent column damage; thus, requiring slow cooling and heating rates and a long stabilization time before analysis can be performed.

Little effort has been applied to investigate chemical composition -molar mass interdependence in the case of polyolefins, and when it was established it demanded off-line FT-IR systems or external flow through cells with liquid nitrogen cooled detectors.

With these considerations in mind, Polymer Char initiated the design of a totally new GPC–SEC system based on a well-known infrared detector (IR4) which provides good stability and sensitivity for the analysis of polyolefins; infrared detection results in equivalent molar mass distributions (MMD) curves to those obtained by DRI and without the presence of negative or spurious peaks after polymer elution.

Today, the use of the IR4 detector in GPC–SEC analysis is common in many laboratories, meaning a significant breakthrough in detection technology. For the first time simultaneous concentration and composition signals were obtained on a single instrument; the composition signal allows the measurement of branches in PE copolymers, ethylene content in EP copolymers and vinyl acetate in EVA resins, all along the molar mass distribution. This new dimension in GPC analysis (chemical composition) comes at a time when new commercial copolymer resins demand such a composition–molar mass interdependence analysis.

Instrument Description: New GPC-IR

In this application note we describe the full integration of the new infrared detector IR5 MCT, which has outstanding sensitivity and stability, into the GPC-IR instrument redesigned in 2012.

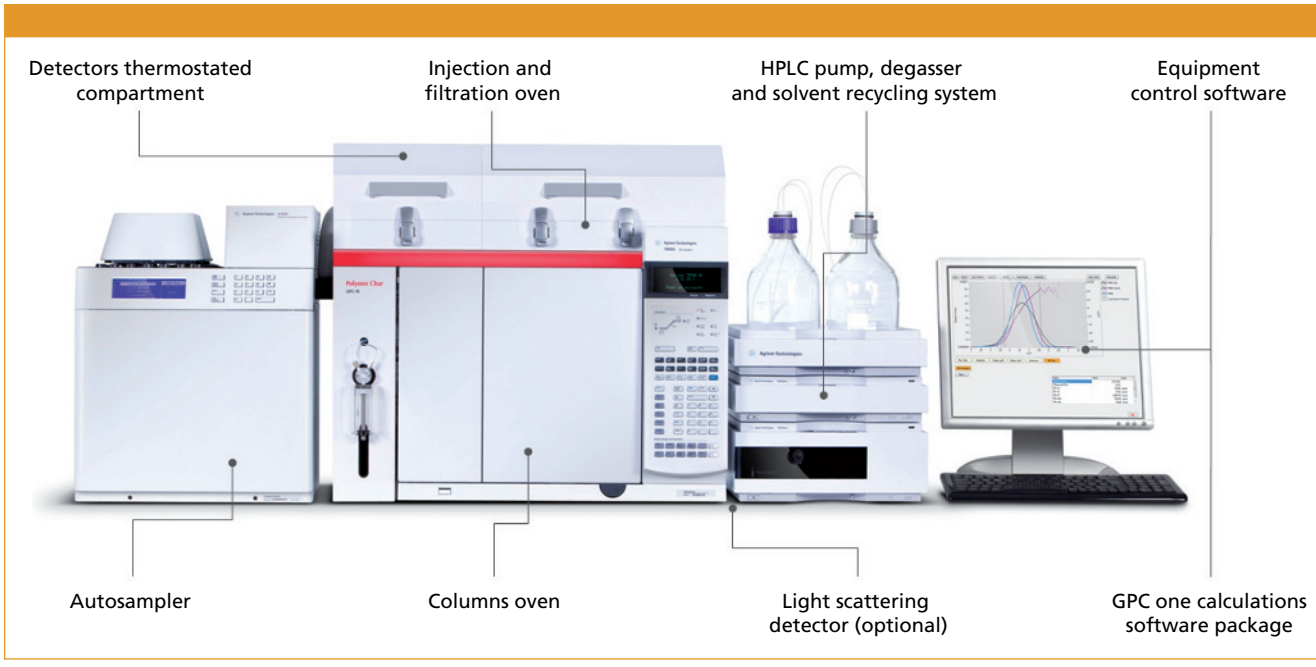

Figure 1: Schematic diagram of Polymer Char high temperature GPC-IR system.

Besides the IR5 MCT detector and the most advanced sample preparation system, the new GPC-IR incorporates other technological developments, (see Figure 1), to fulfill Polymer Char's design philosophy, based on the principles of the most reliable hardware, full automation, user friendly operation, easy maintenance and proper diagnostic tools:

- Three independent thermostated compartments with adjacent walls for reduced length of connecting tubing are included within the main body of the GPC-IR which also holds all the instrument electronics:
  - independent column oven,
  - injector and filter compartment,
  - detectors compartment.
- Separate high temperature autosampler module, in which sample preparation takes place.
- Solvent management system incorporating Agilent HPLC pump and degasser, syringe dispenser and a software-controlled solvent recycling system.
- Differential viscometer and optional DAWN® HELEOS™ II multiple angle light scattering (MALS) detector from Wyatt Technology®, complementing the IR5 MCT detection, and optional IR4.
- Advanced and comprehensive processing software GPC One which integrates all detectors' signals in a single package.
- Virtual instrumentation control software with advanced diagnostics tools and prepared for remote control access.

Sample Preparation

The GPC-IR system has been designed with a fully-automated sample preparation approach, minimizing the user interaction and eliminating manual solvent handling, while at the same time taking care to prevent sample degradation. The analyst needs only to weigh the samples into separate vials and place them in the autosampler tray where they will stay at room temperature waiting for the dissolution step (Figure 2).

The autosampler can handle 70 vials of 10 mL and the new design can accommodate 40 vials of either 10 or 20 mL for a better sample prepartion. In both cases vial inserts of 3 mL can be used when having only a few milligrams of sample.

The control software takes care of the overall solvent-handling process and dissolution time coordination with steps as shown in point 4 of Figure 2. Samples solution stay at a high temperature for just the required time for dissolution.

Solvent preheating before vial filling, a heated carousel with forced airflow and vial shaking results in a very efficient but gentle dissolution process.

Sample degradation in polypropylene and in very high molecular weight (MW) polyolefins is minimized. This was made possible by the elimination of oxygen from the vial and solvent, through the nitrogen purge and installed degasser, the precise control of time and temperature in the dissolution step and the avoidance of stirring bars which may result in shear degradation.

A significant effort was devoted to developing a fully automated operational in-line filter media and filtration process which would be reliable and last for months.

Filtration is performed during the filling of the injection loop; therefore, only a small part of the solution needs to be filtered. Once the injection is performed, the filter is back-flushed with a significant solvent volume from the syringe to have the filter clean and ready for the next sample filtration.

