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Reference chart of phases, dimensions, and part numbers on reverse side.

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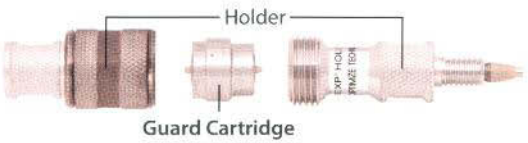
Ordering Information

I.D.	Length	C18	C8	Peptide ES-C18	RP-Amide	Phenyl-Hexyl	ES-Cyano	F5	HILIC	OH5
Ascentis Express Columns										
2.1 mm	2 cm	53799-U	53795-U	—	53797-U	53798-U	53494-U	53592-U	—	53779-U
2.1 mm	3 cm	53802-U	53839-U	53299-U	53910-U	53332-U	53468-U	53566-U	53933-U	53748-U
2.1 mm	5 cm	53822-U	53831-U	53301-U	53911-U	53334-U	53470-U	53567-U	53934-U	53749-U
2.1 mm	7.5 cm	53804-U	53843-U	53304-U	53912-U	53335-U	53472-U	53568-U	53938-U	53755-U
2.1 mm	10 cm	53823-U	53832-U	53306-U	53913-U	53336-U	53473-U	53569-U	53939-U	53757-U
2.1 mm	15 cm	53825-U	53834-U	53307-U	53914-U	53338-U	53475-U	53571-U	53946-U	53764-U
3.0 mm	3 cm	53805-U	53844-U	53308-U	53915-U	53341-U	53476-U	53574-U	53964-U	53766-U
3.0 mm	5 cm	53811-U	53848-U	53311-U	53916-U	53342-U	53478-U	53576-U	53967-U	53767-U
3.0 mm	7.5 cm	53812-U	53849-U	53312-U	53917-U	53343-U	53479-U	53577-U	53969-U	53768-U
3.0 mm	10 cm	53814-U	53852-U	53313-U	53918-U	53345-U	53481-U	53578-U	53970-U	53769-U
3.0 mm	15 cm	53816-U	53853-U	53314-U	53919-U	53346-U	53483-U	53579-U	53972-U	53771-U
4.6 mm	3 cm	53818-U	53857-U	53316-U	53921-U	53347-U	53484-U	53581-U	53974-U	53772-U
4.6 mm	5 cm	53826-U	53836-U	53318-U	53922-U	53348-U	53486-U	53583-U	53975-U	53774-U
4.6 mm	7.5 cm	53819-U	53858-U	53323-U	53923-U	53351-U	53489-U	53584-U	53977-U	53775-U
4.6 mm	10 cm	53827-U	53837-U	53324-U	53929-U	53352-U	53491-U	53590-U	53979-U	53776-U
4.6 mm	15 cm	53829-U	53838-U	53328-U	53931-U	53353-U	53492-U	53591-U	53981-U	53778-U
Ascentis Express Guard Cartridges, Pk of 3										
2.1 mm	—	53501-U	53509-U	53536-U	53514-U	53524-U	53495-U	53594-U	53520-U	53780-U
3.0 mm	—	53504-U	53511-U	53537-U	53516-U	53526-U	53496-U	53597-U	53521-U	53781-U
4.6 mm	—	53508-U	53512-U	53542-U	53519-U	53531-U	53497-U	53599-U	53523-U	53782-U

Capillary Ascentis Express Columns				
75 µm	5 cm	53982-U	53983-U	53543-U
75 µm	15 cm	54219-U	54229-U	53549-U
100 µm	5 cm	53985-U	53987-U	53544-U
100 µm	15 cm	54256-U	54260-U	53552-U
200 µm	5 cm	53989-U	53991-U	53545-U
200 µm	15 cm	54261-U	54262-U	53553-U
300 µm	5 cm	53992-U	53997-U	53546-U
300 µm	15 cm	54271-U	54272-U	53554-U
500 µm	5 cm	53998-U	53999-U	53547-U
500 µm	15 cm	54273-U	54275-U	53558-U
1000 µm	5 cm	—	—	53548-U
1000 µm	15 cm	—	—	53561-U

Ascentis Express Guard Columns

Description	Cat. No.
Universal Guard Holder	
Holder w/EXP Titanium Hybrid Ferrule (cartridge not included)	53500-U



