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June 2016
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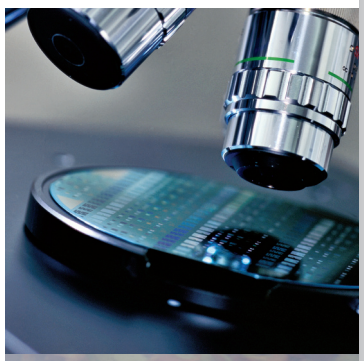
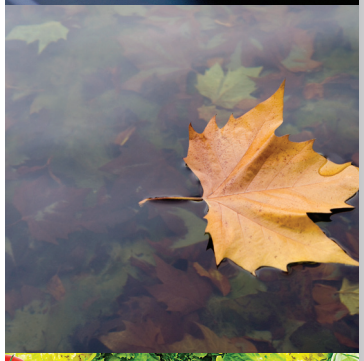
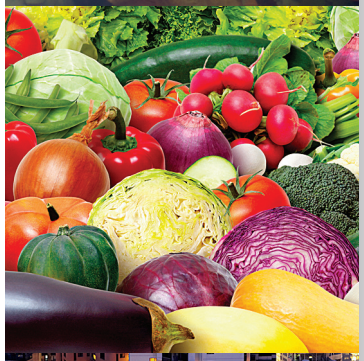

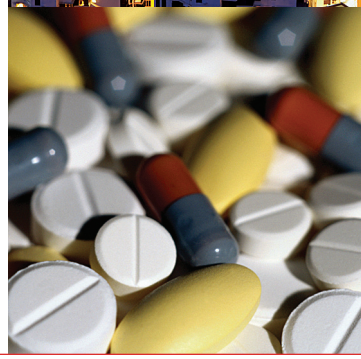

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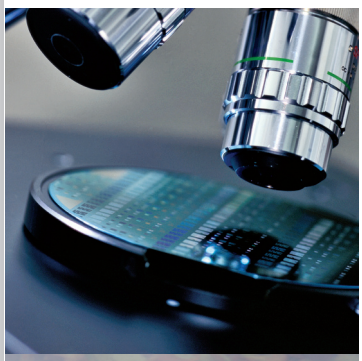
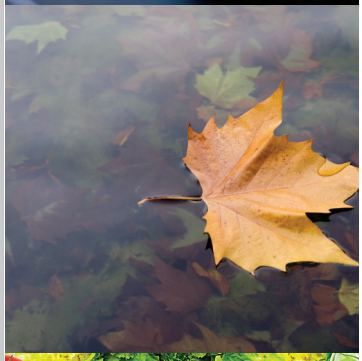
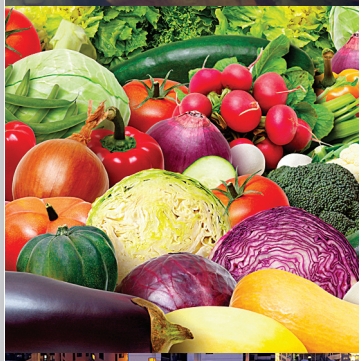

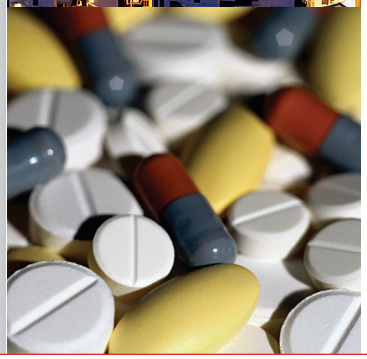

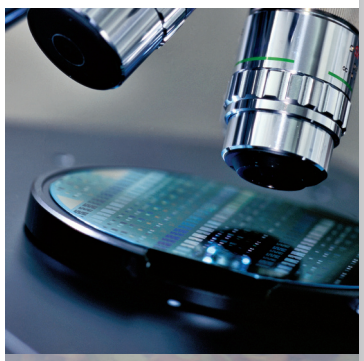

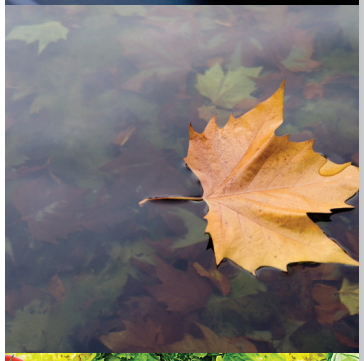

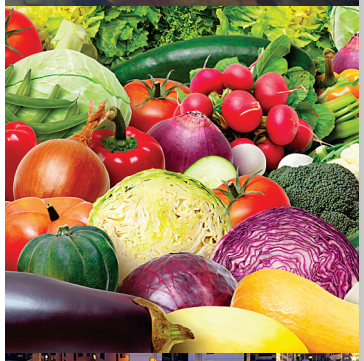

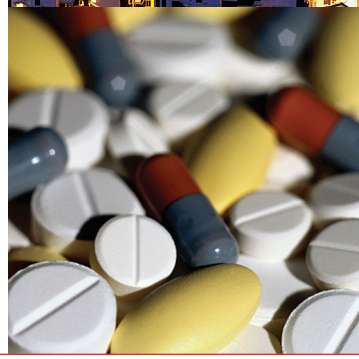

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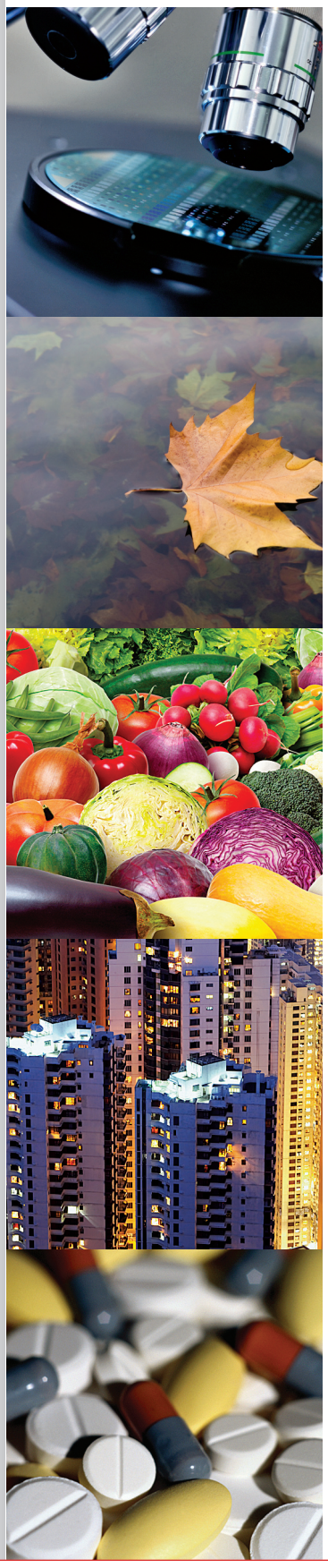

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Z-Drug and Metabolite Panel in Urine Using SPE Extraction and HPLC–MS-MS Analysis

Tina Fanning and Jody Searfoss, UCT, LLC

This application note describes the development of an analytical method for the sensitive and accurate determination of Z-drugs in urine using CLEAN SCREEN® XCEL I SPE columns and HPLC–MS-MS analysis. Chromatographic separation is achieved using a Selectra® DA column. Z-drugs or nonbenzodiazepines are a class of psychoactive drugs whose pharmacological actions are similar to those of the benzodiazepines and have shown efficacy in treating sleep disorders. Clean Screen® XCEL I columns were chosen because of their excellent capability to extract basic compounds while eliminating the need for time consuming column conditioning. This method reduces the amount of time needed to extract a panel of samples and minimizes solvent use for sample cleanup.

Extraction/Analytical Materials	
CSXCE106	CLEAN SCREEN ® XCEL I 130 mg / 6 mL tube
SLDA100ID21-3UM	Selectra® DA HPLC column 100 × 2.1 mm, 3 µm
SLDAGDC21-3UM	Selectra® DA guard column 10 × 2.1 mm, 3 µm

Procedure

1. **Sample preparation:**
   (a) To 1 mL of urine add 3 mL of 0.1% formic acid solution
   (b) Vortex for 30 s
2. **Apply sample to Clean Screen® XCEL I column**
   (a) Load sample directly to column without any preconditioning
   (b) Pull sample through at a rate of 1–2 mL/min
   (c) Dry column thoroughly under full vacuum or positive pressure for 1 min
3. **Wash cartridge**

(a) 1 × 3 mL 75:25 100 Mm acetic acid:MeOH
(b) 1 × 3 mL hexane
(c) Dry column thoroughly under full vacuum or positive pressure for 5–10 min

4. **Elution**
   (a) 1 × 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2)
   (b) Evaporate to dryness/reconstitute in 100 µL of mobile phase

Instrumental

LC–MS-MS: Agilent 1200 Series Binary Pump LC System/ API 4000 QTRAP MS-MS
Column: UCT Selectra® DA LC column, 100 × 2.1 mm, 3 µm
Guard column: UCT Selectra® DA guard column, 10 × 2.1 mm, 3 µm
Injection volume: 10 µL
Mobile phase A: 0.1% formic acid in D.I. H_2O
Mobile phase B: 0.1% formic acid in ACN
Column flow rate: 0.30 mL/min

Table I: Extraction recoveries and matrix effect data for Z-drugs in urine (n=5)

	50 ng/mL		300 ng/mL	
Analyte	Recovery (%)	Matrix Effect (%)	Recovery (%)	Matrix Effect (%)
N-Desmethyl Zopiclone	88	8	89	5
Zopiclone	90	44	91	23
Zopiclone-N-Oxide	71	46	75	37
Zolpidem	80	26	93	26
Zaleplon	90	13	102	20

Conclusion

Excellent recoveries ranging from 71% to 102% were obtained using UCT's XCEL I extraction column (CSXCE106) and procedure in conjunction with the Selectra® DA HPLC column (SLDA100ID21-3UM) for the analysis of Z-drugs and their metabolites in urine. Insignificant loss of Zaleplon was observed using 3 mL 75:25 100 mM acetic acid:MeOH wash in this study. To correct for any residual recovery or matrix issues, the use of deuterated internal standards is strongly recommended.

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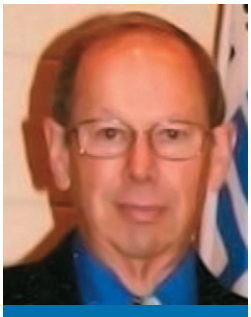
A Q&A

Technological Developments In Ion Chromatography



Dr. Arthur Fitchett
Director, Sales Training,
Thermo Fisher Scientific

The use of smaller particle (4-µm) ion-exchange columns enables higher efficiency separations and faster throughput seen in ultra high-pressure liquid chromatography (UHPLC). A reagent-free IC system with automated eluent generation capabilities and flexible detector configurations contributes to consistent results, operator safety, and fast, high-efficiency separations.

LCGC: When did high pressure become important to ion chromatography, and for what types of analysis is it best suited?

Fitchett: As we've seen with ultra high-pressure chromatography (UHPLC), there's been a move to smaller and smaller particle-size column packings. Smaller particle-size materials give higher efficiency separations and faster throughput. The same is now true for ion chromatography — we want to get higher efficiency separations. Pressure limitations for high-pressure ion chromatography (HPIC) systems are quite different than UHPLC because they are totally inert, metal-free systems. In UHPLC, pressures up to about 22,000 psi (1500 bar) are not uncommon. In HPIC, our maximum operating pressure under most conditions is between 5000 and 6000 psi.

By using a 4-µm particle ion-exchange packing material, we are able to get higher efficiencies and faster throughput. We're getting much better separations across all types of analyses — drinking water, wastewater, food, beverages, cosmetics, pharmaceuticals, and biotechnology compounds.

LCGC: What concerns are there when using 4-µm columns? Do you have to worry about particulates and column plugging?

Fitchett: As a rule of thumb, if you see visible particulates in your sample, we recommend filtering through either a 0.22- or a 0.45-µm filter. These are standard syringe filters that are typically designated as "sterile," but sterile doesn't necessarily mean clean. We highly recommend rinsing them with deionized (DI) water to ensure that there is no contamination coming off the filter and going into the sample. We don't see major issues with column plugging, but if there are particulates in the sample, they can build up at the top of the guard column or the analytical column. They can also cause havoc with the injection valve. So, we recommend filtering.

LCGC: Why is automated eluent generation so important, and what kinds of eluent generation are there?

Fitchett: We call this "reagent-free technology" using eluent generation, and it's revolutionized the field of ion chromatography. The only thing you need to bring to the instrument is a source of clean, deionized water — that's it. The instrument prepares the mobile phases and

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regenerants that will be used in the suppressor. If I want to produce a concentration of 10-mmol potassium hydroxide, I simply type in "10-mmolar potassium hydroxide," and it's made automatically by the system.

The isocratic pump is fed with only DI water, which prolongs the lifetime of the pump and reduces maintenance. The eluent goes into an electrolytic cartridge, in this case, a potassium electrolyte, and by applying a potential across two electrodes, any concentration of potassium hydroxide can be generated.

A device called an Eluent Generator Cartridge (EGC) cleans up that solution so that we generate high-purity potassium hydroxide. Being able to produce a pure mobile phase consistently from day to day and from operator to operator is a big advantage in ion chromatography. The system also eliminates exposure of the operators to corrosive acids or bases.

We also have a device that will generate a carbonate mobile phase as well as one that will generate bicarbonate. For cations, we use methanesulfonic acid.

For gradient separations, when we use an eluent generator, we don't need a gradient pump. We can use an isocratic pump and allow the software to generate the gradient profile simply by typing in the desired gradient parameters. We do the same thing using an electrolytic process to generate the acids and bases that are necessary for chemical suppression technology.

LCGC: How long do eluent generator cartridges last?

Fitchett: It depends on the concentration that you're running. If you're running a very high concentration of, say, potassium hydroxide for anions or methanesulfonic acid, you will deplete the source of ions. Cartridges run under normal conditions and concentrations last between 9 and 12 months.

LCGC: Tell us about IC PEEK Viper fittings. What do they add to the system?

Fitchett: For ion chromatography, we created a new PEEK (polyether ether ketone) high-pressure, fingertight fitting. PEEK is an inert material, and the Dionex Integrion HPIC system is completely configured using the PEEK Viper fittings. You simply screw in these fittings fingertight, and you have a perfect, zero-dead-volume seal that won't leak.

LCGC: What is consumables device monitoring, and how does it help with analysis?

Fitchett: Consumables device monitoring is a new feature recently introduced on the Thermo Scientific Dionex Integrion HPIC system. Each consumable component — whether it's an eluent generation cartridge, a separator, a suppressor, or a guard column — has an RFID tag, which allows us to monitor the components in the system. When we install a consumable, the system automatically registers the device, its serial number, and installation date. It monitors the number of injections and any other parameters we want to monitor. This tracking allows the analyst to know when a consumable is reaching the end of its useful life.

LCGC: Thermo Fisher Scientific recently launched a new high-pressure IC system. What makes it stand out from previous systems?

Fitchett: We just introduced the Dionex Integrion HPIC system, a high-pressure system this February. This system comes in a variety of configurations for various applications and levels of investment.

The Dionex Integrion HPIC system has the flexibility to work with both microbore and standard bore columns. The system has a thermostatted column compartment, a dual-piston pump, and can be used with conductivity and electrochemical detectors.