New Integrated Infrared Detector IR5 MCT

The most important aspect of infrared detection of polyolefins is the good sensitivity, baseline stability and fast stabilization periods as

1 **Weigh sample**

2 **Insert polymer** in the vial

3 **Transport to GPC-IR** carousel or system

4 **Sample Preparation Steps:**

. Vial nitrogen purge to remove oxygen.
. Automated vials solvent filling.
. Automated flow marker injection.
. Sample dissolution by gentle shaking.
. Sample solution staying at high temp. just the required time for dissolution.
. Automated in-line filtration with backflush after every injection.

Figure 2: GPC-IR sample preparation process.

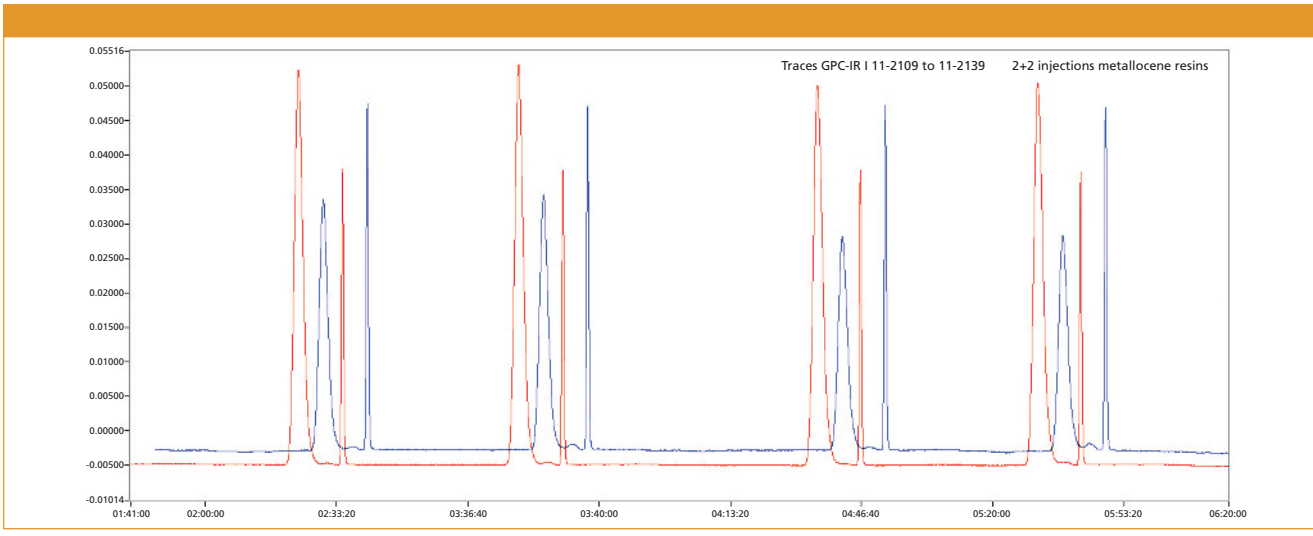

Figure 3: Recording baseline of four injections (two per vial) using three Agilent PLgel Olexis GPC/SEC columns and 2 mg/mL, 200 µL loop. Red signal: concentration. Blue signal: composition (CH_3/1000C emphasis).

has been demonstrated by the extensive incorporation of the IR4 detector in existing GPC systems. Besides sensitivity and stability of the concentration signal, infrared detection provides a continuous and simultaneous measurement of chemical composition along the MMD.

New developments in infrared technology have allowed us to integrate a highly sensitive Mercury Cadmiun Telluride (MCT) detector (thermoelectrically cooled) into the GPC-IR, thereby eliminating the need for heated transfer lines. The results obtained show an outstanding improvement in signal-to-noise ratio over the standard IR4 of around 10 times in both concentration and composition signals. Besides sensitivity improvement, the now integrated IR5 MCT is not affected by variations in laboratory temperature due to the carefully controlled detector environment, resulting in superb long-term baseline stability.

The analysis of two metallocene PE samples, each one by duplicate from the same vial, is shown in Figure 3. An excellent sensitivity and baseline stability can be observed in concentration as well as composition signals for an injection of 200 microlitres of a solution of 2 mg/mL in a 3 Agilent PLgel Olexis column. The good definition of baseline is the basis for the precise calculation of MW moments.

In Figure 4a an expanded view of one of the curves is presented without any smoothing to show the good sensitivity and stability achieved. The calculated MMD is shown in Figure 4b with composition-molar mass interdependance.

The high sensitivity of the IR5 MCT allows us to reduce the injected amount of sample into the column preventing viscous fingering effects and columns damage when analysing high MW polyolefins. The repeated analysis of one of the samples by lowering the concentration

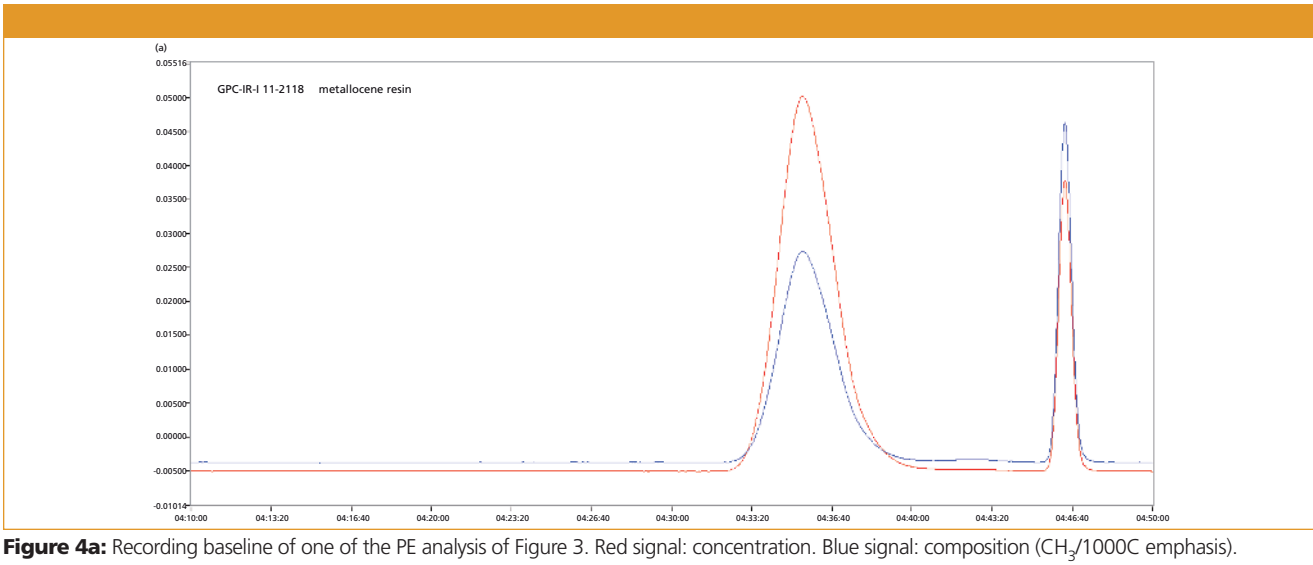

Figure 4a: Recording baseline of one of the PE analysis of Figure 3. Red signal: concentration. Blue signal: composition (CH_3/1000C emphasis).