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ADVANSTAR SCIENCE

Pittcon 2012: New Chromatography Columns and Accessories, Part II

New Gas Chromatography Products for 2012

Characterizing Biologics and Biosimilars

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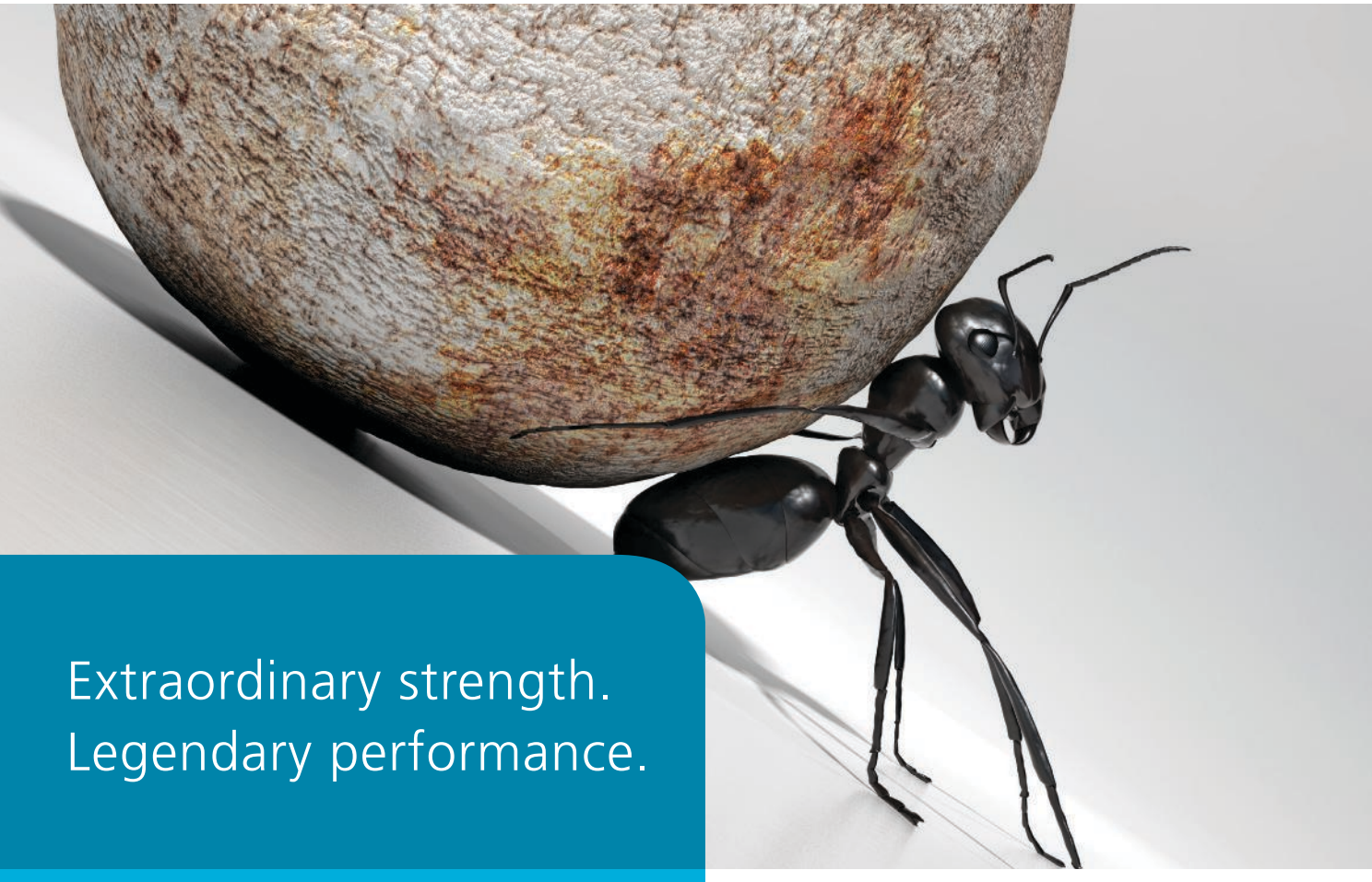