We have two other HPIC systems. One is the Thermo Scientific Dionex ICS-5000+, which is our high-end ion chromatograph. It's a modular system that allows you to work in standard (4-mm column), microbore (2-mm column), or capillary (0.4-mm column) mode. We also have the Thermo Scientific Dionex ICS-4000, a dedicated capillary system that operates only in the capillary 0.4-mm mode.

LCGC: What kind of detection does the Dionex Integrion HPIC system use?

Fitchett: The standard configuration uses a conductivity detector contained in a separate compartment. We can replace that detector with an amperometric detector if required.

LCGC: Does the Dionex Integrion HPIC replace the Dionex ICS-4000 and Dionex ICS-5000+ systems?

Fitchett: No. The Dionex Integrion HPIC system essentially replaces the Thermo Scientific Dionex ICS-1600 and the Thermo Scientific Dionex ICS-2100 systems.

Use of 1.9 µm YMC-Triart C18 and 2.7 µm YMC Meteoric Core C18 BIO Stationary Phases for Fast Peptide Mapping of Monoclonal Antibodies

Jeffrey A. Kakaley and Ernest J. Sobkow, YMC America Inc.

In the quest to increase the speed and efficiency of analytical methods, today's scientists are increasingly turning to innovative products such as UHPLC totally porous as well as superficially porous core-shell materials. Both sub-2-µm totally porous and superficially porous core-shell stationary phases allow for faster analyses and increased throughput while simultaneously providing increased resolution. The advantages are thoroughly evident when applied to peptide mapping runs which are often more than an hour per injection on standard 5 µm and 3 µm size columns. This work highlights improvements in speed, resolution, and solvent consumption offered by YMC-Triart C18 1.9 µm and YMC Meteoric Core C18 BIO 2.7µm stationary phases when used for peptide mapping of monoclonal antibodies.

Operating Parameters

Mobile phase A:	0.1% TFA in HPLC water
Mobile phase B:	0.1% TFA in acetonitrile
Column temp.:	40 °C
Flow rate:	0.2 mL/min (60 min runtime)
	0.6 mL/min (20 min runtime)
Inj. volume:	10 µL
Detection λ:	215 nm
HPLC system:	Waters Acquity

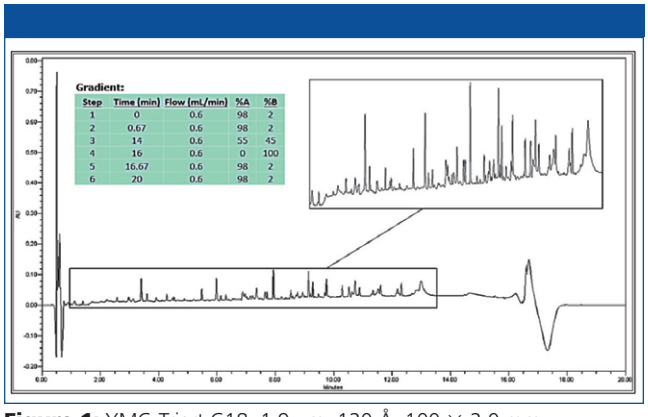

Figure 1: YMC-Triart C18, 1.9 µm, 120 Å, 100 × 2.0 mm.

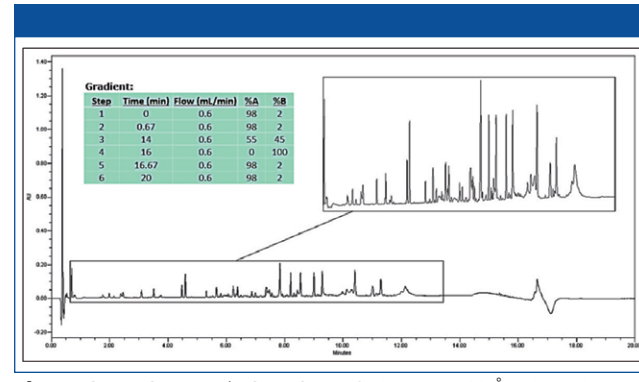

Figure 2: YMC Meteoric Core C18 BIO, 2.7 µm, 160 Å, 100 × 2.1 mm.

The original application began as a typical peptide mapping analysis run on a 5 µm 250 × 2.0 mm C18 column, using a linear gradient spanning 150 min (data not shown). The method was then transferred to a 1.9 µm 100 × 2.0 mm YMC-Triart C18 column with the gradient scaled accordingly, reducing runtime to 60 min. Linear velocity was then increased 3-fold in order to take advantage of the resolving power of the 1.9 µm particle, resulting in a runtime of 20 min, saving 130 min per injection and decreasing solvent usage from 30 mL down to 12 mL per injection. This procedure was then repeated using the core-shell column, exhibiting similar results.

In summary, the results indicate that YMC-Triart C18 1.9 µm and YMC-Meteoric Core C18 BIO 2.7 µm stationary phases are excellent choices for scaling down lengthy peptide mapping runs. These stationary phases allow for faster analyses and increased throughput while simultaneously providing superior resolution.

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Separation of Methyclothiazide Using New Immobilized Column – CHIRALPAK IG

Elena Eksteen, PhD, Chiral Technologies, Inc.

Daicel Corporation has introduced a new and unique chiral selector, CHIRALPAK® IG, to its line of Daicel immobilized chiral stationary phases (CSPs). The IG phase is an amylose polymer derivatized with phenyl moieties (chloro- and methyl-groups) in meta positions. This distinctive phase was developed from our extensive library of > 150 CSPs. Moreover, our studies have shown that the IG chiral selector has the highest selectivity of any of our immobilized CSPs.

Meta-substituted immobilized chiral selectors such as CHIRALPAK IA, IB, and IC have been shown to have remarkable affinity for resolution of chiral compounds from different types of molecules. Based on laboratory results, we have found that the addition of CHIRALPAK IG forms the most effective set of four chiral columns, CHIRALPAK IA, IB, IC, and IG, to be utilized for primary screening. It is noteworthy that the four best columns are all meta-substituted.

Primary Screening Columns

- CHIRALPAK IA: Amylose *tris* (3,5-dimethylphenylcarbamate)
- CHIRALPAK IB: Cellulose *tris* (3,5-dimethylphenylcarbamate)
- CHIRALPAK IC: Cellulose *tris* (3,5-dichlorophenylcarbamate)
- CHIRALPAK IG: Amylose *tris* (3-chloro-5- methylphenylcarbamate)

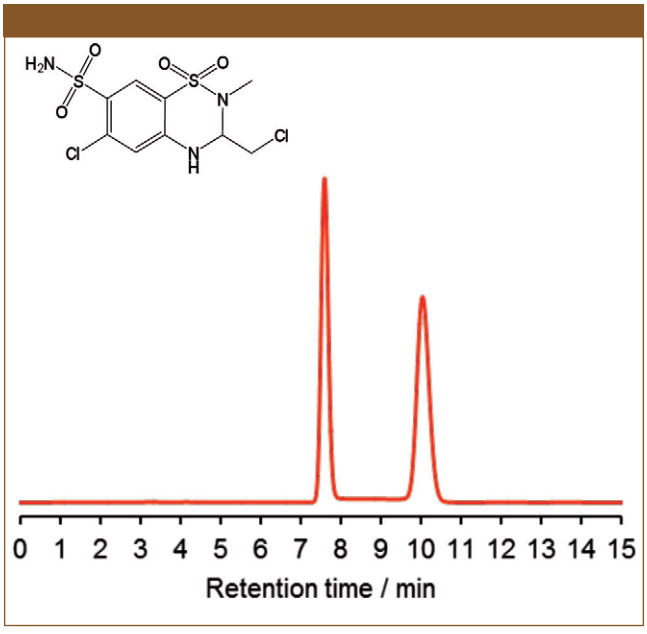

Figure 1: Separation of Methyclothiazide.

Experimental and Discussion

A CHIRALPAK IG column, packed with 5-µm particles, was used to develop the enantioselective separation of methyclothiazide. The mobile phase was a mixture of ammonium bicarbonate and acetonitrile. All chromatographic conditions are given in Figure 1.

Chromatographic Conditions

Column:	Daicel CHIRALPAK IG 4.6 mm i.d. × 250 mm long
Mobile phase:	20 mM NH_3HCO_3 aq/MeCN (pH 9 with DEA, 60/40)
Flowrate:	1 mL/min
UV detection:	254 nm
Column temperature:	25 ºC

Note: Methyclothiazide is a thiazide diuretic that is used to treat high blood pressure (hypertension). Using CHIRALPAK IG provides fast and effective separation of methyclothiazide enantiomers.

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DAICEL GROUP

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Investigation of Chiral Separation Conditions of Omeprazole by Supercritical Fluid Chromatography Method Scouting System

JASCO

In the synthesis of medicinal drugs and agrichemical fields, synthetic compounds with optical activity are continuing to gain more attention. These compounds can show the same physical chemical properties, but show different biological activity.

It has been reported that using only one enantiomer of the optical isomer can enhance the medicinal effects and reduce the side effects when the biological activity varies between the enantiomers.

Our Methods Scouting System makes it simple for users to search and select the appropriate measurement conditions using various solvents and columns for both chiral and achiral separations.

Supercritical fluid chromatography (SFC) is well known for quick separations, easy solvent replacement, easy sample treatment after preparation, decreasing solvent cost over HPLC, and simple scale-up from analytical to preparative.

In this application, method scouting of omeprazole, used as acid suppressant, with three modifiers and six columns is carried out by using SFC and the Method Scouting Program, which is an optional program of ChromNAV Ver.2.

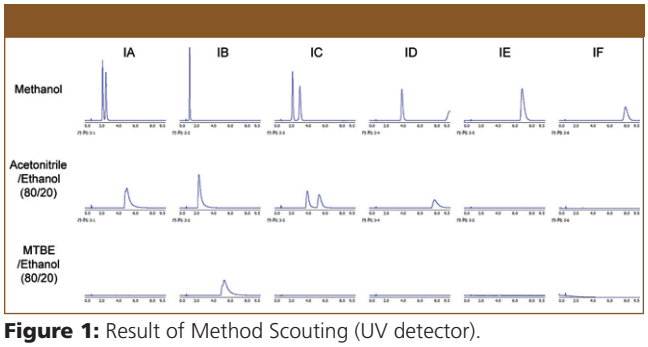

Figure 1: Result of Method Scouting (UV detector).

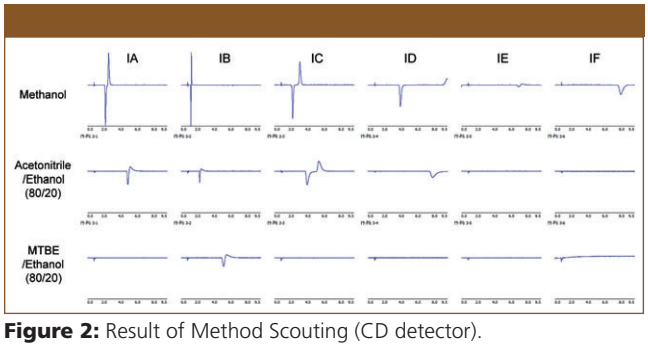

Figure 2: Result of Method Scouting (CD detector).

Experimental

Equipment

CO_2 pump:	PU-4380
Modifier pump:	PU-4180
Modifier pump option:	SV unit, MX unit, LV unit
Autosampler:	AS-4350
Column oven:	CO-4065
Oven option:	1 in 6 out valve unit x 2
Detector:	MD-4010 (H.P. Analytical Cell) CD-4095 (H.P. Analytical Cell)
Back pressure regulator:	BP-4340

Conditions

Column:	CHIRALPAK IA, IB, IC, ID, IE and IF/SFC (i-CHIRAL 6), (4.6 mm I.D. × 150 mmL, 5 µm)
Eluent:	CO_2/modifier (60/40)
Modifier:	1) methanol 2) acetonitrile/ethanol (80/20) 3) methyl tert-butyl ether (MTBE)/ethanol (80/20)
Flow rate:	3.0 mL/min
Column temp.:	40 °C
Wavelength:	UV: 300 nm, CD: 275 nm
Wavelength:	UV: 300 nm, CD: 275 nm
Back pressure.:	15 MPa
Injection volume:	5 µL
Standard:	1.0 mg/mL omeprazole in methanol

Table I: Degree in separation

Column Modifier	IA	IB	IC	ID	IE	IF
Methanol	2.02	N.S.	3.38	N.E.	N.S.	N.E.
Acetonitrile/Ethanol (80/20)	N.S.	N.S.	2.26	N.E.	N.E.	N.E.
MTBE/Ethanol (80/20)	N.E.	N.S.	N.E.	N.E.	N.E.	N.E.

Table I shows the degree in separation of omeprazole. As shown in the table, methanol and CHIRALPAK IC is suited to this measurement.

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Improved Chiral Separations for Enantiopure D- and L-Amino Acids

Claude Lerner and Kristine Biederer, Regis Technologies

Several D-amino acids have been found to be important in brain neurochemistry and changes in the levels of these amino acids coincide with development of different diseases. For example, the D-form of serine is involved in modulation of the NMDA receptor and has been implicated in a broad spectrum of disorders including schizophrenia, ischemia, and epilepsy.

D-amino acids have historically been considered unnatural since naturally produced proteins are composed exclusively of L-amino acids. While bacterial cell walls were known to contain D-amino acids, their presence in humans and higher organisms has been reported more recently.

Crown-ether chiral stationary phases (CSPs) have been found to be especially well suited for separation of D- and L-amino acid enantiomers. Regis Technologies' crown-ether CSP ChiroSil® is a trifunctionally bonded (18-crown-6)-2,3,11,12-tetracarboxylic CSP available in both (+) and (-) conformations which thereby enables inversion of the elution order of the D- and L- isomers.

In this application note, we present separation of the D- and L-isomers of two neurophysiologically relevant amino acids, serine and glutamic acid, by high pressure liquid chromatography (HPLC) using the CSP.

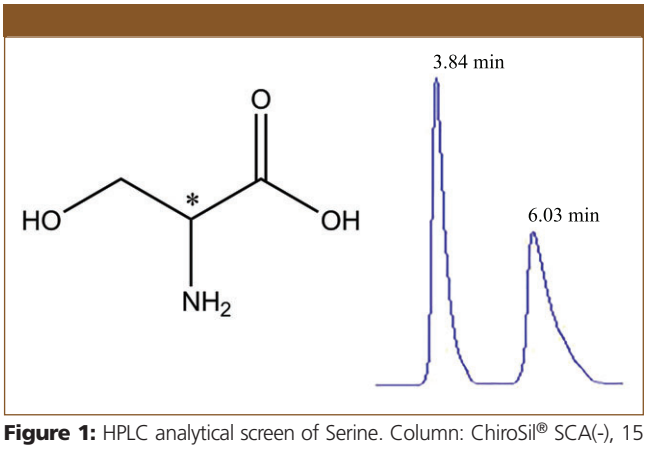

Figure 1: HPLC analytical screen of Serine. Column: ChiroSil® SCA(-), 15 cm × 4.6 mm, 5 µm. Mobile Phase: 84% MeOH/16%H_2O, 5 mM $HClO_4$. k'_1: 1.37 α: 1.99

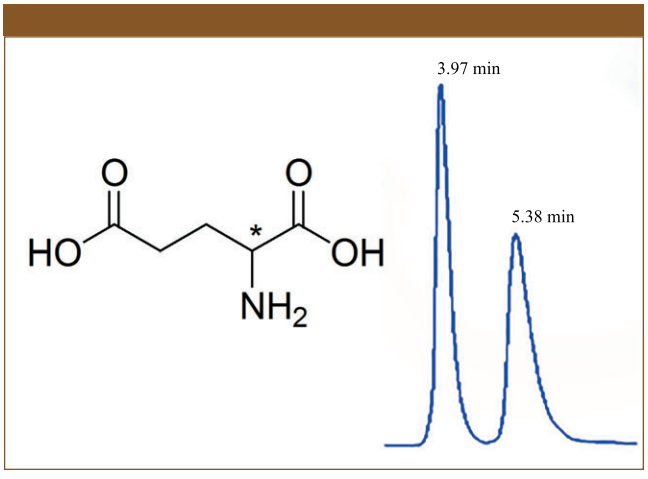

Figure 2: HPLC analytical screen of glutamic acid. Column: ChiroSil® SCA(-), 15 cm × 4.6 mm, 5 µm. Mobile phase: 84% MeOH/16%H_2O, 5 mM $HClO_4$. k'_1: 1.45 α: 1.60.