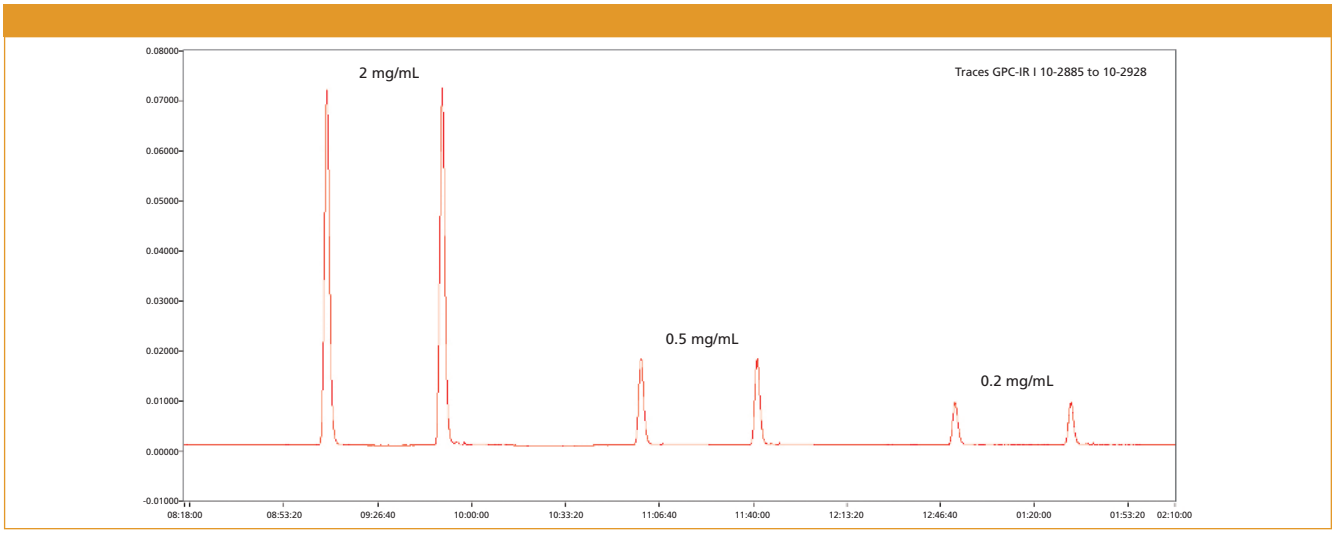

Figure 5: Recording baseline of duplicate PE analysis at different concentrations and with 200 µL loop.

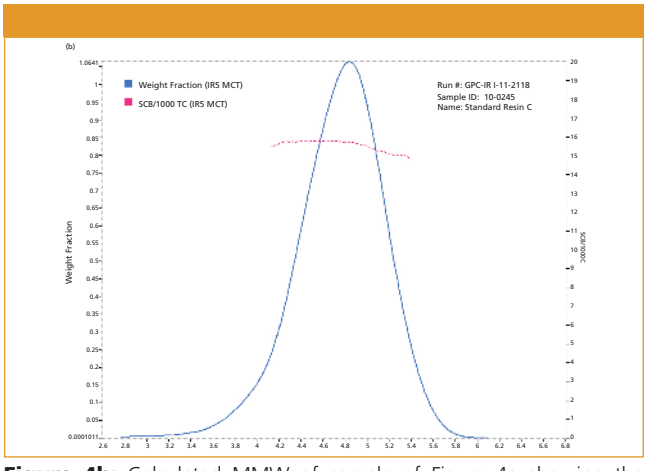

Figure 4b: Calculated MMW of sample of Figure 4a showing the composition – molar mass interdependence.

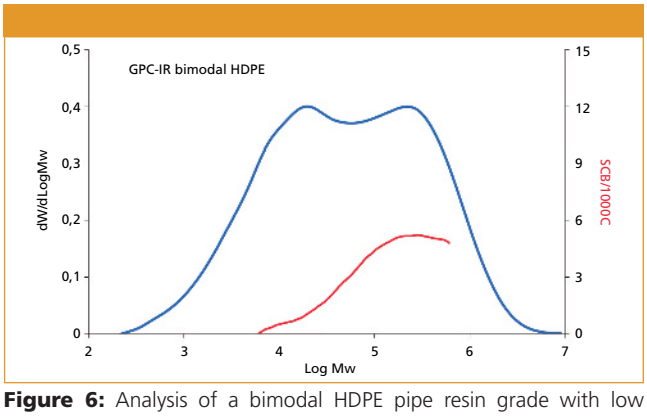

Figure 6: Analysis of a bimodal HDPE pipe resin grade with low number of branches. GPC-IR run at standard conditions 200 µL injection of 2 mg/mL with three Agilent PLgel Olexis columns.

down to 0.2mg/mL is presented in Figure 5 maintaining a good s/n ratio and baseline stability.

A significant advantage of using the integrated IR5 MCT detector is in the composition analysis along the molar mass of PE with a very low number of branches like in the case of HDPE pipe resins grades, which previously required the use of FT-IR with detection at liquid nitrogen temperatures. The analysis of a pipe resin with a low number of branches is shown in Figure 6 with a limit of detection of less than 1 CH_3/1000C.

Conclusions

The integration of the new IR5 MCT detector into the GPC-IR results in an outstanding sensitivity in both concentration and composition signals; this is an order of magnitude better than the existing IR4 detector. The new design of the IR5 MCT makes it also very resilient towards external temperature changes, while an order of magnitude improvement in baseline stability has been achieved as well; never before has the stability and sensitivity of GPC analysis of polyolefins reached such a mark.

A good baseline definition is the basis for precise MMD analysis and calculation of MW moments. Reducing the amount of polymer injected when dealing with high MW materials reduces plugging problems, extends column life and eliminates or minimizes viscous fingering spurious effects.

Full automation of the sample preparation with special care to avoid sample degradation and the incorporation of automated solvent recycling are presented in the new GPC-IR design.