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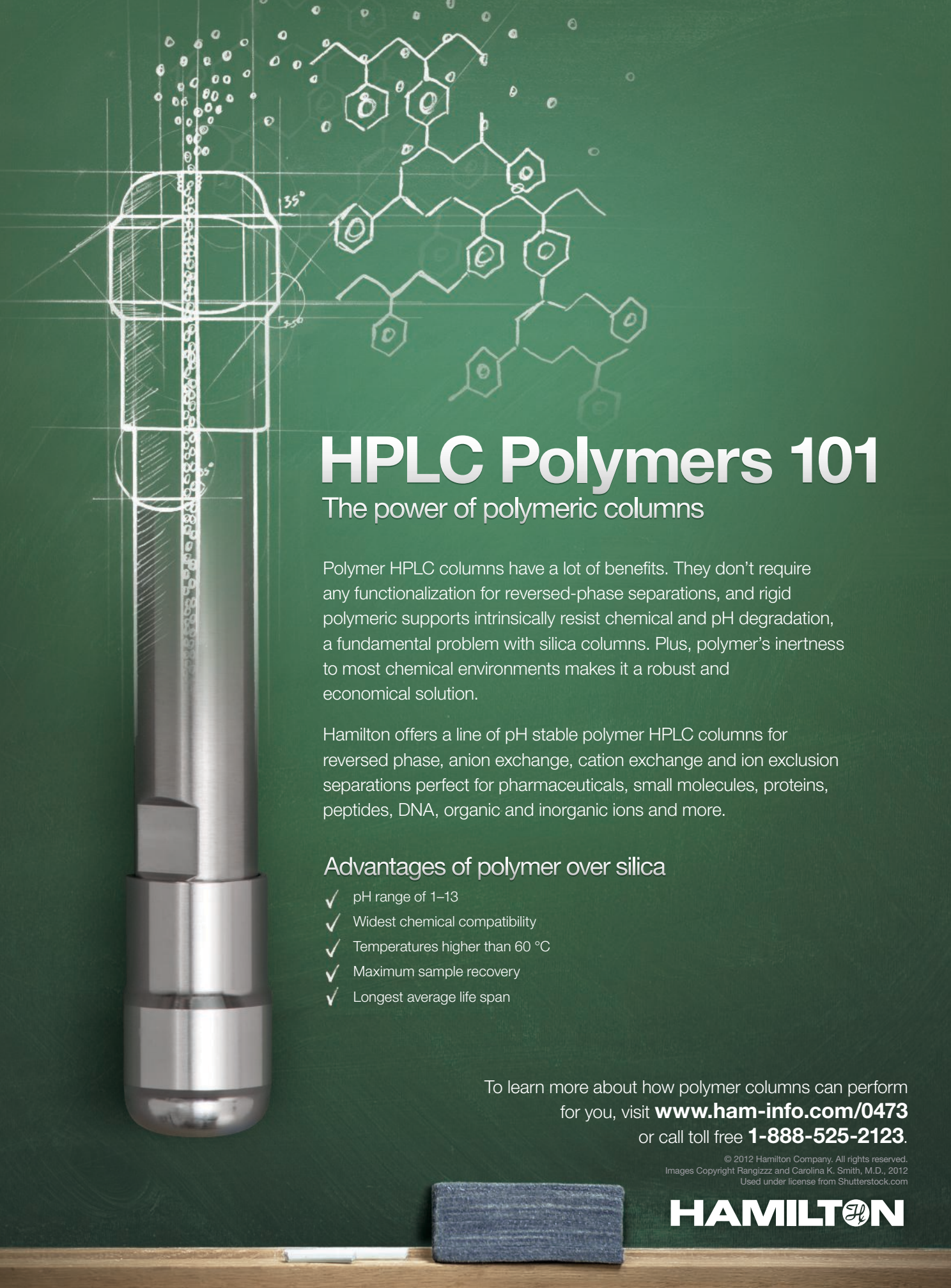

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ON THE WEB

NEW WEB SEMINARS

Advantages of GC-Based Multidimensional Chromatography for Food Analysis
Peter Q. Tranchida, Associate Professor in the School of Pharmacy at the University of Messina (Italy)

Optimizing GC Methods for Speed: More than Just Columns
Nicholas H. Snow, Seton Hall University

chromatographyonline.com/WebSeminar

LC–MS

In a new roundtable, experts discuss how liquid chromatography–mass spectrometry (LC–MS) analysis continues to evolve in ways that make it a versatile technique in laboratories worldwide.