Experimental Conditions

Compounds were separated on ChiroSil® SCA(-) chiral columns using standard dimensions, 15 cm × 4.6 mm i.d. packed with 5 micron particles. ChiroSil® is a registered trademark of RStech Corporation of Daejeon, South Korea.

Conclusions

Baseline enantiomeric resolution of serine and glutamic acid amino acids was achieved in less than 10 min using the crown-ether CSP method described here. The ChiroSil® CSP is a beneficial column in analyzing amino acids in the neurochemistry field.

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Website: www.registech.com

Automated Low Background Solid-Phase Extraction System for Perfluorinated Compounds in Water

FMS, Inc.

Perfluoralkylated substances is a general term used to describe substances which are largely comprised of or contain a perfluorinated or polyfluorinated carbon chain moiety such as $F(CF_2)_n$- or $F(CF_2)n$-$(C_2H_4)_n$. PFOS and other perfluorinated compounds are widely used in industrial and consumer applications including stain-resistant coatings for textiles, leather, and carpets, grease-proof coatings for paper products approved for food contact, firefighting foams, mining and oil well surfactants, floor polishes, and insecticide formulations. In recent years, there has been increasing concern over the levels of perfluorinated and polyfluorinated chemicals, such as PFOS (perfluorosulfonate) and PFOA (perfluoro-octanoicacid), in the global environment and their fate and possible adverse effects in the environment.

In animal studies, some PFCs disrupt normal endocrine activity; reduce immune function; cause adverse effects on multiple organs, including the liver and pancreas; and cause developmental problems in rodent offspring exposed in the womb. Data from some human studies suggests that PFCs may also have effects on human health, while other studies have failed to find conclusive links. Additional research in animals and in humans is needed to better understand the potential adverse effects of PFCs for human health.

Two compounds in particular, perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA), represent the final environmental degradation products of (and contaminants in) a wide range of other perfluorinated products and have been most extensively studied. PFOS is now subject to varying but increasing levels of control in a number of countries. PFOA, also a widespread contaminant but with a far lower bioaccumulation potential is still under evaluation.

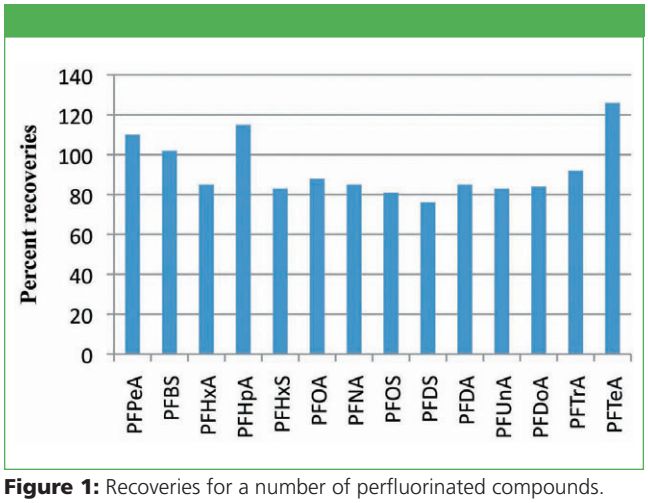

Figure 1: Recoveries for a number of perfluorinated compounds.

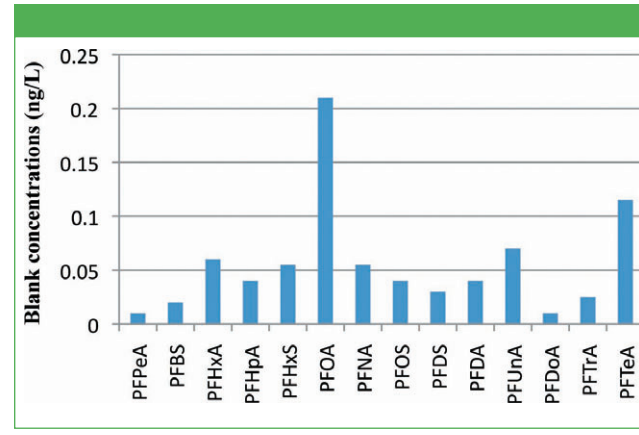

Figure 2: Background concentrations for a number of perfluorinated compounds.

Procedure

Five hundred mL water samples were spiked with 25 µL of 1 µg/mL PFC standard solution. Samples were then loaded onto the FMS TurboTrace® PFC SPE system and passed across a FMS, Inc. PFC cartridge under -12 psi vacuum. After loading, the bottle was rinsed with 25 mL of water and loaded onto the cartridge under negative pressure. The cartridges were dried using nitrogen until no residual water was present, and the cartridges were subsequently eluted with methanol. The extracts were concentrated to 500 µL, after which internal standard was added.The samples were diluted to a final volume of 1 mL of water for LC–MS analysis.

Conclusion

The FMS TurboTrace PFC SPE system and the FMS, Inc. PFC cartridge both specifically designed for the analysis of perfluorinated componds produces reliable, reproducible results for perfluorinated compounds in water. The system, by design, has very low background PFC allowing for analysis of samples without any significant interference.

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THE APPLICATION NOTEBOOK

Call for Application Notes

LCGC is planning to publish the next issue of *The Application Notebook* special supplement in September. The publication will include vendor application notes that describe techniques and applications of all forms of chromatography and capillary electrophoresis that are of immediate interest to users in industry, academia, and government. If your company is interested in participating in these special supplements, contact:

Michael J. Tessalone, Vice President/ Group Publisher, (732) 346-3016
Edward Fantuzzi, Associate Publisher, (732) 346-3015
Stephanie Shaffer, East Coast Sales Manager, (774) 249-1890
Lizzy Thomas, Account Executive, (574) 276-2941

Application Note Preparation

It is important that each company's material fit within the allotted space. The editors cannot be responsible for substantial editing or handling of application notes that deviate from the following guidelines:

Each application note page should be no more than 500 words in length and should follow the following format.

Format

- **Title:** short, specific, and clear
- **Abstract:** brief, one- or two-sentence abstract
- **Introduction**
- **Experimental Conditions**
- **Results**
- **Conclusions**
- **References**
- **Two graphic elements:** one is the company logo; the other may be a sample chromatogram, figure, or table
- **The company's full mailing address, telephone number, fax number, and Internet address**

All text will be published in accordance with *LCGC*'s style to maintain uniformity throughout the issue. It also will be checked for grammatical accuracy, although the content will not be edited. Text should be sent in electronic format, preferably using Microsoft Word.

Figures

Refer to photographs, line drawings, and graphs in the text using arabic numerals in consecutive order (Figure 1, etc.). Company logos, line drawings, graphs, and charts must be professionally rendered and submitted as .TIF or .EPS files with a minimum resolution of 300 dpi. Lines of chromatograms must be heavy enough to remain legible after reduction. Provide peak labels and identification. Provide figure captions as part of the text, each identified by its proper number and title. If you wish to submit a figure or chromatogram, please follow the format of the sample provided below.

Tables

Each table should be typed as part of the main text document. Refer to tables in the text by Roman numerals in consecutive order (Table I, etc.). Every table and each column within the table must have an appropriate heading. Table number and title must be placed in a continuous heading above the data presented. If you wish to submit a table, please follow the format of the sample provided below.

References

Literature citations must be indicated by arabic numerals in parentheses. List cited references at the end in the order of their appearance. Use the following format for references:

(1) T.L. Einmann and C. Champaign, *Science* **387**, 922–930 (1981).

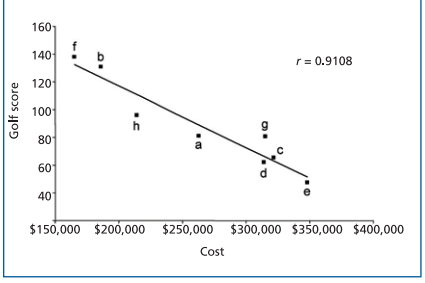


Figure 1: Chromatograms obtained using the conditions under which the ion suppression problem was originally discovered. The ion suppression trace is shown on the bottom. Column: 75 mm × 4.6 mm ODS-3; mobile-phase A: 0.05% heptafluorobutyric acid in water; mobile-phase B: 0.05% heptafluorobutyric acid 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

The deadline for submitting application notes for the September issue of *The Application Notebook* is:

July 29, 2016

This opportunity is limited to advertisers in *LCGC North America*.
For more information, contact:
Mike Tessalone at (732) 346-3016, Ed Fantuzzi at (732) 346-3015, Stephanie Shaffer at (774) 249-1890, or Lizzy Thomas at (574) 276-2941.

Analysis of Drinking Waters for a Suite of Pesticides Using GC with Dual ECD (US EPA Method 508.1) after SPE Concentration

Eric Scott and Keith Ewing, Department of Environmental Protection, Frankfort, KY

Pesticides, including insecticides, herbicides, and fungicides are used extensively to increase agricultural yields. US EPA Method 508.1 is a sensitive method to detect chlorinated pesticides in drinking water using solid phase extraction (SPE) and gas chromatography (GC) coupled with ECD detection. Method 508 includes 29 chlorinated pesticides, three herbicides, and four organohalides that can be determined in drinking water in any stage of treatment, and groundwater. EPA Method 508 is a good method for measurement of regulated levels and for screening of other pesticides that may be present. This work will demonstrate the performance of method 508 using solid phase extraction with an automated system.

Drinking water samples from the county are obtained using usual tap collection procedures. Table I lists a selection of analytes and their recoveries from lab-fortified blank samples and duplicates, fortified at 100 µg/L. The recoveries are excellent and the precision between runs is very good. Table II shows the lab fortified blank and lab-fortified blank duplicate for selected analytes. The agreement is well within the 20% relative percent difference limit specified in the method.

Table I: Lab fortified blank, four runs, selected analytes

	Run 1	Run 2	Run 3	Run 4	%RSD
2,4'-DDD	102	96	118	99.5	9.4
Aldrin	84	82.5	102	77	12.6
cis-Chlordane	106	86	120	95.5	14.3
Dieldrin	113	104	122	109	6.8
Endrin	110	108	126	108	7.7
gamma-BHC (Lindane)	95.5	98.5	93.5	104	4.7
Hexachlorobenzene	95.5	87.5	84	77.5	8.7
Methoxychlor	99.5	99	113	98.5	6.8

Table II: Relative percent difference (RPD) between duplicates for selected analytes

	LFB	LFB Dup	RPD
2,4'-DDD	102	102	0
Aldrin	84	85	1.2
cis-Chlordane	106	105	0.9
Dieldrin	113	112	0.9
Endrin	110	108	1.8
gamma-BHC (Lindane)	95.5	93.5	2.1
Hexachlorobenzene	95.5	93.5	2.1
Methoxychlor	99.5	97.5	2.0

Conclusion

Solid phase extraction has become well established and most of the US EPA drinking water methods include SPE as the primary extraction technique or as an alternative. This work demonstrates the good performance of automated SPE for a wide suite of chlorinated pesticides, herbicides, and organic compounds. Recoveries are good and agreement between duplicates is well within the method requirements of 20%. In addition to good reproducibility, automation of the technique requires less attention than a manual extraction technique

Reference

(1) Analysis of Drinking Waters for a Suite of Pesticides using GC with dual ECD (US EPA Method 508.1) after SPE Concentration, AN1031602_01, available from Horizon technology at www.horizontechinc.com (2016).

Improving the Analysis of Carbohydrates Using Temperature and Sample Preheating

Bart Poulsen and Daniel Etcheto, Benson Polymeric

The use of elevated temperatures is a common tool for the analysis of carbohydrates using ligand-exchange chromatography. Sample preheating can further enhance peak efficiency and improve the analysis of carbohydrates.

Ligand-exchange chromatography is a common technique for the analysis of carbohydrates using polymeric based columns. Since these types of HPLC columns require only water as the eluent, laboratories that prefer to not introduce solvents into their environment will use this type of "green technology" as a viable alternative for the analysis of many common samples containing carbohydrates. Columns packed with gels (low cross-linked, microporous, polystyrene-divinylbenzene polymer) typically require elevated temperatures for optimal peak efficiency. Peak efficiency can be further enhanced with the use of a column oven equipped with a sample preheat device or a plug-in preheat coil.

Table I: Temperature effect on glucose efficiency

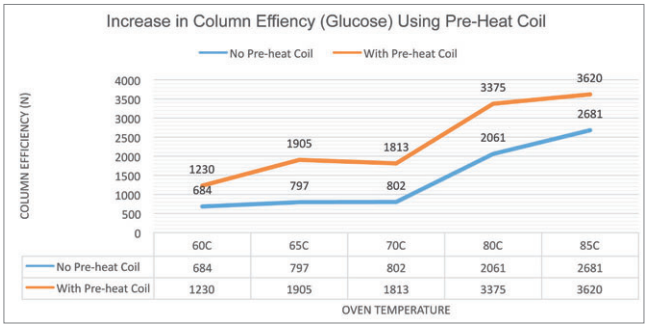
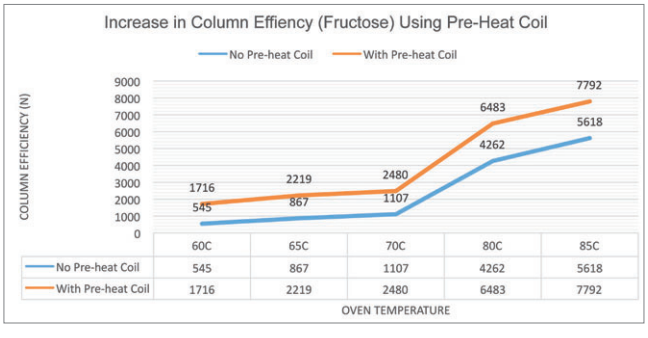


Table II: Temperature effect on fructose efficiency



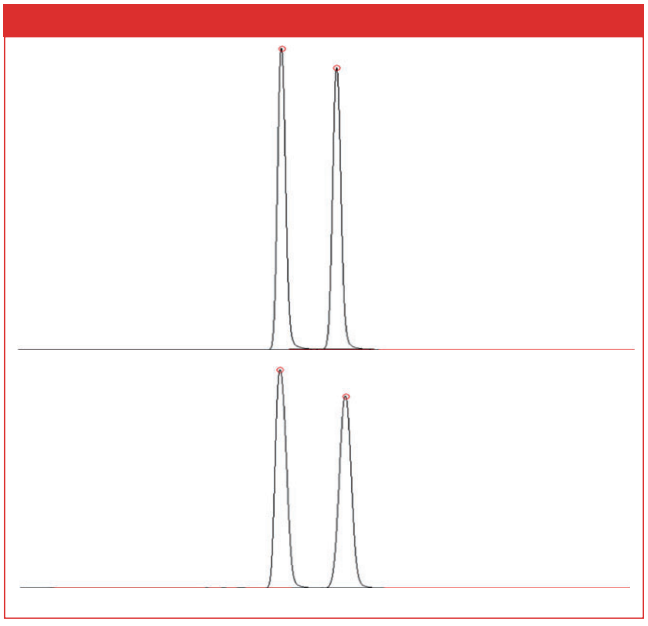


Figure 1: Glucose and fructose peaks with pre-heat (BP-800 Ca, (300 × 7.8 mm), 85 °C, 0.5 mL/min, water eluent).
Figure 2: Glucose and fructose peaks without pre-heat coil (BP-800 Ca, (300 × 7.8 mm), 85 °C, 0.5 mL/min, water eluent).