Polymer Char

Polymer Characterization S.A.
Gustave Eiffel 8, Paterna, Valencia E-46980, Spain
tel. +34 96 131 81 20, fax +34 96 13181 22
E-mail: info@polymerchar.com Website: www.polymerchar.com

Synthesis Monitoring and Oligomeric Analysis of PEGylated Polymers

Amandaa K. Brewer, PhD, Tosoh Bioscience LLC

New roles and applications in the areas of science and technology are continuously being found for synthetic polymers. Specifically, the use of synthetic polymers in medicine is growing, as these polymers offer unique and versatile platforms for applications such as implants, medical devices, surgical adhesives, drug delivery vesicles, and injectable polymer-drug conjugates (1,2). As the applications of synthetic polymers increases, there is a manifested need for methods to accurately and precisely characterize the materials.

One of the most common and valuable tools employed for the analysis and characterization of polymers is size exclusion chromatography (SEC or gel permeation chromatography, GPC). The principle use of SEC, even a half-century after its inception, remains as determining the molar mass averages and distributions of natural and synthetic polymers through the application of calibration curves (3). The applicability of SEC for synthetic polymers also extends into the realms of synthesis monitoring and oligomeric quantification. Synthesis monitoring using SEC not only allows for separation of polymeric material based on size but also provides information about the reactions, e.g., did the reaction go to completion, is the product uniform in terms of molar mass or size, did a by-product form, etc. Oligomeric SEC plays an important role in the quantification of oligomeric content (i.e., low-molar mass species) of a polymer sample for the purposes of pre-manufacture notification (PMN) regulations for new chemical substances as well as import and export purposes (3).

The utility of SEC for synthesis monitoring and oligomeric analysis makes it an invaluable tool for characterizing synthetic polymeric material for use in medicine, as these materials require thorough characterization amongst other validations. Here we report on the use of an all-in-one dedicated GPC system, the EcoSEC® GPC System, to monitor the synthesis and to quantify the oligomeric content of two PEGylated synthetic polymers intended for use in medical applications.

Experimental Conditions

Sample analysis was performed on a system consisting of an EcoSEC GPC System (HLC-8320) equipped with a refractive index detector (RI). Separation of unfiltered 40 µL injections occurred over a column bank consisting of two 6.0 mm i.d.× 15 cm, 3 µm particle size TSKgel® SuperH3000 columns (exclusion limit 60,000 g/mol) preceded by the appropriate guard column (Tosoh Bioscience LLC). The solvent and mobile phase were tetrahydrofuran (THF) (Fisher Chemical) at a flow rate of 0.3 mL/min. Detector, pump oven, and column oven were maintained at 35 °C. Two PEGylated polymers and a starting material were analyzed: PEG-A, PEG-B and starting material C, respectively. For all chromatographic determinations, results are averages of three injections from two separate sample solutions. Sample solutions were prepared by diluting the sample (98% purity) with THF for a final sample concentration of approximately 10 to 15 mg/mL. Samples were shaken manually for a minute and allowed to sit for 3 h before analysis was performed. Data was processed with the EcoSEC GPC Workstation Software version 1.08.

A calibration curve was created using PStQuick Kit-L polystyrene standards (Tosoh Bioscience LLC) ranging in molar mass from 266 to 37,900 g/mol. Calibration curve data was fitted with a cubic function and error values were less than 5%.

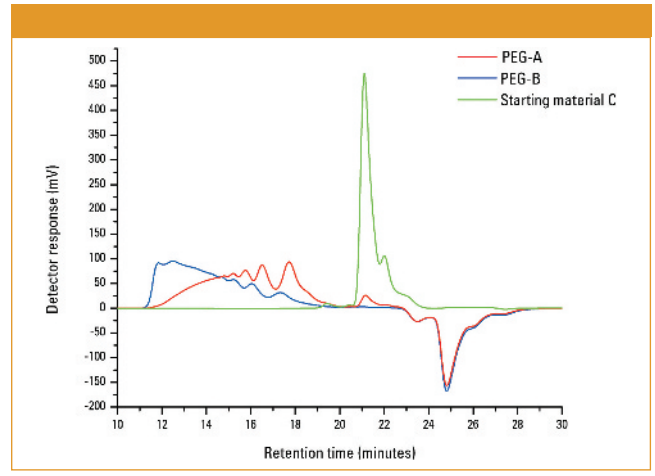

Figure 1: Synthesis monitoring by SEC. SEC elution profile of PEG-A (red), PEG-B (blue), and starting material C (green) as monitored by RI.

Results and Discussion

As described above, an EcoSEC GPC System equipped with an internal dual-flow refractive index (RI) detector was used for the characterization of two PEGylated polymers. The detector response of the RI detector was used to monitor the synthesis of the formation of the two PEGylated polymers and to compare the chromatograms of both materials to that of one of the starting materials. Additionally, a polystyrene relative calibration curve was used to determine the peak-average molar masses M_p and the oligomeric content of the two samples.

Synthesis Monitoring

SEC is an ideal tool for monitoring the formation of PEGylated polymers, as the method separates the polymers based on size while simultaneously providing information about the molar mass of the newly synthesized species. The two polymers analyzed here, PEG-A and PEG-B, are composed of the same basic components which vary in molar mass between the two samples. The molar mass difference of the starting material of PEG-A and PEG-B is reflected in the end-products, as PEG-A and PEG-B produce different chromatograms when separated by SEC, Figure 1.

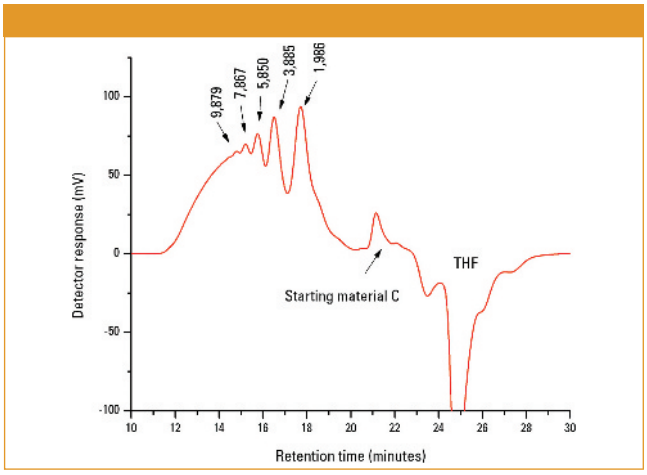

Figure 2: SEC analysis of PEG-A as monitored by RI. Numbers on graph represent polystyrene relative peak-average molar mass M_p values of each mode.