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MAY 2012

DEPARTMENTS

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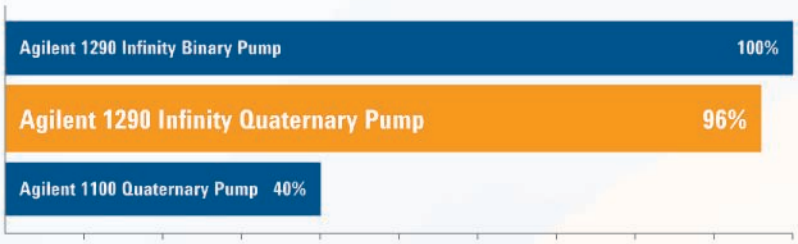

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GAS CHROMATOGRAPHY

Innovation with Integrity

PEAKS of Interest

Schug to Lead New Shimadzu Center at UT Arlington

Kevin Schug, an associate professor of chemistry and biochemistry at The University of Texas (UT) at Arlington, has been named the university's new Shimadzu Distinguished Professor of Analytical Chemistry. He will also oversee a new Shimadzu Center for Advanced Analytical Chemistry, which has been established through an in-kind gift valued at nearly $3 million from Shimadzu Scientific Instruments (Columbia, Maryland).

Schug said the facility will provide many new capabilities that were not previously available. These include enhanced capabilities for matrix-assisted laser desorption–ionization (MALDI), including ion trap–time-of-flight (IT–TOF), TOF-TOF, and high mass systems; proteomics and bioinformatics capabilities for use following liquid chromatography–mass spectrometry (LC–MS) and MALDI–MS analysis; polymer characterization by gel-permeation chromatography–MALDI with automated fractionation and spotting; MS surface imaging with chemical inkjet printer technology; and comprehensive metals analysis by inductively coupled plasma–optical emission spectroscopy (ICP-OES).

"In general, we have built a facility that can handle virtually any molecular analysis scenario," said Schug.

Schug also noted that the central location of the Shimadzu Center would allow researchers in the UT College of Science and the College of Engineering to access the enhanced capabilities for trace qualitative and quantitative analysis.

"Kevin Schug is one of the leading young scientists in the country, and it will be a pleasure to work with him and the entire team at UT Arlington on future projects," said Shuzo Maruyama, president of Shimadzu Scientific Instruments.

The instruments, which are now up and running, will be used for research into the prevention and treatment of illnesses such as cancer and malaria, as well as in the development of nanofabrication materials for industry. Examples of some of the cutting-edge research that will be aided by the instruments available in the new center include

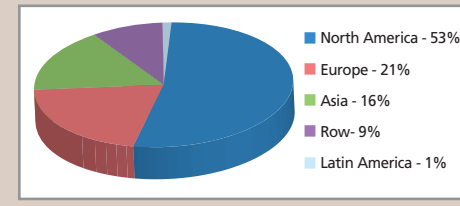
- Research led by Schug using MALDI-TOF-MS equipment to analyze cuticular lipids that can reveal age in a species of mosquito known for spreading malaria.
- Subhrangsu Mandal, associate professor of chemistry and biochemistry, is currently looking for chemicals in the environment that could interfere with normal hormone functions and, possibly, fuel cancer growth. The MS equipment will help him better analyze test items such as commonly used growth hormones, water from various sources, and milk.
- Jian Yang, associate professor of bioengineering, will use the new high performance liquid chromatography and MS equipment in his work on biodegradable fluorescent polymers.

Schug won the 2010 Eli Lilly Young Analytical Scientist Award, which recognizes a researcher doing work relevant to the pharmaceutical industry. He also is a member of the *LCGC North America* Editorial Advisory Board. ■

Chromatography Market Profile

Laboratory Information Management Systems

With accountability and regulatory compliance being such a large part of the modern laboratory, the necessity for laboratory information management systems (LIMS) cannot be understated. LIMS allow laboratories to manage both logistic and analytical data, as well as the ability to interface with corporate reporting software, instruments, and a host of other software modules.



Regional distribution of LIMS survey respondents (*n* = 552).

LIMS are used in both small and large companies to allow its users to automatically input, store, and archive information, track samples, and easily generate reports. LIMS products vary widely in cost, sophistication, and industrial focus.