Experimental Conditions

Column:	Benson Polymeric BP-800 Ca (PN 8000-0)
Mobile phase:	DDI water
Oven temperature:	60 °C, 65 °C, 70 °C, 80 °C, 85 °C
Flow rate:	0.5mL/min
Detector:	Refractive Index
Column oven:	Benson Polymeric column oven
Sample preheat:	Benson BP-PH Preheater (PN 5050-0)

Results

Tables I and II show the increase in peak efficiency for both glucose and fructose as temperature is increased when using a calcium form ligand-exchange HPLC column (Benson BP-800 Ca). Peak efficiency is further enhanced with the use of a preheat device for sample heating prior to entering the column. Figures 1 and 2 are a visual presentation of the increase in efficiency for glucose and fructose.

Conclusion

Elevated temperatures are required for optimal peak efficiency when using polymeric ligand-exchange HPLC columns. By supplementing elevated temperature with a sample pre-heat device, peak efficiency is further enhanced, sometimes dramatically.

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Comprehensive Analysis of Raw Foodstuffs Using Dynamic Headspace Sampling with Thermal Desorption–GC–MS Analysis

Caroline Widdowson, Hannah Calder, and David Barden, Markes International

Herbs are widely used in many food products, but substantial variations in aroma can result from differences in growing conditions or preparation of the plant material, which can affect product quality.

In this application note we show the wide range of aroma chemicals that can be detected in the headspace of basil leaves using a micro-chamber sampling device with analysis by thermal desorption (TD) and gas chromatography–mass spectrometry (GC–MS).

Micro-Chamber/Thermal Extractor

Of the numerous TD-compatible sampling instruments, the Micro-Chamber/Thermal Extractor™ (μ-CTE™) from Markes International is one of the most versatile. It is a compact, stand-alone unit comprising cylindrical chambers suitable for sampling chemical emissions from larger samples, or from materials that are not entirely homogeneous.

Operation is simple—materials are placed in one of the chambers, and the headspace vapors are dynamically extracted onto a 3½-inch × ¼-inch sorbent-packed TD tube by a flow of heated air or gas. This tube is then placed into the thermal desorber and analyzed as described below. Sampling times are short (typically < 60 min), and the instrument can analyze up to four or six samples at once, depending on the model chosen.

Thermal Desorption

Thermal desorption (TD) uses heat and a flow of inert gas to desorb volatile and semi-volatile organic compounds (VOCs and SVOCs) from sorbents or sample materials. Extracted vapors are swept onto an electrically-cooled focusing trap, which is then rapidly heated to inject them into a gas chromatograph (GC).

TD offers many advantages over conventional solvent-based sample preparation methods such as liquid extraction. These include wider analyte range (from acetylene to n-C_{44} and reactive species on one platform), quantitative re-collection of split flows for repeat analysis and simple method validation, and enhanced sensitivity.

In this study, the TD-100™ automated cryogen-free thermal desorber from Markes International was employed, which has capacity for 100 industry-standard tubes.

Analysis of Fresh Basil Leaves

Figure 1 shows the results obtained by dynamic headspace sampling of fresh basil leaves with analysis by TD–GC–MS. As well as the rapidity with which the entire vapor profile can be collected using the Micro-Chamber/Thermal Extractor, the inertness and adjustable flow-path temperature of Markes' TD systems ensure reliable analysis of a wide range of analytes, including reactive or difficult-to-analyze species such as sulphur species and certain monoterpenoids.

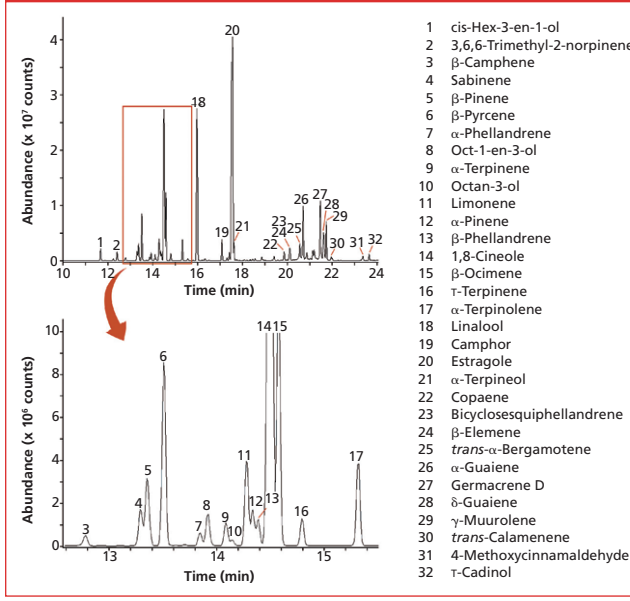

Figure 1: Dynamic headspace sampling of fresh basil leaves, with analysis by TD–GC–MS. The inset highlights some of the lower-level compounds identified.

The information obtained in this case illustrates the power of TD and associated sampling techniques to provide quick yet comprehensive analyses of foodstuffs, for improved understanding of aroma profiles and product quality.

Typical Analytical Conditions

Sample: 5 g pre-packaged fresh basil leaves.

Dynamic headspace (Micro-Chamber/Thermal Extractor): Flow rate: 50 mL/min for 20 min. Chamber temperature: 40 °C.

TD (TD-100): Tube (Tenax TA): Desorbed at 280 °C (10 min). Trap (Tenax TA): Analytes trapped at 20 °C, desorbed at 290 °C (3 min). Split ratio: Inlet 2:1, Outlet: 16:1.

Analysis: Single-quadrupole GC–MS operated in full-scan mode (*m/z* 45–600).

MARKES international

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Quick and Convenient Comparison of Curry Powders Using Direct Thermal Desorption with GC–MS Analysis

Caroline Widdowson, Gareth Roberts, and David Barden, Markes International

Herbs and spices are used in many food preparations, and identifying the differences between samples is of particular interest to manufacturers, both for ongoing quality control and to compare their products against competitors. However, the volatile organic compound (VOC) profiles of such samples often differ in the relative abundance of key components, and these differences can be difficult to assess by traditional methods such as solvent extraction, equilibrium headspace, or solid-phase microextraction (SPME).

In this application note we show the value of direct thermal desorption (TD) with analysis by gas chromatography–mass spectrometry (GC–MS) for assessing aroma profiles from small samples of curry powder.

Thermal Desorption

TD uses heat and a flow of inert gas to desorb VOCs and semi-volatile organic compounds (SVOCs) from sorbents or sample materials. Extracted vapors are swept onto an electrically-cooled focusing trap, which is then rapidly heated to inject them into a gas chromatograph (GC).

TD offers many advantages over conventional solvent-based sample preparation methods such as liquid extraction. These include wider analyte range (from acetylene to n-C_{44} and reactive species on one platform), quantitative re-collection of split flows for repeat analysis and simple method validation, and enhanced sensitivity.

In this study, the TD-100™ automated cryogen-free thermal desorber from Markes International was employed, which has capacity for 100 industry-standard tubes.

Direct Desorption

Of the numerous TD-compatible sampling procedures, direct thermal desorption is the most straightforward and cost-effective for small quantities of relatively homogeneous, finely-divided materials—for example, therapeutic drugs, packaging materials, resins, spices, ointments/creams, polymers, water-based paints, and edible fats.

The material is simply weighed into an empty 3½-inch × ¼-inch TD tube, and heated directly within a thermal desorption instrument, followed by direct injection into the GC system. In this way, sample preparation is essentially reduced to zero, and the associated risk of introducing errors is eliminated.

Analysis of Curry powder

To illustrate the usefulness of direct desorption, Figure 1 shows the results obtained by direct desorption and TD–GC–MS analysis of two brands of curry powder. The range of analytes is very similar, but there are substantial differences in relative abundance. In particular, the cheaper brand (top) shows much higher quantities of linalool (#11), camphor (#12), and estragole (#13) compared to a mid-range brand (bottom), but lower concentrations of cuminaldehyde (#14) and caryophyllene (#19).

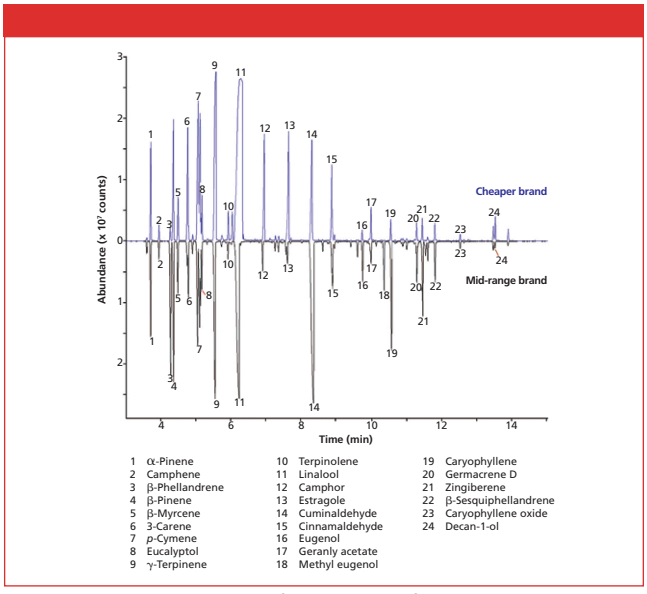

Figure 1: Direct desorption of two brands of curry powder, with analysis by TD–GC–MS.

This analysis exemplifies how direct desorption can enable quick, robust analysis of multiple samples, which make it of considerable value to food analysts for quality control and product comparisons.

Typical Analytical Conditions

Sample: Curry powder (~50 mg), placed in an empty TD tube.

TD (TD-100): Tube: Desorbed at 50 °C (3 min). Trap (Material emissions): Analytes trapped at 10 °C, desorbed at 280 °C (5 min). Split ratio: Outlet 25:1.

Analysis: Single-quadrupole GC–MS operated in full-scan mode (*m/z* 45–600).

MARKES international

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Glyphosate Analysis in Soy Beans, Corn, and Sunflower Seeds by HPLC with Post-Column Derivatization and Fluorescence Detection

Maria Ofitserova, Rebecca Smith, and Michael Pickering, Pickering Laboratories, Inc.

Glyphosate is a broad-spectrum herbicide widely used around the world. Monitoring of glyphosate in crops and water is mandated in many countries. We describe a sensitive and robust HPLC method for analysis of glyphosate in soy beans, corn, and sunflower seeds. This method utilizes a simplified sample preparation procedure that has proven to be effective even for challenging matrices.

Method

Analytical Conditions

Column:	Cation-exchange column, K+ form, P/N 1954150
Guard column:	Cation-exchange GARD™ Column Protection System or Cation-exchange guard column P/N 1953020
Column temperature:	55 °C
Flow rate:	0.4 mL/min
Mobile phase:	K200, RG019
Injection volume:	100 µL

Post-Column Conditions

Post-column system: Pinnacle PCX or Vector PCX

Heated reactor volume:	0.5 mL
Temperature:	36 °C
Ambient reactor:	0.1 mL
Reagent 1:	100 µL of 5% NaOCl (Bleach) in 950 mL of GA116 Diluent
Reagent 2:	100 mg of OPA and 2 g of Thiofluor in 950 mL of GA104 Diluent
Reagent flow rate:	0.3 mL/min each reagent
Detection:	λ_{EX} 330 nm, λ_{EM} 465 nm

Supplies for Sample Preparation

Methylene chloride, HPLC grade

Acidic modifier solution (16 g KH_2PO_4, 160 mL of water, 40 mL of methanol, 13.4 mL of conc. HCl)

Elution solution (160 mL of water, 40 mL of methanol, 2.7 mL of HCl)

RESTORE™

SPE sample clean-up cartridges P/N 1705-0001

Sample Preparation

Extraction

To 25 g of homogenized sample, add enough water (after estimating moisture content) such that the total volume of water is 125 mL. Blend at high speed for 3–5 min and centrifuge for 10 min. Transfer 20 mL of the aqueous extract into a centrifuge tube and add 15 mL of methylene chloride. Shake for 2–3 min and centrifuge for 10 min. Transfer 4.5 mL of aqueous layer to another centrifuge tube and add 0.5 mL of acidic modifier solution. Shake and centrifuge for 10 min. Filter through a 0.45 µm filter.

Matrix-Specific Modifications

Matrix with high 1) Water; 2) Protein; 3) Fat Content:

1) For samples that absorb large amounts of water, reduce test portion to 12.5 g while keeping water volume the same.
2) For samples with high protein content, add 100 µL of concentrated HCl to 20 mL of crude extract. Shake and centrifuge for 10 min.
3) For samples with high fat content, do the methylene chloride partitioning twice.

SPE Cleanup

Remove the top cap first, then the bottom cap of the SPE columns and place them into the manifold. Drain the solution to the top of the resin bed. Transfer 1 mL of extract into the column and elute to the top of the resin bed. Add 0.7 mL of the elution solution and discard the effluent. Repeat with a second 0.7 mL portion of the elution solution and discard the effluent. Elute glyphosate with 12 mL of the elution solution and collect the effluent in a round bottom flask. Evaporate to dryness at 40 °C using a rotary evaporator. Dissolve the residue in 2.0 mL of a solution of 10% RESTORE™ in water (use 1.5 mL for dry samples), filter through a 0.45 µm syringe filter and inject onto the HPLC column. Extracts can be stored refrigerated for up to seven days before the evaporation step.

Table I: HPLC gradient		
Time (min)	K200 (%)	RG019 (%)
0	100	0
15	100	0
15.1	0	100
17	0	100
17.1	100	0
25	100	0

Table II: Recoveries for glyphosate			
Spike Level	Soy Beans	Corn	Sunflower Seeds
0.2 ng/g	109%	102 %	70%
0.1 ng/g	90%	93%	82%
0.05 ng/g	93%	93%	71%

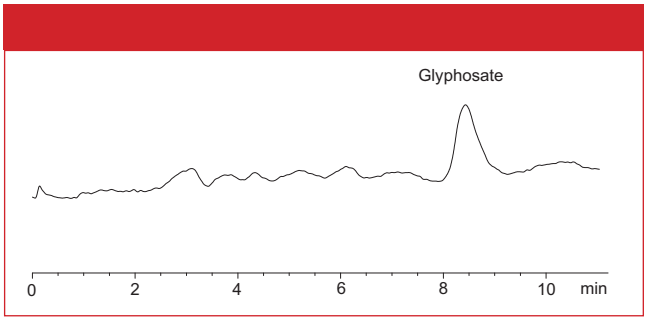

Figure 1: Chromatogram of soy beans sample spiked with glyphosate at 0.1 ppb level.