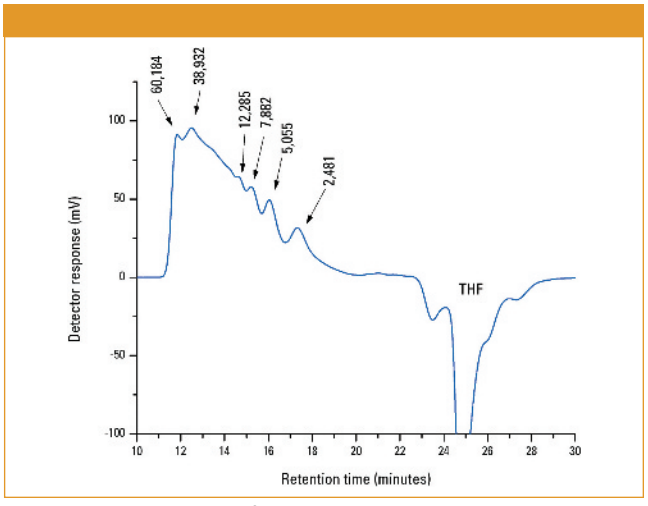

Figure 3: SEC analysis of PEG-B as monitored by RI. Numbers on graph represent polystyrene relative peak-average molar mass M_p values of each mode.

The ability to identify all species within the PEGylated sample is essential in the validation of polymers intended for medical applications. Thus, the role of synthesis monitoring here is not only to compare the difference between the two species produced from similar procedures but to compare the two species to the starting material(s). As seen in Figure 1, by comparing the SEC chromatograms of starting material C with that of PEG-A and PEG-B, a fairly substantial amount of starting material C remains in PEG-A while all of starting material C has reacted in PEG-B.

Oligomeric Analysis

The characterization of the oligomers present in the PEGylated samples is best achieved by the peak-average molar mass M_p. The polystyrene relative peak-average molar mass values for each mode in the chromatograms for the PEG-A and PEG-B samples are given in Figures 2 and 3, respectively. The values for the peak-average molar mass between the two samples differ significantly. The M_p values of PEG-A range from approximately 2000 to 10,000 g/mol indicating that most, if not all, of the species present are oligomeric in nature. Conversely, the M_p values of PEG-B range from approximately 2500 to 60,000 g/mol indicating that low- and high-molar mass species are present.

Conclusions

The EcoSEC GPC System was used for synthesis monitoring and oligomeric content analysis. The synthesis process for two PEGylated synthetic polymers intended for use in medical applications was analyzed by comparing the SEC chromatograms of the two PEGylated polymers with that of one of the starting materials. From this comparison it was concluded that starting material remained in one of the PEGylated samples, PEG-A, and was absent in the other PEGylated sample, PEG-B. The SEC chromatograms of the PEGylated polymers also provided indication of differences in the molar mass distribution between the two PEGylated samples. Additionally, based on the peak-average molar masses M_p the oligomeric content of the two PEGylated polymers were shown to differ, with PEG-A containing mainly oligomeric species and PEG-B containing both low- and high-molar mass species. Combining the oligomeric content information with the SEC chromatograms was shown to provide a more detailed picture about the distribution of the low-molar mass species within the two PEGylated samples, information beneficial in the validation and regulation of synthetic polymers. Finally, the advanced engineering design of the EcoSEC GPC System, e.g., low-dead volume, minimal extra-column band broadening, etc., is an added advantage of using the system for synthesis monitoring and oligomeric analysis as it provides increased resolution and separation efficiency in the oligomeric region compared to traditional GPC systems.

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TOSOH BIOSCIENCE

Tosoh Bioscience LLC
3604 Horizon Drive, Suite 100, King of Prussia, PA 19406
tel. (484) 805-1219, fax (610) 272-3028
Website: www.tosohbioscience.com

Creation of a New IMS Analyzer Platform with High Resolving Power

Paula Holmes, PhD, and Bruce N. Laprade, PHOTONIS USA

Ion mobility spectrometry (IMS) has become widely accepted for the detection of chemical warfare agents, explosives, and narcotics as well as for pharmaceutical quality control and pesticide screening of food. Most currently available commercial IMS units have a resolving power of 10–60.

In order to minimize the frequency of false positive and false negative results that create delays and cost overruns, it has become necessary to design and construct an ion mobility spectrometer with high separation power without compromising instrument simplicity, serviceability, and cost. Such an instrument can be used in applications where high volume, accuracy, and speed of specific contaminant identifications are critical, such as those involving product safety, composition analysis and environmental control.

PHOTONIS has designed a new IMS analyzer platform designed for simple integration with most mass spectrometers, GCs and other analytical instruments. The new IMS analyzer unit combines PHOTONIS' patented Resistive Glass products with a novel robust ion gate technology to create an IMS analyzer which improves resolution and simplifies serviceability. This new unit provides significantly higher resolving power (64–150) when compared to most currently available IMS instruments.

In this new IMS analyzer platform, key components made from PHOTONIS Resistive Glass were designed to replace conventional lens assemblies. Reaction and drift regions of the ion mobility spectrometer were fabricated using Resistive Glass technology, as they enhance ion throughput and simplify construction.

Resistive Glass is manufactured in a patented process by PHOTONIS in which an electric field is created to guide or direct charged particles. It consists of alkali-doped lead silicate glass that has been reduced to make the surface a semiconductor. Resistive Glass can be used to manufacture capillary inlet tubes, drift tubes, ion guides, ion mirrors, collision cells, conversion diodes or voltage dividers.

The use of Resistive Glass drift tubes in an IMS Analyzer provides key benefits, such as uniform electric fields with minimal radial inhomogeneities and ease of construction. In addition, the single-piece construction allows uniform flow of drift gas without the need for additional containment.

A new novel and robust ion gating assembly was also created for this IMS Analyzer. It comes equipped with a Bradbury-Nielsen type electronic gate, built using photo-etching technology. The unique approach of the ion gate design results in a device that is robust, high-performance, low cost, and simple to assemble when compared to the conventional winding technique. The ability to handle entire grid sets rather than individual wires to insure precise positioning of all wires provides a distinct advantage to traditional approaches in terms of resolution and serviceability.

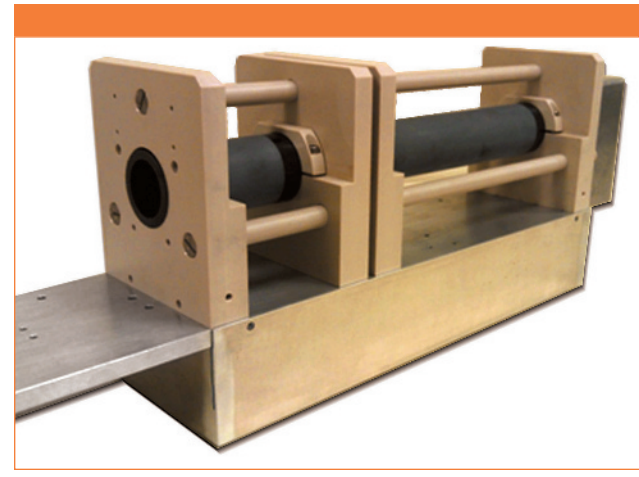

Figure 1: IMS analyzer prototype.