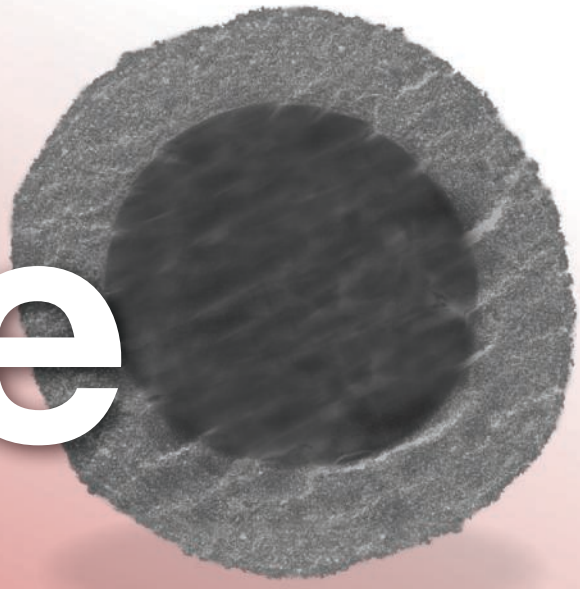
In a recent survey from SDi, more than 550 LIMS managers and knowledgeable users were questioned about their needs, capabilities, and trends relating to the LIMS market. These surveys provided a great deal of insight into user opinions about leading companies. In fact, when asked to indicate the top criteria when choosing a LIMS vendor, the two most frequently mentioned responses were service and support, and responsiveness and reliability of the LIMS vendors. These criteria have become increasingly important over the years, partly because the magnitude of LIMS installations and the vital role it plays in everyday laboratory decisions.

The respondents were also asked to rate their satisfaction with a wide array of product and vendor factors using a set rating scale. Vendor's service expertise and long-term company commitments were among the highest rated vendor factors.

The accompanying chart shows the regional distribution of respondents to the survey. North America represented the largest segment of respondents. Europe accounted for over one-fifth, while Asia, the rest of the world (ROW), and Latin America combined for the remaining 26% of the respondent base.

The foregoing data were extracted from SDi's Tactical Sales and Marketing (TSM) report entitled: *2012 Lab Informatics Survey of End-Users.* For more information, please contact Glenn Cudiamat, VP of Research Services, Strategic Directions International, Inc., 6242 Westchester Parkway, Suite 100, Los Angeles, CA 90045, tel. (310) 641-4982, fax (310) 641-8851, e-mail cudiamat@strategic-directions.com.

It's What's Inside that Counts



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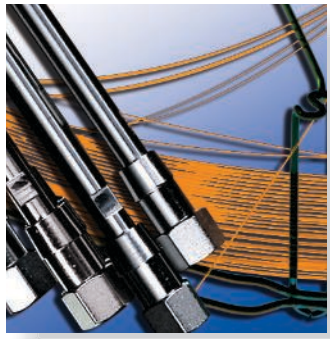

COLUMN WATCH

New Chromatography Columns and Accessories at Pittcon 2012, Part II

Part II of our yearly report on new products introduced at Pittcon. This month, gas chromatography columns, thin-layer chromatography plates, sample preparation products, as well as chromatography and sample preparation accessories (including small benchtop instruments) are covered.

Ronald E. Majors
Column Watch Editor

Pittcon 2012 (more formally known as the 63rd Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy) returned to the Orange County Convention Center in Orlando, Florida, March 11–15, 2012, after a one year absence. This year's event hosted 948 instrument manufacturers and laboratory suppliers in 1854 booths. In addition to attending the exposition, conferees listened to more than 2000 technical presentations (orals, posters, workshops, invited and contributed talks, and award symposia), checked on numerous company seminar rooms, or attended one of 95 short courses.

Although the average attendance has been shrinking over the last several years, undoubtedly, Pittcon still remains the most important yearly international analytical exhibition where companies introduce their latest instruments, instrument accessories, software, columns, sample preparation, and other consumable products. Because many past attendees have purchased one or more new products within three months after attending the show, most exhibitors attempt to maximize their booth traffic to meet as many potential customers as possible.

The purpose of this report is to provide information about many of the new separation consumables and accessory products that were displayed at Pittcon 2012. In some cases, products that were introduced during 2011 but after Pittcon 2011 (1,2) may be included for reasons of completeness. The information is based on manufacturers' responses to a questionnaire mailed in early 2012. Because of space limitations and the fact that some manufacturers did not respond to the questionnaire, this report cannot be considered an exhaustive listing of all new products that were introduced since last year's event in Atlanta. However, over the years, these Pittcon introduction summaries have provided a good source of information that would be difficult for one individual to gather during the four days of the exhibition. In addition, the products introduced have shown definite correlations to current research, development, and application activity in the separation sciences.