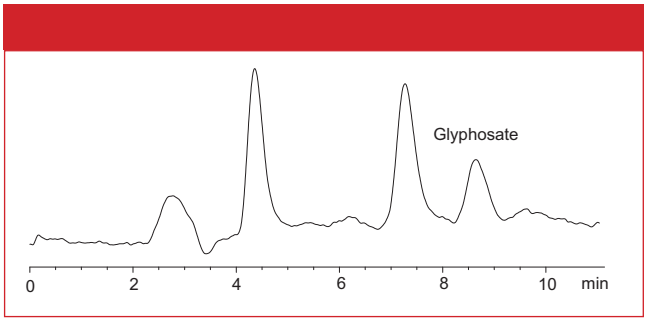

Figure 2: Chromatogram of corn sample spiked with glyphosate at 0.1 ppb level.

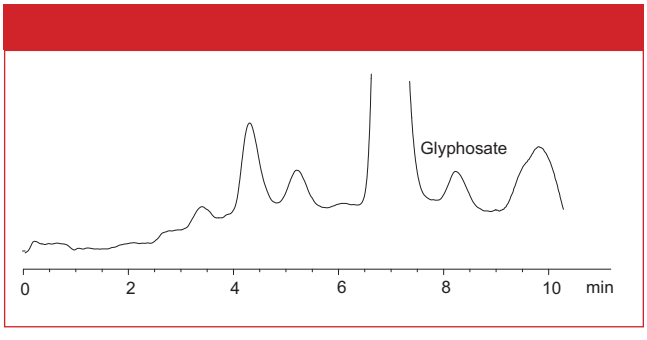

Figure 3: Chromatogram of sunflower seeds sample spiked with glyphosate at 0.1 ppb level.

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Determination of Pesticides Residues in Food Matrices—An Automated Versus Manual QuEChERS Extraction

Tyler Trent, Teledyne Tekmar

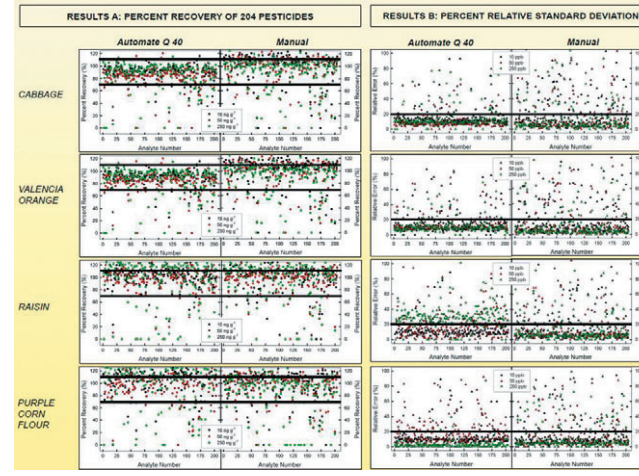
An ever growing area of interest for pesticide residue laboratories is the ability to automate manual steps, particularly in workplaces where they can see as many as 500 samples per day. These analyses typically involves the use of multi-residue methods (both GC–MS-MS and LC–MS-MS), to test for pesticide residues. QuEChERS has proven to be the extraction method of choice for numerous laboratories, due to its ease of use and robustness.

Teledyne Tekmar's AutoMate-Q40 is a robotic system designed to optimize and automate the QuEChERS sample preparation workflow. This revolutionary system automates the following sample extraction requirements: solvent, internal standard and matrix spike addition, vial shaking, vortex mixing, addition of solid reagents (salts, buffers), centrifugation, identifying liquid levels, pipetting, dSPE cleanup, and preserving the final extract.

The United States Food and Drug Agency (US FDA) evaluated the extraction performance of the AutoMate-Q40 against the manual process. They compared the automated and manual QuEChERS extraction, using the AOAC 2007.01 unbuffered method. All samples in this evaluation were analyzed using liquid chromatography-tandem mass spectrometry (LC–MS-MS).

AutoMate-Q40 Method Parameters	
AOAC 2007.01 Unbuffered	
Extraction Salts	
	Weight (g)
Unbuffered Salts	**5.0**
Extraction Solvents	
	Volume (mL)
Acetpmorile	**10.0**
Solvent 2	**0.0**
Water	**0.0**
Extraction Mix/Vortex	
	Duration (min)
50 mL Sample Mix	**1.0**
15 mL dSPE Mix	**1.0**
Sample Vortex Before Extraction Salts	**0.5**
Centrifuge	
	Duration (min)
50 mL Sample Centrifuge Time	**1.0**
15 mL dSPE Centrifuge Time	**1.0**
Extraction Volumes	
	Volume (mL)
dSPE Volume	**5.0**
Final Extract Volume	**2.0**

Figure 1: AutoMate-Q40 method parameters.



Experimental Instrument Conditions

Figure 1 shows the parameters for the QuEChERS extraction procedure for fruits and vegetables using the AutoMate-Q40.

Results

The scatter plot shows the precision and accuracy recoveries of the 204 pesticides spiked onto the samples of cabbage, valencia oranges, raisins, and purple corn flour. The samples were spiked at 10.0, 50.0, and 250.0 ng/mL. The AutoMate-Q40 system is capable of adding these standards directly to the sample, however in this study; the analyst added the spike directly to the sample manually and allowed 15 min of interaction, before placing inside the AutoMate-Q40.

Acknowledgements

Teledyne Tekmar would like to thank the United States Food and Drug Agency CFSAN Group, namely Kelli A. Simon, James B. Wittenberg, Jon W. Wong, and Alexander J. Krynitsky. Their exceptional work evaluating the system is greatly appreciated.

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High pH and Elevated Temperatures for Fast Separation of Strongly Basic Drug Compounds

Derek A. Jensen and Mark Carrier, Hamilton Company

Two important but frequently underutilized tools in methods development are mobile-phase pH and elevated temperatures. Hamilton's PRP-C18 column is well-suited for high pH and high temperature applications because the polymer-based stationary phase is chemically inert and has excellent thermal stability above 100 °C.

In modern drug discovery, where analytical HPLC can be a bottleneck, production is streamlined through the use of shorter columns with smaller particles, operated at higher flow rates. The flexibility to employ a high pH mobile phase and elevated temperatures represents further valuable tactics in methods development. These tools, often underutilized or not practical with silica-based columns, enable rapid separation of closely-related basic solutes in their charge-neutral forms that would otherwise be coeluted under non-alkaline, ambient temperature conditions.

In this study, nine structurally-diverse, strongly basic drug compounds are resolved in less than 2 min using a high pH mobile phase and a fast acetonitrile gradient at 80 °C.

Column:	Hamilton PRP-C18, 5 µm, 2.1 x 33 mm
Flow rate:	1.0 mL/min
Temperature:	80 °C
Injection volume:	5 µL
Flow rate:	1.0 mL/min
Mobile phase:	A) 30 mM diethylamine (pH 11.5) B) Acetonitrile + 30 mM diethylamine
Gradient:	2 to 99% B in 1 min
Detection:	UV at 254 nm

Analytes	
1.	Ephedrine
2.	Norephedrine
3.	Nicotine
4.	Metoprolol
5.	Quinine
6.	Doxylamine
7.	Diphenhydramine
8.	Nortriptyline
9.	Amitriptyline

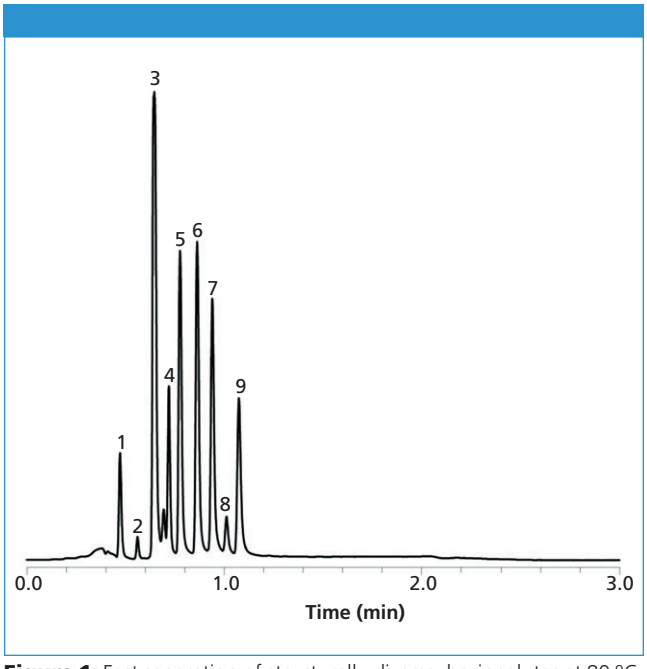

Figure 1: Fast separation of structurally-diverse, basic solutes at 80 °C.

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DAR Analysis of Antibody Drug Conjugates Using a TSKgel® HIC Column

Tosoh Bioscience LLC

The monoclonal antibody (mAb) drug market has continued to expand in recent years, and several of the top 10 blockbuster antibody drugs currently on the market include antibody drug conjugates (ADCs). Antibodies, when conjugated with cell-killing cytotoxic drugs, are called antibody drug conjugates. These drugs are designed to be used for chemotherapeutic cancer treatments. ADCs have a structure in which a low molecular weight cytotoxic drug is chemically bonded to an antibody. Because there are numerous binding sites for a low molecular weight drug on the antibody (Cys, Lys residues, etc.), heterogeneity arises with respect to the number of bonds and binding sites. It is necessary to study in detail the effect that this heterogeneity has on the medicinal effects and safety of drugs containing ADCs. Since low molecular weight drugs are strongly hydrophobic, differences in hydrophobicity of antibodies arise when the bonding number of these types of drugs differs. This property can be utilized to determine the drug-to-antibody ratio (DAR) by hydrophobic interaction chromatography (HIC). In HIC, a weakly non-polar stationary phase is used with an aqueous mobile phase containing a high concentration of a chaotropic salt. The technique is mainly applied to the separation of biomolecules, such as proteins, which are eluted by gradually reducing the salt concentration.

In this application note, an ADC was separated using a TSKgel Butyl-NPR column, the least hydrophobic of the TSKgel HIC columns. TSKgel HIC columns consist of polymethacrylate based material and a choice of three ligands (butyl, ether, and phenyl) with varied hydrophobicities from low to high, respectively. TSKgel Butyl-NPR is the best choice for high-speed separations with excellent recovery, even for more hydrophobic samples. The non-porous resin requires lower sample loading and leads to faster analysis time because the binding kinetics occur only on the bead's surface. The ultra-efficient 2.5 µm particles allow for fast and highly efficient separations. Thus, the TSKgel Butyl-NPR columns (4.6 mm ID × 3.5 cm and 10 cm) are ideal for time-critical quality control (QC) analysis or sample-limited applications. Because of the polymethacrylate based material, the TSKgel Butyl-NPR columns are stable in either acid or caustic cleaning regimes up to 1 mol/L.

An ADC (trastuzumab-vcMMAE) consisting of an antineoplastic drug (monomethyl auristatin E, MMAE) bonded via a linker to trastuzumab was used in this application note. Hence, an organic solvent (2-propanol) was added to eluent B for optimizing the elution of ADC peaks.

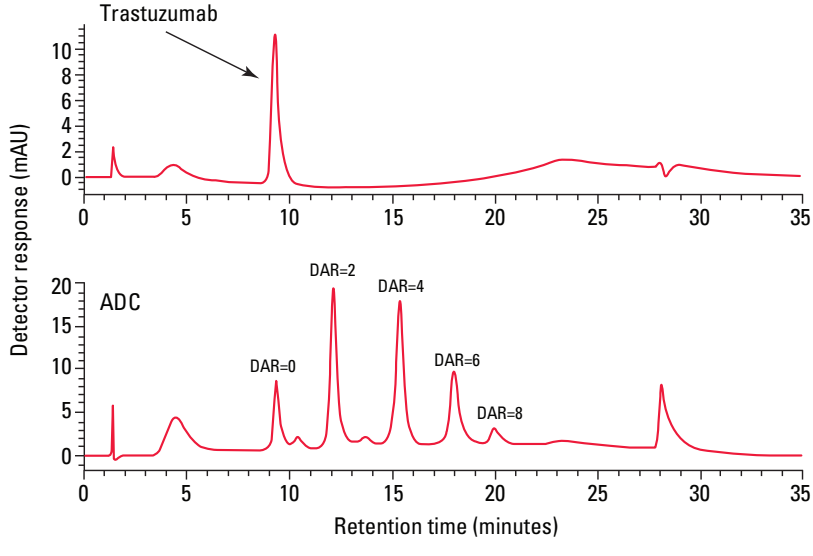

Figure 1: Analysis of unconjugated trastuzumab (upper panel) and drug-conjugated trastuzumab (lower panel) using TSKgel Butyl-NPR column.

Experimental Conditions

Column:	TSKgel Butyl-NPR, 2.5 µm, 4.6 mm ID × 10 cm
Eluent:	A: 25 mmol/L sodium phosphate buffer, pH 7.0, + 1.5 mol/L ammonium sulfate
	B: 25 mmol/L sodium phosphate buffer, pH 7.0, + 2-propanol (80:20)
Gradient:	0–100% B (0–20 min)
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm
Injection vol.:	10 µL
Samples:	Trastuzumab, 0.24 g/L
	ADC (trastuzumab-vcMMAE), 2.2 g/L

Results and Discussion

Both unconjugated monoclonal antibody (trastuzumab) and drug-conjugated trastuzumab (trastuzumab-vcMMAE) samples were independently injected onto a TSKgel Butyl-NPR, 2.5 µm, 4.6 mm ID × 10 cm column. After samples were injected onto the column, they were eluted with the organic solvent mixed in with a low concentration of sodium phosphate buffer, as shown in the experimental conditions.

The unconjugated trastuzumab sample was eluted as a major single peak at approximately 9.5 min (Figure 1, upper panel). This single peak indicated that the unconjugated trastuzumab consisted of mostly homogeneous molecules. The profile of the drug-conjugated trastuzumab (an ADC) exhibited well-resolved peaks with different retention times than that of the unconjugated drug and with baseline separation (Figure 1, lower panel). These well-resolved peaks were suggested to have different drug-to-antibody ratio (DAR). These peaks corresponded to DAR ranging from 0 to 8, estimated based on the mobility of the peaks. The heterogeneity of this sample was due to the addition of different drug loads. Consequently, this caused a decrease in mobility which resulted in differing elution times; the lower drug-loaded peaks eluted first and the higher drug-loaded peaks eluted later.

The ADC peak with a retention time of 9.5 min displayed the same retention time as the unconjugated trastuzumab peak (compare Figure 1 lower panel to upper panel). The retention time similarity of the two peaks indicated that it contained a group of unconjugated trastuzumab; therefore, it had the same mobility (retention time) as that of the unconjugated trastuzumab. This peak was called DAR = 0. When trastuzumab was conjugated with 2 cell-killing drugs, it eluted later than the non-conjugated trastuzumab and was called DAR = 2. DARs = 4, 6, and 8 peaks were labeled due their retention times, respectively.