The new IMS unit can be operated at room temperature but also features integral heaters that can be operated at temperatures up to 150 °C.

This new IMS Analyzer is designed to be scaled or customized to interface with a wide variety of instruments to simplify IMS analysis in applications where a narrow range of contaminants need to be quickly identified. Its unique design allows the user to quickly and inexpensively perform a simple IMS analysis and receive fast, accurate results. Its higher resolving power (64–150) lowers the probability of false positive and false negative results for a more efficient analysis.

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Next-Generation Stationary Phases: Properties and Performance of Core–Shell Columns

This short review focuses on the physicochemical properties of core–shell particles and their resulting chromatographic efficiencies as they relate to the comparison of their mass transfer kinetics to those of columns packed with sub-2-µm fully porous particles.

Jesse O. Omamogho, Ekaterina Nesterenko, Damian Connolly, and Jeremy D. Glennon

In recent years, column technologies have diversified for a multitude of separation applications. The improvement in separation efficiency attainable today on modern liquid chromatography (LC) columns results from the evolution of new packing materials and sorbents that offer analysts the choice to use fully porous particles, core–shell particles, or silica–polymer monolithic columns, depending on the application (1). Among the new column packing materials, core–shell particles, also called *superficially porous particles*, of sub-3-µm diameter have gained considerable attention because of significant improvements in separation efficiency. Columns packed with core–shell particles can generate a minimum plate height below $H \sim 3.5$ (2,3) for small molecules, a similar efficiency to that of columns packed with fully porous sub-2-µm particles.

The use of core–shell particles as column packing materials is not new; in fact, they have been in existence and have been used since the early days of high performance LC (HPLC) (4–6). The main purpose of pellicular particles in the early days of HPLC was to improve the mass transfer resistance of large molecules to facilitate faster separation (6). However, pellicular particles later met their demise because such particles were not as robust and refined as the fully porous particles that were introduced later. For detailed historical accounts of the development of various pellicular particles as packing materials for HPLC columns, readers should refer to reference 7. The new-generation core–shell particles display significant improvements in important physicochemical parameters, such as particle-size distribution; thickness of the porous shell, providing substantial increase in surface area for retention (8); surface roughness or smoothness; and the purity and robustness of the base core–shell silica. A number of current manufacturers and academic researchers currently are investing a great deal of time in the preparation of core–shell silica particles (9); a list appears in Table II in reference 10. The new-generation core–shell particles have been solely designed to compete with the sub-2-µm fully porous particles by providing a substantial increase in column permeability; this results in faster separations while maintaining the same high column efficiencies. Recently, another set of core–shell particles (Phenomenex's Aeris core–shell silica particle) has been released and is claimed to be a rival to the sub-2-µm fully porous particles for separating large molecules (11). This short review will summarize the key physiochemical properties of the new generation of core–shell particles that results in superior performance of HPLC columns.

Physical Properties of Core–Shell Particles

Shell Thickness

In contrast to earlier core–shell packing materials, the volume fraction of the porous permeable layers around the solid impermeable core of the new generation of core–shell particles (which are generally 2.6, 2.7, or 1.7 µm in diameter) is 29–37%, as opposed to <10% for the earlier core–shell particles (5,12). For the separation of small molecules, increasing the shell thickness is of paramount importance because it contributes to solute retentivity and minimizes the *A*-term contribution to band broadening (13). For example, the 2.7- and 2.6-µm commercially available core–shell particles provide an excellent peak capacity under gradient elution with holdup time as low as 10 s, which requires an inlet pressure of 400 bar (14,15).

Figure 1 shows electron micrographs of how porous shell thickness is controlled while keeping the overall core–shell particle diameter constant. Gritti and Guiochon (16) have examined in detail the improvement in column separation efficiency brought by the new core–shell particles as a result of their thicker porous shell. Modern-day core–shell particles now have larger surface areas (~100–150 m^2/g) compared to their predecessors (< 1–13 m^2/g), which results in significant gains in sample loading capacity and enhanced stability of the stationary phase.

Surface Roughness

Literature reports have shown that the surface roughness of core–shell particles plays a major role in enhanced film mass transfer resistance, an independent contribution to band broadening caused by the rugosity of the particle surface (17). A recent study by Gritti and Guiochon has shown that core–shell particles with large pore sizes can alleviate the film mass transfer resistance (18).

Figure 2 shows the external surface structure of two sub-3-µm core–shell particles: prototype 2.6-µm core–shell

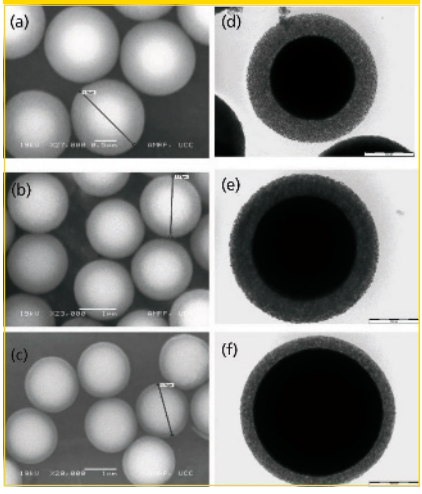

Figure 1: Electron micrograph of three Erioshell 1.7-µm particles: (a) SEM of EiS-350 and (d) TEM of EiS-350; (b) SEM of EiS-250 and (e) TEM of EiS-250; (c) SEM of EiS-150 and (f) TEM of EiS-150. Note the stratified structure that is clearly visible on the EiS-350 shell particle, indicating uniform layering of the silica shells. Adapted from reference 8 with permission.

particles synthesized in the ISSC Cork laboratory (Figure 2a); Kinetex 2.6-µm core–shell particles (Phenomenex) (Figure 2b); and 3.5-µm fully porous particles (Figure 2c). The prototype material has a similar smooth surface in comparison to the fully porous particles. Conversely, the Kinetex 2.6-µm material is unique with its rough surface. The impact of the surface roughness on the packed bed porosity heterogeneity is still a matter of debate.

Particle-Size Distribution and Column Packing

The degree of the packed-bed radial heterogeneity of the external porosity across the column is directly proportional to the *A* term of the van Deemter equation. Traditionally, there is a general consensus that a narrow particle-size distribution is the major source of improvement of the *A* term of columns packed with core–shell particles (19,20), it was recently reported that subtle changes in the column packing methodology itself can greatly improve packed beds made of particles with a broader size distribution (21), provided fines of particles of the same fractional size of the external porosity are eliminated. Using a numerical packing algorithm, Tallarek and colleagues (22) studied the relationship of particle-size distribution in unconfined packing (without a wall) generated over a range of bed porosities (external porosities) to flow permeability and the *A* term of the van Deemter equation. The *A* term is expected to be more sensitive to bed porosity heterogeneity than to particle-size distribution. An example of the relationship of particle-size distribution and efficiency was demonstrated at the ISSC Cork laboratory. As shown in Figure 3, two batches of prototype 2.6-µm core–shell particles were characterized and differ significantly in particle size distribution (Figure 3a and 3b). These different packing materials were identically packed in 100 mm × 2.1 mm columns and their corresponding height equivalent to a theoretical plate (HETP) plot is given in Figure 3c, which shows no discernible differences. This result implies that particle-size distribution has a minimal effect on overall column efficiency.