As in previous years, products recommended by their manufacturers primarily for biomolecule and biofluids sample preparation are denoted in the tables with the designation *BIO*. Some of these products may be used for general sample prep as well, but their main emphasis is for biological samples.

In this month's coverage, I will look at gas chromatography (GC) columns, thin-layer chromatography (TLC), sample preparation products and hardware, accessories, and small tabletop instruments, mainly for sample preparation.

Gas Chromatography Columns

Although GC is considered a relatively mature separation technique, new GC columns continue to be introduced each year at Pittcon (Table I). This year, we saw the introduction of eight new GC columns: seven wall-coated open tubular (WCOT) columns and one porous layer open tubular (PLOT) column. Columns were introduced by six different vendors. In addition, Agilent Technologies introduced an entire line of J&W packed columns for laboratories still using this technology.

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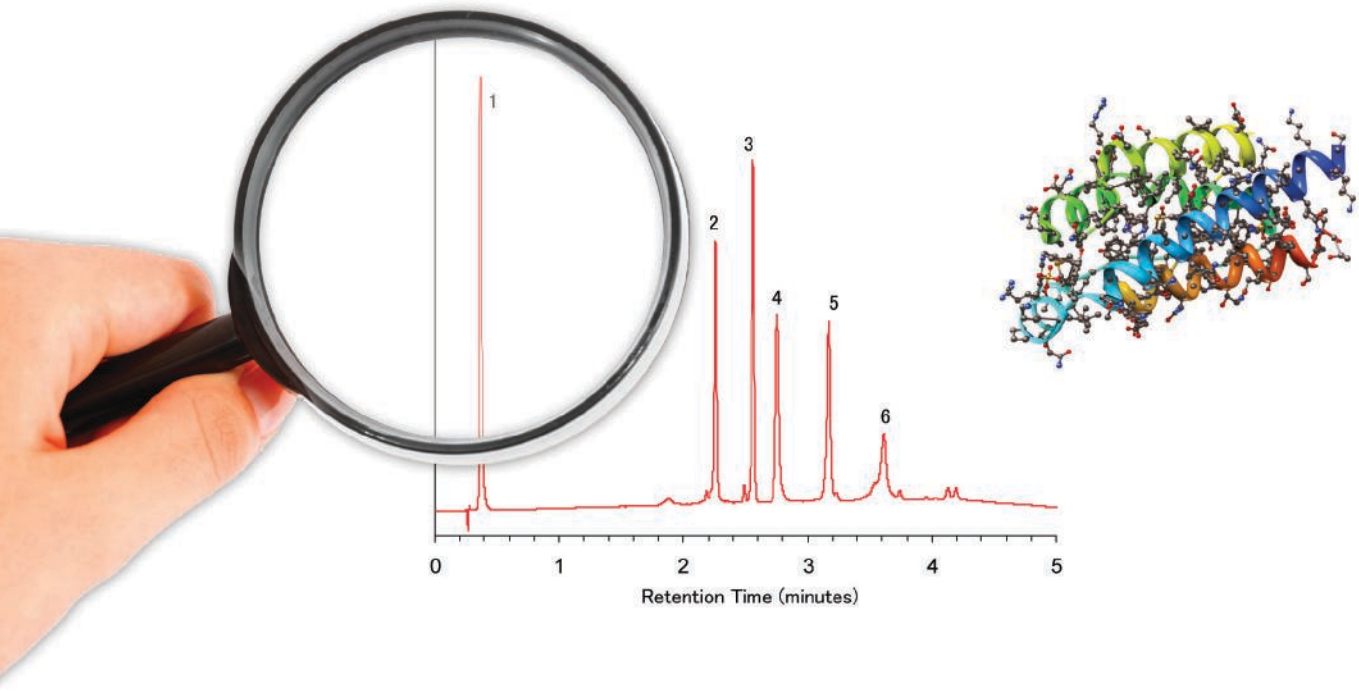