Based on the baseline resolution of these peaks, with further method optimization (including modification of gradients, analysis time, and flow rates), analysis time can be significantly reduced. Indeed, the analysis time could be reduced by 50%.

Conclusion

Both unconjugated and drug-conjugated trastuzumab samples were successfully separated with baseline resolution using a TSKgel Butyl-NPR column. The baseline resolution enabled an easy integration and quantification of different drug payloads in ADC characterization. Using a modified elution buffer (mixture of phosphate and organic solvent), the DAR peak can be easily collected for LC–MS analysis. With 2.5 µm particles and 4.6 mm ID × 3.5 cm and 10 cm lengths, TSKgel Butyl-NPR columns can be used with both conventional HPLC and UHPLC systems.

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High-Speed Process Aggregate Monitoring with UHP-SEC-MALS

S. Kenrick, Wyatt Technology Corp.

At-line process monitoring (ALPM) of protein aggregation during processing and purification is made feasible by the 5-min runs typical of UHP-SEC. ALPM is particularly valuable in production because it can minimize the losses encountered when a process begins to fail. The addition of multi-angle light scattering (MALS) analysis to UHP-SEC deepens the quality and reliability of information obtained in near-real-time.

We demonstrate rapid UHP-SEC-MALS analysis under conditions mimicking different manufacturing and purification processing steps as well as stresses applied to test stability. MALS, RI, and dynamic light scattering (DLS) data provided robust, reproducible, and reliable quantification of molar mass, size, and amount of each species in a given sample.

Materials and methods

UHP-SEC was performed with an Acquity UPLC pump and autosampler (Waters Corporation) and BEH SEC column (200 Å, 1.7 µm, 4.6 mm × 150 mm; Waters). The effluent of the SEC column flowed through an inline UV–vis detector (Waters), µDAWN multi-angle light scattering detector with internal WyattQELS dynamic light scattering detector, and Optilab UT-rEX dRI detector (all three from Wyatt Technology). For each chromatogram, 2 µL to 5 µL sample was injected with an overall run time of 5 min per sample. Data collection and analysis were performed with ASTRA software (Wyatt). Monoclonal antibody 1 (mAb1) represents various phases of the purification process, ending with purified sample. The suffixes -C1, -C2, and -C3 represent different fractions collected during the purification process.

A different monoclonal antibody, mAb2, was subjected to stresses representing stability conditions. In addition to MALS analysis similar to mAb1, DLS measurements were performed to assess any conformational changes.

Results

Light scattering chromatograms for two replicates of each of the three conditions are overlaid in Figure 1. The starting material (mAb1-C1) contains approximately 70% monomer, with the rest of the mass represented by aggregate and fragment species. Sample mAb1-C2 represents an intermediate purification process, resulting in a solution of 85% monomer. The final purified antibody (mAb1-C3) is >95% monomer by mass. UHP-SEC-MALS provided rapid quantitation of each mAb sample to verify the identity of SEC peaks, making this technique perfect for ALPM. In addition, the replicates overlay perfectly as do the molar masses determined from each replicate, indicating the robustness of this method.

Five different species were defined based on the mAb1-C1 chromatogram. The peak definitions for these species are shown in Figure 2. The table in Figure 2 shows how effectively the different aggregate fractions are removed, while the monomer is enriched, as the purification process progresses. The molar mass of each species also appears consistent for collected fraction.

The molar mass of eluting species measured by the µDAWN and UT-rEX is extremely robust and reliable, despite the low concentration of certain peaks. For example, in the case of the purified mAb1, even though the maximum concentration of aggregate is only 0.8 µg/mL as it elutes from the column, the measured molar mass is within 2% of the molar mass measured for conditions 1 and 2, where the eluting concentration is significantly higher (Peak 2 Mw, Figure 2).

In another test, mAb2 was subjected to three different stress conditions and characterized by UHP-SEC-MALS-DLS, with results shown in Figure 3. Both conditions 1 and 2 caused mAb2 dimer formation, where condition 2 was shown to produce about 20% more dimer than condition 1. On the other hand, mAb2 subjected to condition 3 underwent quite different changes as a result of the applied stress: little dimer formation, but a shift in the molar mass of the fragment, and a shift in elution time of the monomer. DLS data indicate no conformational change of the monomer, suggesting that the later elution time is the result of a chemical modification leading to column interaction.

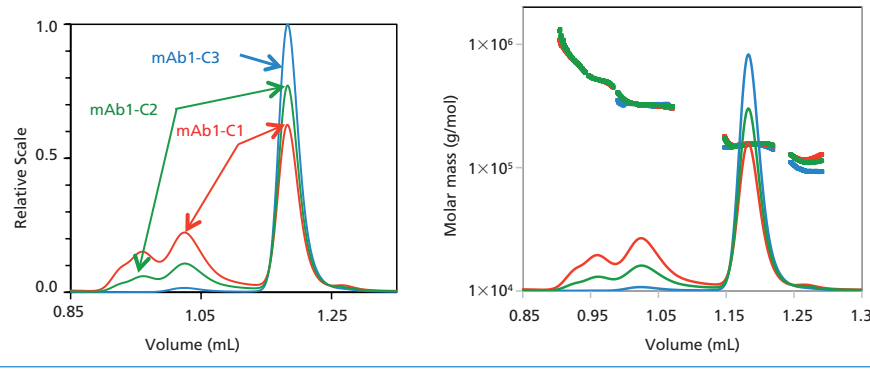
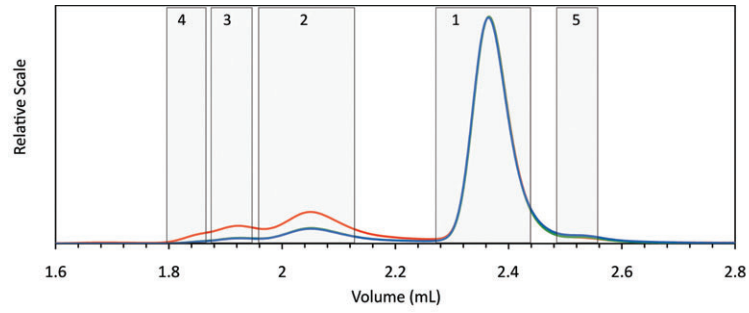


Figure 1: Left: Overlay of light scattering chromatograms for mAb1 undergoing different stages of purification for condition 1 (red), condition 2 (green), and condition 3 (blue). Right: Overlay of refractive index concentration signals and molar masses calculated from light scattering.



		Peak 1 (monomer)		Peak 2		Peak 3		Peak 4		Peak 5 (fragment)	
		Mw (kDa)	Mass (%)	Mw (kDa)	Mass (%)	Mw (kDa)	Mass (%)	Mw (kDa)	Mass (%)	Mw (kDa)	Mass (%)
mAb 1-C1	1	155.4	70.7	328.1	18.5	507.3	4.7	720.5	2.0	120.0	4.2
	2	155.3	70.6	329.7	18.6	510.9	4.8	735.9	2.0	119.9	4.0
mAb 1-C2	1	153.6	85.2	329.7	8.8	510.5	1.9	729.2	0.7	114.3	3.5
	2	153.3	85.1	329.9	8.9	510.8	1.9	730.0	0.7	115.4	3.4
mAb 1	1	152.4	96.0	323.4	1.1	--	<0.1	--	<0.1	98.2	2.8
	2	152.4	96.1	335.9	1.2	--	<0.1	--	<0.1	102.9	2.7

Figure 2: Peak definitions (top) and resulting molar mass and mass fraction (bottom) of each species. Plots correspond to light scattering (red), RI (blue), and UV (green) signals. RI and UV signals are barely distinguishable from each other for monomer and aggregates, indicating identical extinction coefficients, but differ for the fragment.

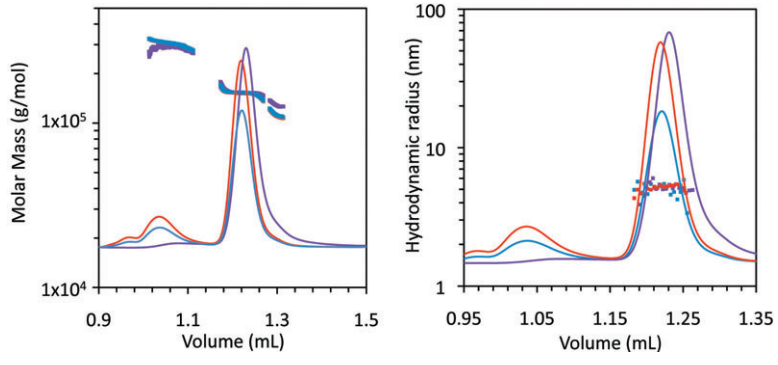


Figure 3: Left: Refractive index chromatograms for mAb2 under stability conditions 1 (blue), 2 (red), and 3 (purple). Molar mass values from MALS of the monomer, dimer, and fragment peaks have been overlaid. Right: Light scattering chromatograms for mAb2, plus hydrodynamic radii values of the monomer measured by online dynamic light scattering.

Conclusions

In summary, we demonstrated the use of high-speed UHP-SEC combined with MALS and DLS for process monitoring of antibody samples. With this technique, measurements may be performed in as little as 5 min, enabling monitoring of purification and other processing steps, as well as high-throughput stability screening. The µDAWN and UT-rEX provided essential and reliable quantification of molar mass and hydrodynamic radius of aggregates and fragments that would not be possible with analytical UHPLC alone. These data provide important information about the purification process, such as whether the aggregates are sheared during the process or whether new aggregate species are formed. The addition of MALS, DLS, and RI to UHP-SEC is critical to ascertain conclusively species identification and conformation.

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Separations of Intact IgGs Using Non-Porous, 2 µm Particle, C18 Presto Column, with a View Towards ADC Analysis

Piotr Macech, Robert Puryear, and Itaru Yazawa, Imtakt USA

Intact and reduced IgGs have been successfully retained and separated using a non-porous column. Potential applications of Presto columns for a single-injection separation of free drug, antibody, and antibody-drug complexes (ADCs) is also discussed.

Introduction

Strategies for chromatographic separation of large proteins typically use wide pore (300 Å) particles with C4 alkyl chains. However, this is fundamentally limited by the pore size in that large proteins may experience significant steric hindrance. Another disadvantage is that short chains usually adversely affect resolution of small molecules, such as when a sample contains both large and small molecules (for example, antibody-drug conjugates, ADCs).

One solution to these challenges is our Presto FF-C18 column, which is packed with non-porous, 2 µm, C18 modified silica particles. The advantages of this design are trifold. Firstly, since there are no pores, there is virtually no size limit for molecules that can be injected on the column. Secondly, small molecules can also be retained more efficiently on C18 and produce sharper elution peaks. Thirdly, both of the large and small molecules' separations can occur in a single injection—ideal for those who want to determine fractions of free drug, free antibody, and antibody-drug complex (ADC).

Experimental Conditions

Presto FF-C18 (250 × 4.6 mm length × ID); mobile phases: (A) water + 0.1% trifluoroacetic acid, (B) acetonitrile + 0.1% trifluoroacetic acid; 400 µL/min; 85 °C; sample 2 mg/mL; 2 µL injection volume. Gradients are 0 to 60 min for: 32–43% for IgG1#1, 34–41% for IgG2#1, 33–42% for IgG4#1. UV detection at 220 nm, operating pressure 15 MPa.

Results and Discussion

Chromatograms for intact and reduced IgGs are shown in Figure 1a–c and Figure 1d–f, respectively.

The IgGs #1 and 4 presented in Figures 1b and 1c show excellent peak shape. The IgG #2 (Figure 1a) exhibits multiple, partially co-eluting peaks indicating multiple isoforms within the IgG #2 subclass. Despite that, it is clear that these proteins can be easily separated using Presto FF-C18 column as their retention times are sufficiently distinctive.

Figure 1d–f presents chromatographic separations of IgGs in their reduced forms. The light chain fraction peaks (L) in all cases, elute earlier compared to intact IgGs. In contrast, heavy chain fraction (H) elute at approximately the same time as intact IgGs indicating their primarily role in intact protein retention time. Further, light chains elute in a single peak, while heavy chains are somewhat asymmetrical and exhibit shoulders. This observation is consistent with the possibility that variations in charge on the chains, and their folding dynamics are likely to exist.

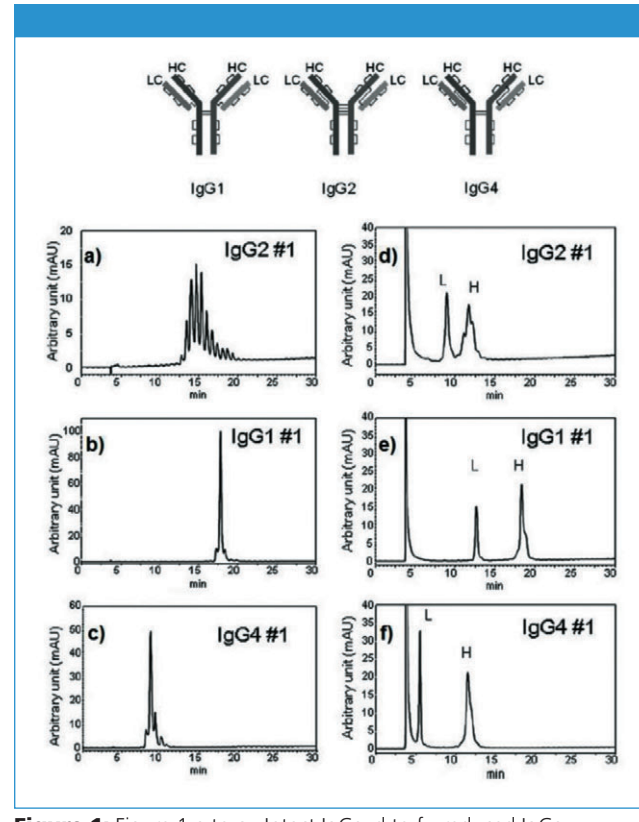

Figure 1: Figure 1 a to c - Intact IgGs, d to f - reduced IgGs.

Conclusion

Presto FF-C18 showed excellent separation capabilities for several IgGs. Proteins were eluted with high resolution for both intact proteins and their reduced forms (light and heavy chains), which has not been previously possible on reversed phase columns. The quality of the separation was shown to extend over a wide range of molecular sizes making this column a potentially attractive strategy for many applications, for example, ADCs analysis.

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A New SEC Column to Be Used with MALS

Leah Block, Showa Denko America, Inc.

With the recent increase of polymer research, there has been a growing need for polymer analysis. The best way to determine the size and estimated molecular weight of polymers and biopolymers is achieved using size exclusion chromatography (SEC). Within SEC, the most commonly used detectors are differential detectors immediately followed by light scattering detectors. When coupling a light scattering detector with a refractometer, a very powerful mode of advanced detection for SEC analysis is the result. Specifically, multiangle light scattering (MALS) allows for the collection of useful information including the molecular weight, average number molecular weight, and distribution of the polymer.

Shodex introduces the LB-806M column with a durable polymer based packing material for aqueous SEC analysis. This column has also been shown to be suitable for light scattering due to the controlled column bleed. Shodex LB-806M is appropriate for polymer and biopolymer analysis, has demonstrated the determination of molecular weight for sodium alginate.