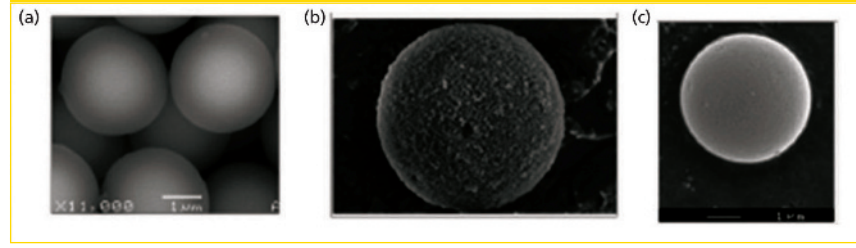

Figure 2: Scanning electron micrograph (SEM) images of HPLC particles: (a): prototype core–shell particles from ISSC, 2.6-µm; (b) Phenomenex Kinetex core shell particle, 2.6-µm; and (c) Phenomenex LunaPorous particle, 3.5-µm.

Core–Shell Particle Chromatographic Efficiency

Height Equivalent to a Theoretical Plate

Columns packed with 2.7- and 2.6-µm core–shell particles consistently generate reduced plates heights of 1.5 and 1.3, respectively, in 4.6 mm i.d. column formats, generally outperforming columns packed with fully porous particles (h > 2.0) in the same column internal diameter under identical conditions. This improvement in core–shell column efficiency is attributed to the mechanism of the mass transfer kinetics, which ultimately plays a pivotal role in the contributions to the HETP. The HETP is a combination of eddy and longitudinal diffusion and resistance to solute mass transfer in the stationary phase present inside the pores and outside the particle surface (the latter is referred to as *film mass transfer resistance*) and is represented as

$$H_{(\mathrm{HETP})} = A + B/u + C_{\mathrm{p}}u + C_{\mathrm{f}}u \qquad [1]$$

where *A* and *B* are the coefficients of the eddy and longitudinal diffusion, respectively, $C_{\mathrm{p}}u$ and $C_{\mathrm{f}}u$ are the trans-particle and film mass transfer resistance coefficients, respectively. For detailed description of the physicochemical aspects of the different mass transfer coefficients and their contributions to HETP, readers are referred to reference 23.

Eddy Diffusion

Conventional wisdom generally accepts that the *A* term of solute mass transfer is unaffected by flow velocity of the mobile phase and that the particle-size distribution is the determinant factor of the *A* term. The evolution of modern core–shell particles appears to confirm a close relationship between particle-size distribution and the *A* term. However, recent studies on packing reconstruction using computer simulation have elucidated the structure–transport relationship of packed beds and demonstrated that the current state of particle-size distribution commonly found in commercial packings of fully porous particles had only a minor effect on the *A* term (24,25). The relationship between the slurry packing methodology and the randomness of interparticle pore spaces present in the packed bed from the column center to the wall is now becoming an avenue to explore (26), because this is the true parameter that influences eddy diffusion. Because the currently existing commercial

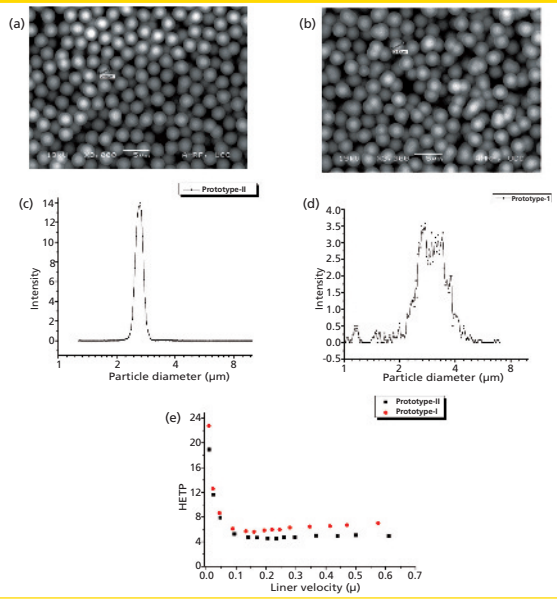

Figure 3: The relationship of particle-size distribution to column HETP. (a): The SEM image of a prototype-II 2.6-µm core–shell particle with narrow particle-size distribution, and (b) a prototype-I non-uniform 2.6-µm partricle with broad particle-size distribution. (c) and (d): the particle-size distribution measured using electric sensing zone (ESZ) of (a) and (b), respectively; and (e): the corresponding HETP plot for the core–shell particle shown in (a) and (b). Column dimensions: 100 mm × 2.1 mm C18, solute: naphthalene. The surface area and pore size of both particles are 145 m^2/g and 120 Å respectively. Note the small difference on the HETP plot only visible at the high linear velocity branch.

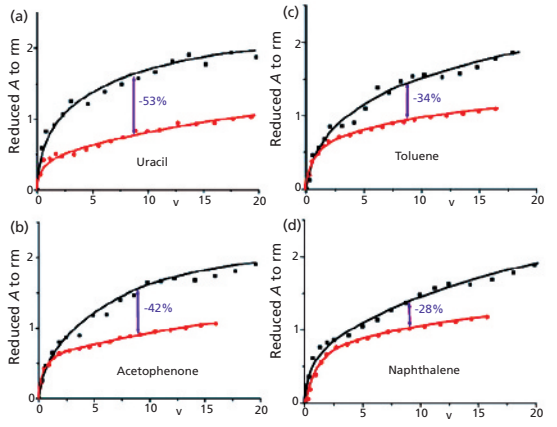

Figure 4: Plots of reduced *A* term vs. ν for (a) uracil, (b) acetophenone, (c) toluene, and (d) naphthalene. ■ Fully porous Atlantis-C18, 150 mm × 4.6 mm column, 3-µm particles. ● Kinetex shell particles, 100 mm × 4.6 mm column, 2.5-µm particles. Adapted from reference 15 with permission.

sub-3-µm core–shell particles seem to generate bed porosity that mimics loose packing and can still generate very high efficiency, it can be said that the bed porosity is far less heterogeneous from the center of the column to the wall in comparison to columns packed with fully porous particles. Gritti and Guiochon have demonstrated that there is a strong link between retention and the *A* term for small molecules at $k' = 0$ to $k' = 3$ (16,27).