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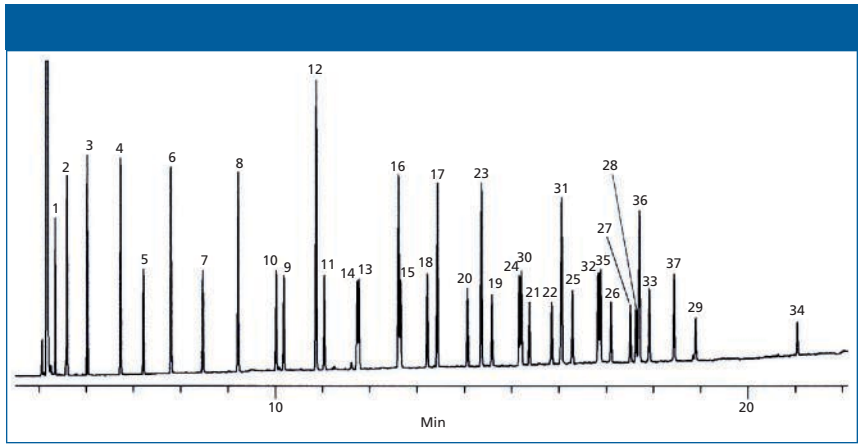

Figure 1: Separation of FAMEs on an ionic liquid stationary phase: Column: 100 m × 0.25 mm, 0.20 µm d_f Supelco SLB-IL111 GC; carrier gas: hydrogen at 40 cm/s; oven temperature program: 140 °C for 5 min, 140–180 °C at 8 °C/min, then 180–260 °C at 5 °C/min; injector temperature: 250 °C; detector: flame ionization, 260 °C; injection volume: 1 µL, 100:1 split; liner: 4 mm i.d., cup design; sample: Supelco 37-component FAME mix, analytes at concentrations indicated in methylene chloride. Peaks: 1 = butyric acid methyl ester (C4:0) at 4 wt %, 2 = caproic acid methyl ester (C6:0) at 4 wt %, 3 = caprylic acid methyl ester (C8:0) at 4 wt %, 4 = capric acid methyl ester (C10:0) at 4 wt %, 5 = undecanoic acid methyl ester (C11:0) at 2 wt %, 6 = lauric acid methyl ester (C12:0) at 4 wt %, 7 = tridecanoic acid methyl ester (C13:0) at 2 wt %, 8 = myristic acid methyl ester (C14:0) at 4 wt %, 9 = myristoleic acid methyl ester (C14:1) at 2 wt %, 10 = pentadecanoic acid methyl ester (C15:0) at 2 wt %, 11 = *cis*-10-pentadecenoic acid methyl ester (C15:1) at 2 wt %, 12 = palmitic acid methyl ester (C16:0) at 6 wt %, 13 = palmitoleic acid methyl ester (C16:1) at 2 wt %, 14 = heptadecanoic acid methyl ester (C17:0) at 2 wt %, 15 = *cis*-10-heptadecenoic acid methyl ester (C17:1) at 2 wt %, 16 = stearic acid methyl ester (C18:0) at 4 wt %, 17 = oleic acid methyl ester (C18:1n9c) at 4 wt %, 18 = elaidic acid methyl ester (C18:1n9t) at 2 wt %, 19 = linoleic acid methyl ester (C18:2n6c) at 2 wt %, 20 = linolelaidic acid methyl ester (C18:2n6t) at 2 wt %, 21 = γ-linolenic acid methyl ester (C18:3n6) at 2 wt %, 22 = α-linolenic acid methyl ester (C18:3n3) at 2 wt %, 23 = arachidic acid methyl ester (C20:0) at 4 wt %, 24 = *cis*-11-eicosenoic acid methyl ester (C20:1n9) at 2 wt %, 25 = *cis*-11,14-eicosadienoic acid methyl ester (C20:2) at 2 wt %, 26 = *cis*-8,11,14-eicosatrienoic acid methyl ester (C20:3n6) at 2 wt %, 27 = *cis*-11,14,17-eicosatrienoic acid methyl ester (C20:3n3) at 2 wt %, 28 = arachidonic acid methyl ester (C20:4n6) at 2 wt %, 29 = *cis*-5,8,11,14,17-eicosapentaenoic acid methyl ester (C20:5n3) at 2 wt %, 30 = heneicosanoic acid methyl ester (C21:0) at 2 wt %, 31 = behenic acid methyl ester (C22:0) at 4 wt %, 32 = erucic acid methyl ester (C22:1n9) at 2 wt %, 33 = *cis*-13,16-docosadienoic acid methyl ester (C22:2) at 2 wt %, 34 = *cis*-4,7,10,13,16,19-docosahexaenoic acid methyl ester (C22:6n3) at 2 wt %, 35 = tricosanoic acid methyl ester (C23:0) at 2 wt %, 36 = lignoceric acid methyl ester (C24:0) at 4 wt %, 37 = nervonic acid methyl ester (C24:1n9) at 2 wt %. (Courtesy of Supelco/Sigma Aldrich.)