The molecular weight of sodium alginate, a common biopolymer extracted from algea, was determined by OHpak LB-806M, a column for aqueous SEC. The LB-800 series are compatible with MALS detectors. By minimizing the baseline noise level, LB-800 series enables the MALS detector to detect a wider range of molecular weight compounds which are difficult to be detected by conventional columns.

Experimental Conditions

The analysis of sodium alginate was accomplished with Shodex LB-806M (8.0 mm ID × 300 mm, 13 µm), a polymer-based column for aqueous SEC analysis. Column temperature was 30 °C and flow rate was 1.0 mL/min. Eluent conditions were 0.1 M $NaNO_3$ aq. Injection volume of 5 µL of dissolved sodium alginate was used for the experiment. The HPLC system was coupled with Shodex RI MALS detector.

Results

The sodium alginate sample was analyzed successfully by SEC and RI MALS detection with LB-806M (Figure 1). From this one run Mw, Mn, and Mw/Mn were all determined. The average molecular weight (Mw) was found to be 166,200. The number average molecular weight (Mn) was found to be 58,790. With these two values determined from the data, the polydispersity index (Mw/Mn) was calculated to be 2.83.

Conclusions

Shodex LB-806M, a size exclusion chromatography (SEC) chromatography column is suitable for polymer and biopolymer analysis, has demonstrated the determination of molecular weight, number average molecular weight, and polydispersity index.

Column:	Shodex OHpak LB-806M (8.0 mmI.D. x 300 mm) x 2
Column temperature:	30 ºC
Injection volume:	5 µL
Eluent:	0.1 M $NaNO_3$ aq.
Flow rate:	1.0 mL/min
Detector:	Shodex RI MALS
Sample:	Sodium alginate

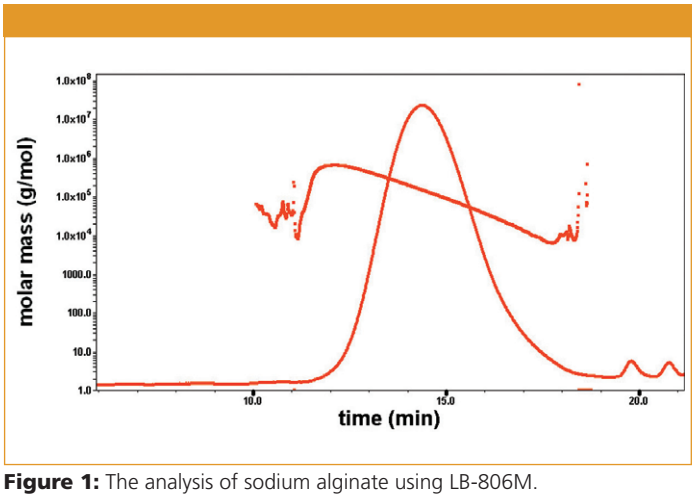

Figure 1: The analysis of sodium alginate using LB-806M.

Replacing Stacked Ring Reflectron Lenses with Segmented Monolithic Resistive Glass

Photonis USA

Reflectron lenses are used in time-of-flight (TOF) mass spectrometers to create an electrostatic field to alter ion flow, providing for a longer flight path and therefore greater resolution. Current reflectron-type TOF-MS instruments use complex multi-piece stacked ring assemblies that require time consuming assembly and cleaning processes. They also require the use of a voltage divider in each layer to control the electric field.

Segmented monolithic lenses made from resistive glass can replace traditional stacked ring reflectron lenses currently used in mass spectrometers. Resistive glass tubes are designed to guide charged particles by generating a highly uniform electric field. Resistive glass products are composed of a proprietary lead silicate glass that has been specially processed to create an integral semiconductive layer on the surface.

Prior studies detailing the use of a resistive glass reflectron tube in an orthogonal TOF system showed the resistive glass tube had lower FWHM values, indicating a better energy focus, while comparative spectra between a traditional stacked ring assembly and the resistive glass tube were nearly identical (1).

An additional patent was granted to Photonis in 2012 for the manufacture of varied, nonlinear electric fields in resistive glass tubes. This new manufacturing capability enables instrument designers to produce nonlinear and dynamic fields within the lens for better instrument performance. Axial lines can be applied for use as a collision cell, or rings can be applied for use as a segmented reflectron lens.

A reflectron lens made with resistive glass provides a solid assembly replacement for a stacked ring assembly yet provides the same ability to alter ion flow. This innovation is a form-fit-function replacement for the multi-piece stacked ring assemblies, while providing the ability to manipulate the electric field inside the resistive glass tube.

The single-piece assembly also greatly simplifies the cleaning and assembly process currently required for stacked ring lenses. Resistive glass can be easily cleaned with water, acetone, methanol, or IPA without degrading performance, and is resistant to scratches from light to moderate abrasions.

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Extending the Hydrocarbon Range for the Analysis of Soil Gas Samples Using Automated Thermal Desorption Coupled with Gas Chromatography–Mass Spectrometry

This study describes the recovery of compounds above the boiling point of naphthalene achieved by optimizing the thermal desorption chemistry for the determination of volatile organic compounds ranging from C_3 to C_{26} in soil gas samples using Method TO-17. Figures of merit such as breakthrough, precision, linearity, and detection capability are presented, in addition to an evaluation of its real-world capability at sites with moderate diesel and semivolatile polynuclear aromatic hydrocarbon (up to pyrene) contamination, in the presence of high humidity. This research has provided a means to determine a more representative composition of soil gas.

Lee Marotta, Stephen Varisco, Miles Snow, Tom Kwoka, and Robert Thomas

In 1994 the United States Environmental Protection Agency (US EPA) published a report that an average person breathes approximately 20,000 liters of air per day (1). Since then, the need to optimize methods for the analysis of toxic compounds in air to understand their impact to human health has increased significantly. The scientific and regulatory communities have long been aware of the potential for migration of vapors from contaminated groundwater or soil into buildings, but until recently (with the exception of radon and major fuel leaks) soil vapor intrusion has not been a major concern. Then in the late 1990s, two sites in Colorado with chlorinated solvent plumes were found to have contributed to the contamination of a number of residential buildings. In 2002, the EPA issued draft guidance that provided technical and policy recommendations for determining if the vapor intrusion pathway posed a risk to human health at cleanup sites. Since then, the majority of American states (2) and several Canadian provinces (3) have introduced vapor intrusion guidelines and legislation.

Current EPA methods for volatile organic compounds (VOCs) in air are Method TO-15 using a Summa canister (4) and Method TO-17 using a sorbent tube (5). However, the target analytes specify a boiling point range of only C_3 to C_{12}. For that reason, efficient methods are needed for the analysis of soil gases for toxic compounds above this range. For example, diesel fuel components are of great concern because they can be found in soil gas and are known to have an impact on human health. Additionally, the sampling and analysis of soil gas samples poses several unique challenges when compared to indoor or ambient air monitoring. For instance, soil gas often has a higher moisture content and possibly a broader range of compounds. Because the compounds are typically confined, some sites can be very contaminated. Thus, sorbent tubes and the analytical systems used need to deal with this while providing accurate data at the low detection limits required for the toxic regulated compounds.

It's also important to emphasize the difference between the sampling procedures of these two EPA methods to fully understand these complex issues. Method TO-15 uses a large stainless steel vessel called a Summa canister, which collects approximately 6 L of air. A fraction of this sample volume, typically 500 mL, is withdrawn from the canister and sent to a concentrator sorbent trap. The sample is then desorbed from the trap and focused onto a gas chromatographic (GC) analytical column to be separated and analyzed by mass spectrometry (MS).

There are several limitations of the TO-15 approach. This method only reliably recovers up to naphthalene (C_{12}) while several regulatory directives require measurements up to at least C_{13}. In addition, many air samples might contain higher boiling substances that can adsorb onto the sides of the canister and condense. Other challenges include analyzing air samples with high moisture content and the requirement for a greater number of analytes (in some cases up to C_{40}) over a wide range of concentrations. Method TO-17 overcomes many of these challenges by using a thermal desorption tube instead of a Summa canister to collect the sample. The thermal desorption process utilizes a sorbent tube, which contains adsorbent mate-

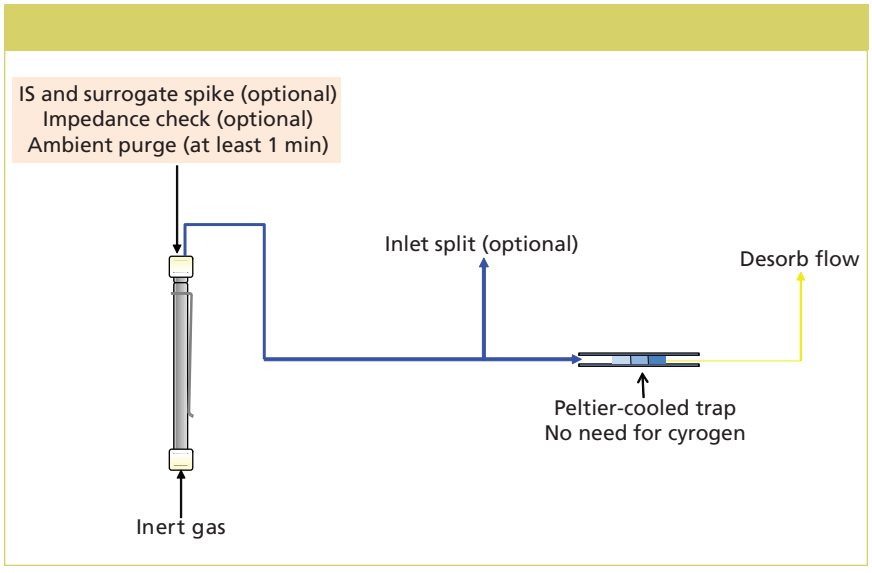

Figure 1: The contents of the tube are transferred to the concentrator (cold) trap.

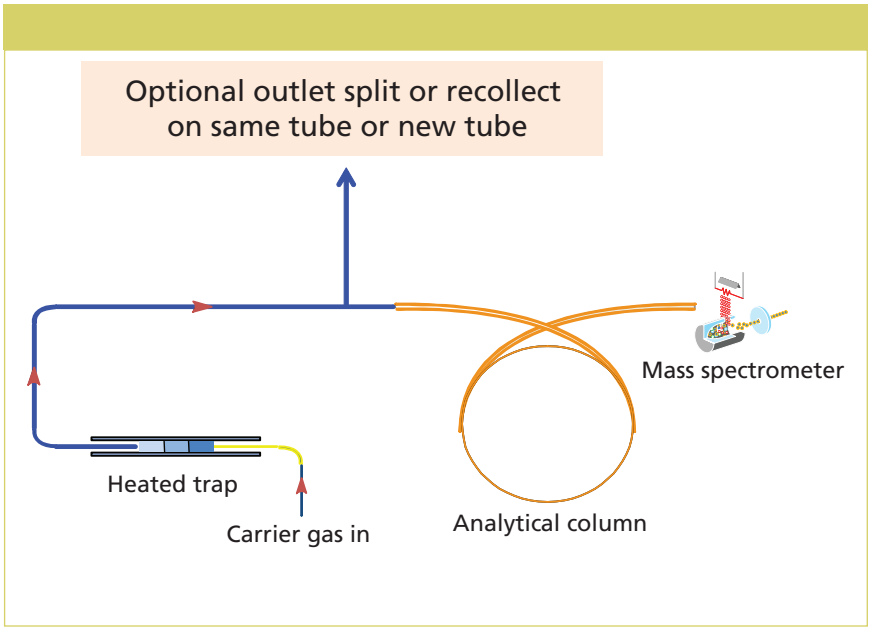

Figure 2: The contents of the trap are introduced into the GC analytical column.

rial specifically selected to trap the range of analytes of interest. In active sampling, a known volume of air is sampled through the tube, where the contents are then desorbed onto a secondary trap into the analytical column to be analyzed by GC–MS (6).

This study therefore describes the need to recover compounds above the boiling point of naphthalene by optimizing the thermal desorption chemistry for the determination of VOCs from C_3 to C_{26} in soil gas samples using Method TO-17. Figures of merit such as breakthrough, precision, linearity, and detection capability are presented, in addition to evaluating its real-world capability at sites with moderate diesel and semivolatile polynuclear aromatic hydrocarbon (up to pyrene) contamination, in the presence of high humidity. Because compounds with boiling points higher than naphthalene were added to the analyte list, experiments were also performed to ensure that lighter compounds, such as vinyl chloride, remained on the tube during sampling. This research has provided a means to determine a more representative composition of soil gas.

Study Objectives

The selection of the tube material and the optimization of the thermal desorption chemistry were based on the following objectives:

- Extend the analyte range past naphthalene (the limit of typical sorbent tubes)
  - Many contaminated sites have diesel contamination. The sorbent tubes need to adsorb these compounds separately from (that is, before) the more volatile ones during sampling
  - Achieve good recovery for the higher boiling components during desorption.
- Ensure the most volatile compounds such as vinyl chloride do not break through the sorbents during sampling
- Ensure the sorbents selected did not produce target artifacts that may result in false positives
- Enable "quick clean up" of the tubes so that the primary desorption process would make them available for resampling, reducing analytical cost
- Maintain good water management inherent with using hydrophobic sorbents
- Increase sampling volumes to attain low reporting limits while enabling the recollection of the sample in case reinjection of the same sample is required.

Before we describe the experimental details of this study, let's first describe the basic principles of processing samples using thermal desorption.

Fundamental Principles of Automated Thermal Desorption for Air Monitoring

After samples have been collected (or standards injected) onto the sorbent tubes, they are loaded onto the automated thermal desorption autosampler. The instrument inserts the tube into the primary desorption path. A leak check is performed on both the sample tube and the concentrator trap to ensure sample integrity. Addi-

Table I: Thermal desorption, GC, and MS analytical parameters used for this study

Thermal Desorber Parameters	
Tube desorb. temp.	325 °C
Tube desorb. time	10 min
Tube desorb. flow	50 mL/min
Concentrator trap low	10 °C
Concentrator trap high	330 °C
Concentrator trap hold	8 min
Trap desorb. time	0.0 min
Dry purge time	10 min (depending on moisture content)
Dry purge flow	50 mL/min
Dry purge temp.	Ambient
Column flow	1.8 mL/min
Recollect (or outlet split)	20 mL/min
Column flow during trap D time	1.8 mL/min
GC Parameters	
Initial oven temp.	35 °C for 3 min
Ramp 1	8 °C/min
Second oven temp.	55 °C no hold
Ramp 2	15 °C/min
Third oven temp.	175 °C
Ramp 3	20 °C/min
Final hold	275 °C hold for 1.5 min
MS Parameters	
Mass range (amu)	35–270
Filament delay	None
Scan time	0.25 min
Interscan delay	0.03 min

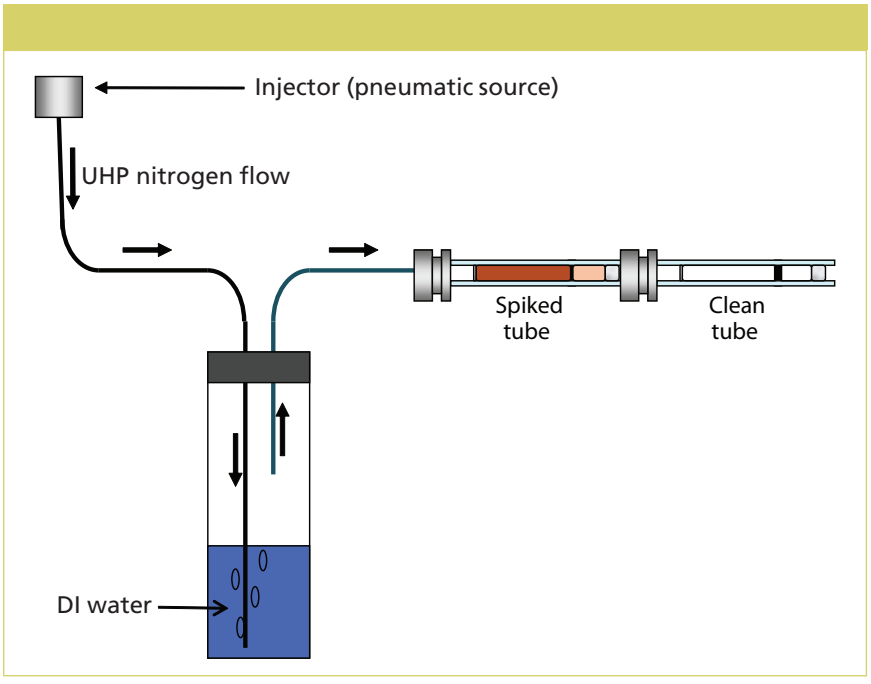

Figure 3: An illustration of breakthrough experiment.

tionally, an impedance check may be performed on the tube at this time to validate that the tube is packed properly (that is, there are no preferential pathways or the sorbents are not packed too tightly).