Figure 4 illustrates the relationship between retention factors of different solutes and the reduced *A* term for columns packed with fully porous particles and core–shell packing (16). The *A* term for nonretained solute (uracil) is far superior for the core–shell column than for the fully porous column at high flow rates. The improvement of the *A* term becomes more significant for well-retained solutes as a result of the impact of retention on the *A* term, which ultimately is larger for fully porous particles.

Longitudinal Diffusion

The *B* term is related to the bulk molecular diffusivity, D_m, according to the following equation:

$$H = B/u = 2\gamma_e D_m/u \quad [2]$$

where γ_e is the obstruction factor. The *B* term in the HETP equation is systematically reduced in core–shell particles compared to the fully porous packing. Given that solute diffusion in the stationary phase is bound to be slower than that in the bulk eluent, the kinetics of diffusion are enhanced at the interface of the solute and stationary phase and results in additional mass transfer effects referred to as *surface diffusion* (28), which increases with stationary-phase surface area. Surface diffusion is minimal in core–shell particles by virtue of the presence of the impermeable solid core particles; thus the *B* term is smaller for the core–shell particles and at zero to very low flow rates. The *B* term can be measured experimentally by the peak parking method (3) and the same method (after subtle modification) also has been reported to estimate the bulk molecular diffusivity, D_m (29).

Trans-Particle and Film Mass Transfer Resistance

The improvement in trans-particle mass transfer resistance (the *C* term) in columns packed with core–shell particles is not significantly greater than it is with the fully porous particles for the separation of small molecules. This is because of the larger contribution of surface diffusion (28) found in fully porous particles (which have a larger specific surface area in comparison to core–shell particles of the same pore size), which increases the particle diffusivity, D_p (a contribution of both pore and surface diffusion) for molecules with faster D_m. With large molecules, however, the pore size of the core–shell particles greatly improves D_p (18). This can afford an improved *C* term for large molecules. The Aeris WidePore core–shell particles serve this purpose, allowing even faster separation of

macro-molecule and proteins without loss of efficiency (30).

The film mass transfer resistance coefficient is larger for the new generation of core–shell particles, especially for large molecules, as a result of the particles' surface roughness, a property that fully porous particles, with their smooth surfaces, lack (17). A substantial increase in pore size has been reported to alleviate the film mass transfer resistance (18); this is the advantage of the Aeris core–shell particles that are designed with large pores.

Frictional Heat Effects

The frictional heat effect of columns packed with core–shell particles is only severe with sub-2-µm particles. However, in comparison to the fully porous sub-2-µm particles, the solid core present in the core–shell particles promotes better heat conductivity and frictional heating become preponderant on the fully porous sub-2-µm particles, especially when operated at high linear velocity (31). The low permeability of columns packed with sub-2-µm particles inherently causes large heat generation that changes the properties of the mobile phase that carries the solute through the columns. The sub-3-µm core–shell particles are now the solution to heat effect when very fast analysis is desired.

Concluding Remarks

A brief summary of the relationship of the physicochemical and mass transfer kinetic properties of the recently evolved core–shell particles has been given, based on recent scientific reports. The superior performance of sub-3-µm core–shell particles is largely attributed to the significant improvement in their manufacture, resulting in improvement in their physicochemical properties compared to the early day core–shell particles. The advantage of the lower permeability brought by the sub-3-µm core–shell particles greatly surpasses those of the sub-2-µm fully porous particles to allow a significant increase in speed of analysis. The future of core–shell particles will see further dramatic changes in porous shell design and configuration, including possible decreases in particle diameter to increase the speed of analysis even further (11).

Acknowledgment

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Ekaterina Nesterenko and Damian Connolly are with the Irish Separation Science Cluster (ISSC) at Dublin City University in Glasnevin, Dublin, Ireland.

Jesse O. Omamogho and Jeremy D. Glennon are with the Irish Separation Science Cluster (ISSC) and Analytical and Biological Chemistry Research Facility (ABCRF) in the Department of Chemistry at the University College Cork, in Cork. Ireland. Please direct correspondence to: j.glennon@ucc.ie and j.omamogho@ucc.ie. ■

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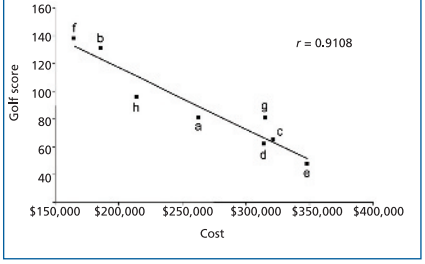

Figure 1: Chromatograms obtained using the conditions under which the ion suppression problem was originally discovered. The ion suppression trace is shown on the bottom. Column: 75 mm × 4.6 mm ODS-3; mobile-phase A: 0.05% heptafluorobutyric acid in water; mobile-phase B: 0.05% heptafluorobutyric acid in acetonitrile; gradient: 5–30% B in 4 min. Peaks: 1 = metabolite, 2 = internal standard, 3 = parent drug.

Table I: Factor levels used in the designs

Factor	Nominal value	Lower level (−1)	Upper level (+1)
Gradient profile	1	0	2
Column temperature (°C)	40	38	42
Buffer concentration	40	36	44
Mobile-phase buffer pH	5	4.8	5.2
Detection wavelength (nm)	446	441	451
Triethylamine (%)	0.23	0.21	0.25
Dimethylformamide	10	9.5	10.5

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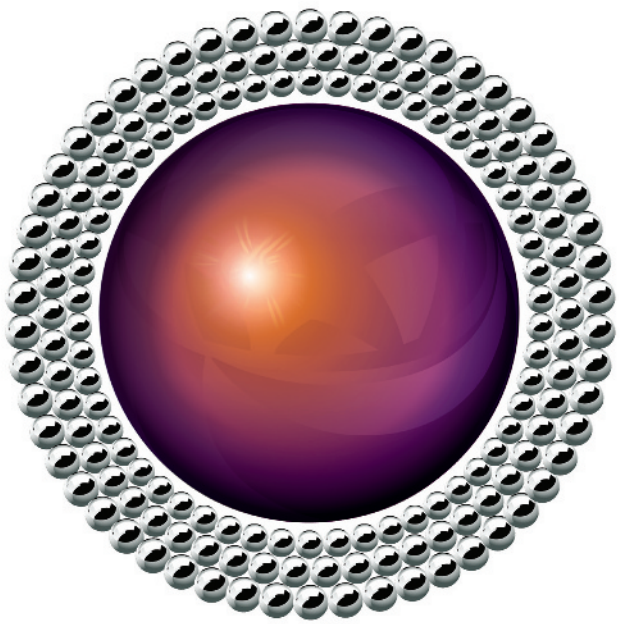

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