After these steps are performed, an inert gas flows through the tube, automatically introducing a gaseous internal standard onto the tube (this step is optional) while performing a dry purge to rid the tube of oxygen and water. Following the dry purge, a heater is placed onto the tube. Using a combination of heat, flow, and time, the contents of the tube are transferred to the concentrator (cold) trap, which is represented in Figure 1.

The concentrator trap uses a Peltier (electronic) cooler instead of the traditional liquid cryogen to achieve the trap's lowest temperature of -35 °C if required. The low dead volume trap contains the same hydrophobic sorbents as the soil vapor intrusion tube; therefore, the analytes are focused in such a way that detectable breakthrough is prevented. After the contents of the tube are adsorbed onto the concentrator trap, it is heated rapidly and the contents of the trap are introduced into the GC analytical column in a narrow band, as shown in Figure 2.

For highly concentrated samples, two splitters may be used: an inlet split between the sample tube and the concentrator trap, and an outlet split between the concentrator trap and the analytical column, enabling split ratios of several orders of magnitude. The inlet split may be disabled, and the outlet split may be used to recollect the sample onto the same tube or onto a different tube, preserving the sample for another injection (such as a sample dilution). For trace-level samples, splitless injection may be performed. Additionally, surrogates can be automatically spiked onto all tubes before sampling to provide additional data quality assurance.

Experimental

The instruments used in this application were a TurboMatrix Thermal Desorber 650, a Clarus 680 gas chromatograph, and a Clarus SQ8 mass spectrometer (both from PerkinElmer Inc.). Details of analytical parameters are described in Table I.

A 60 m × 0.25 mm, 1.4-µm d_f Restek–624Sil MS column was chosen for this work because it allows a maximum temperature of 320 °C, which is required to elute the polynuclear aromatic hydrocarbons (PAHs) that are heavier than

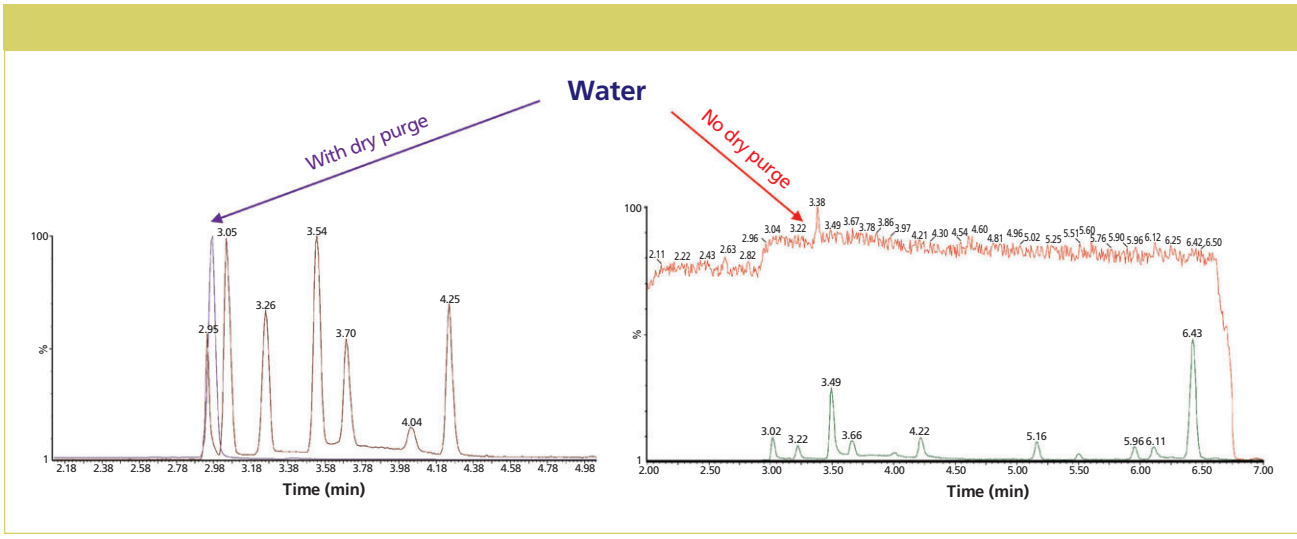

Figure 4: Dry purge efficiently removes moisture (this was performed on a different column).

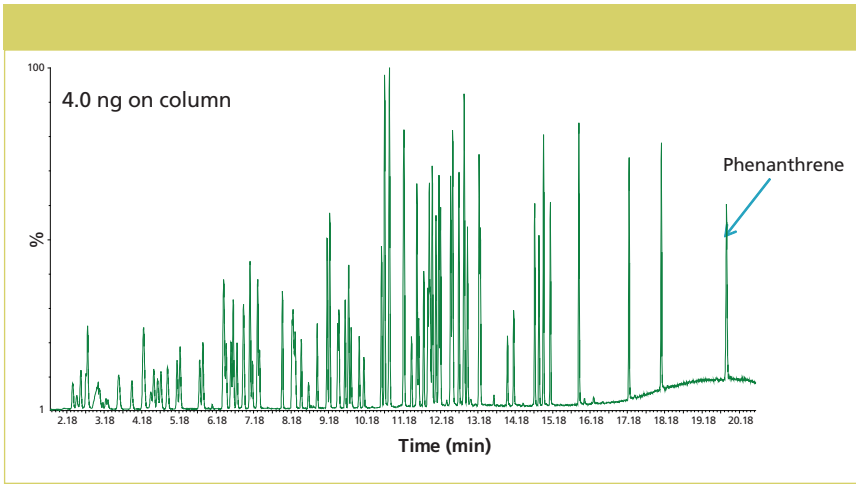

Figure 5: Chromatogram of 4.0 ng of analytes on column (chromatography on a 60 m × 0.25 mm, 1.4-µm d_f Rxi-624Sil MS column).

naphthalene. The mass spectrometer was operated in full scan mode, achieving the necessary detection limit criterion without the need for selective ion monitoring (SIM).

A total of 86 target compounds and diesel was investigated. Diesel was purchased from a nearby gas station. The following standards were purchased from Restek Corporation:

- 502.2 calibration mix 1 containing six gases
- 1,3-Butadiene
- 8260B Mega Mix containing 76 VOCs
- 2-Methylnaphthalene, anthracene, fluorene, and phenanthrene from separate ampules

The standards were diluted with purge-and-trap-grade methanol to attain the required concentrations for the following experiments.

Breakthrough and Recovery

The *breakthrough volume* is a critical factor in the performance of an adsorbent. It is defined according to EPA TO-17 as the volume sampled when the amount of analyte collected in a backup sorbent tube reaches 5% of the total amount collected by both sorbent tubes. Another very important performance criterion is spike recovery, which involves the analysis of a spiked tube from the breakthrough test, by first analyzing a blank tube and then reanalyzing the spiked tube to see if all analytes were desorbed off the tube from the first desorption. This analysis was performed by spiking the thermal desorption tubes with a high concentration of the following analytes to mimic a contaminated site:

- 300 ng of the 502.2 calibration mix (six gases)
- 300 ng of 1,3-butadiene
- 300 ng of 8260B Mega Mix
- 300 ng of the four PAHs
- 10 µg of diesel

After spiking three tubes, each tube was connected to a clean tube referred to as the *breakthrough check tube*. Each set (spiked tube connected to breakthrough check tube) was placed on a manifold that accommodated three sets. Then 100 mL/min of humidified nitrogen (85%) was simultaneously passed through the tubes for 100 min to simulate a 10-L sampling volume. This process is exemplified in Figure 3, which provides an illustration of the experiment.

Table II: Percent spike recoveries of a group of polynuclear hydrocarbon compounds

PAH Compounds	% Recovery
1-Methyl napthalene	99.7
Anthracene	99.8
Fluorene	99.4
Phenanthrene	98.8
Diesel	99.8

Table III: Precision, linearity, and reporting limits attained in this study

Class of Compound	Number of Analytes per Group	Linearity (0.05 to 250 µg/m³)		Precision	Reporting Limit
		r^2	Average RF	(n = 10)	S/N at 0.05 (µg/m³)
Gases	7	0.9994	9.07	7.39	530:1
Aliphatic hydrocarbons (halogenated)	35	0.9996	14.00	4.80	560:1
Aromatics (halogenated)	9	0.9997	13.30	2.58	1350:1
Aromatics (non-halogenated)	14	0.9996	10.27	1.91	1220:1
Polynuclear aromatic hydrocarbons	5	0.9997	8.69	3.56	570:1
Others	13	0.9996	9.26	3.19	560:1

Table IV: Precision of the automated internal standard (n = 15)

Internal Standard	Fluorobenzene	1,4 Difluorobenzene	Chlorobenzene-d_5	BFB
Quant ion	96	114	117	95
%RSD (n = 15)	1.34	1.29	0.53	0.98

The goal was that none of the target compounds would be detectable in the breakthrough check tube (the second tube in the series). An assessment of the recovery of the desorption process was confirmed by reanalyzing the spiked tube. A trap test was performed first to ensure the targets were recovered from the trap. Then an empty (blank) tube was analyzed before the reanalysis of the spiked tube to confirm instrument cleanliness.

Water Management

The retention of water was determined by starting the MS scan at 15 amu instead of 35; therefore, enabling the mass characteristic of water at 18 amu to be acquired. Figure 4 illustrates the level of water management achieved by using this dry purge system, essentially reducing moisture content to instrument background levels.

Instrument Precision

Instrument precision was investigated by spiking 10 tubes with a 20-µg/mL VOC standard (4 ng on column).

Chromatography

Figure 5 represents a total ion chromatogram (TIC) of a standard representing 4.0 ng on column.

Method Dynamic Range

The method dynamic range was evaluated over four orders of magnitude, from 0.05–250 ng spiked onto the tube. For a 1-L sample, this equates to a concentration range of 0.05–250 µg/m³; therefore, with a 1-L sample volume, a reporting limit of 0.05 µg/m³ is achieved across the compound target list. However, several targets can achieve a lower reporting limit.

Detection Capability

The detection capability of the method was also investigated as outlined in the summary of results below.

Internal Standard Precision

Internal standard precision was also investigated. This process is automated by the thermal desorber; 15 tubes were inserted onto the instrument's carousel and spiked with an internal standard.

Tuning Criterion

The tuning criterion was met for TO-17 using 4-bromofluorobenzene (BFB).

Summary of Results

With 10 L of humidified nitrogen flowing through the tubes, the results of the breakthrough experiment were minimal, with only one of the most volatile compounds, dichlorodifluoromethane (Freon-12), observed at less than 1%. Vinyl chloride, one of the most toxic volatile gases, exhibited no detectable breakthrough, despite the fact that the concentration and humidity of the tubes were very high. Retaining this compound is critical since its toxicology is well documented. The full set recovery data for a group of polynuclear aromatic hydrocarbon compounds is shown in Table II.

It should be noted that pyrene was also investigated at 325 °C, which resulted in a 90% recovery (a 99.3% recovery was achieved when the sample temperature was increased to 350 °C). However, since the goal of the study was to use a temperature of 325 °C for these experiments, pyrene was not investigated any further.

The data collected on target precision, linearity, reporting, and method detection limits are demonstrated in Table III, which easily meets Method TO-17 performance criteria for the solid adsorbent sampling of ambient air (2). The reporting limits are calculated using a 1-L sample volume. The dynamic range achieved was at least four orders of magnitude across the target component list.

The results from the internal standard precision study are presented in Table IV.

Table V displays the results for the BFB tune and the requirements set forth in EPA Method TO-17.

Discussion

Thermal desorption is a very cost effective and accurate technique for the sampling and analysis of air samples at very low detection capability. Thermal desorption has many environmental applications such as soil gas, studying healthy building syndrome, fenceline monitoring, and indoor–outdoor air analysis as well as addressing industrial hygiene concerns since 2009 (7). Sorbent tubes

Table V: Precision of the automated internal standard (n = 15)			
Mass	Reference Mass	Criterion	Relative Abundance (%) (achieved)
50	95	≥ 8% and ≤ 40%	17.8
75	95	≥ 30% and ≤ 66%	45.1
95	BPI	100%	100.0
96	95	≥ 5% and ≤ 9%	6.1
173	174	< 2%	0.5
174	95	≥50% and ≤120%	88.2
175	174	≥ 4% and ≤ 9%	6.0
176	174	≥93% and ≤ 101%	97.1
177	176	≥ 5% and ≤ 9%	6.5

are small and light, making them easy to transport, thus reducing shipping costs compared to other techniques. In addition, the tubes are cleaned during the desorption process, rendering them available for immediate resampling, which can be verified with a short GC–MS analysis.

Water management is rigorous and automatic. Eliminating or reducing water entering the analytical system prevents the "quenching" of the response of target analytes, yielding accurate data and enhanced detection limits. Sample integrity is preserved using the following automated processes:

- A surrogate may be spiked onto the tube before sending the tube into the field for sampling.
- The sample tube and the concentrator trap is leak checked before desorption.
- An optional internal standard can be automatically spiked onto the tube.
- If needed, a tube impedance check can be performed on the tube to ensure packing is consistent.
- The sample may be recollected onto a new tube or the same tube if there is a need to reanalyze, or if the sample needs to be preserved for legal purposes.

Conclusion

In conclusion, the TO-17 method using the soil vapor intrusion sorbent tube designed for this investigation has demonstrated the capability of retaining the gaseous volatiles while extending the analyte range to phenanthrene. All US EPA method criteria were met and the management of moisture in the soil gas was adequately addressed. The achievable linear dynamic range of 0.05–250 µg/m^3 was acceptable for the vast majority of soil samples encountered. If dilution is required, this can be accomplished by modifying the split ratios on the thermal desorber, or by recollection of the sample. The same calibration curve can be used and the dilution factor can be applied in the processing sequence. A reporting limit of 0.05 µg/m^3 was achieved, which is below the required limit for regulatory agencies.

Acknowledgments

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