These columns are still accepted in many environmental and pharmaceutical applications.

Application-specific GC columns are always popular introductions at Pittcon. This year, a majority of the columns were for specific application areas. GC has found widespread usage in the petroleum industry. A single PLOT column was introduced by Restek for the trace-level analysis of volatiles in hydrocarbon streams, particularly for downstream products such as ethylene, propylene, the butylenes, and butadiene. Supelco/Sigma Aldrich also introduced a hydrocarbon processing industry (HPI) column for the analysis of benzene and other aromatics in gasoline, but the proprietary ionic, very polar liquid stationary phase also can be used for fatty acid methyl ester (FAME) profiles in biodiesel and edible oils. Ionic liquids are a new breed of GC phases that have higher temperature limits than typical coated or bonded GC phases. To illustrate the capability of this new type of phase, Figure 1 shows the separation of a standard 37-component FAME mix (Supelco/Sigma Aldrich) containing methyl esters of fatty acids ranging from C_4 to C_{24}, including key monounsaturated and polyunsaturated fatty acids. Although not shown, Supelco's standard column for FAME analysis has been a cyanopropyl siloxane phase (SP-2560) and it was compared with the SLB-IL 111 ionic liquid phase column. The newer SLB-IL 111 column gave a slightly better signal-to-noise ratio, had a reduction in analysis time because of weaker dispersive interactions, and showed stronger dipole-induced dipole interactions resulting in increased retention of polarizable analytes (those with double bonds) relative to nonpolarizable analytes. The higher the degree of unsaturation, the stronger the retention; thus, an alternative selectivity is noted with the SLB-IL 111 column relative to the SP-2560 cyanopropyl siloxane column.

The workhorse column in many GC and gas chromatography–mass spectrometry (GC–MS) laboratories is the 5% phenyl–95% polysiloxane phase. Three companies introduced a 5% phenyl column, but one of them has a polysilphenlene siloxane backbone that is said to increase the thermal stability and decrease column bleed. Also, Thermo Fisher Scientific's version was available in stainless steel metal configurations.

Thin-Layer Chromatography

TLC (and high performance TLC [HPTLC]) is a simple technique that many chromatographers overlook, even when they only need a cursory separation or purity check. Macherey-Nagel is one of the few companies that produces thin-layer products. They have expanded their Alugram Xtra line of TLC and HPTLC products to a wide variety of formats. The base product is unmodified standard silica layers on aluminum. The silica gel has a specific surface area of 500 m^2/g, a mean pore size of 60 Å, and a specific pore volume of 0.75 mL/g. The particle size is 5–17 µm. The binder holding the silica to the aluminum plate is a highly polymeric product so that the plate can be cut easily without flaking the silica. The layer is stable in almost all organic solvents including pure aqueous eluents. The HPTLC plate has a smaller particle size of 2–10 µm, which gives sharper separations, shorter developing times, and increased detection sensitivity compared to the regular TLC

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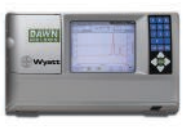

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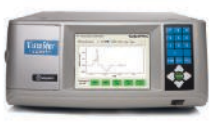

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Table I: Gas chromatography columns

Product Name	Supplier	Column Type	Phase	Film Thickness (µm)	Internal Diameter (mm)	
Optima WAXplus	Macherey-Nagel	WCOT	PEG with optimized crosslinking	0.25–0.50	0.25, 0.32	
BP5MS	SGE Analytical Science	WCOT	5% phenyl–95% polysil-phenlene-siloxane	0.25	0.15, 0.18, 0.25, 0.32	
J&W packed GC columns	Agilent Technologies	Packed columns	Wide range of coated and solid sorbents	Not applicable	1, 2	
Nano Stationary Phase NSP-5	J & K Scientific	WCOT	5% phenyl–95% methylpolysiloxane	0.1–2	0.10–0.25	
Rt-Alumina Bond/MAPD PLOT	Restek	PLOT	Alumina	5, 10	0.32, 0.53	
Rtx-BAC Plus1 and Plus2 column set	Restek	WCOT	Proprietary	0.6, 1.0, 1.8, 3.0 (phase dependent)	0.32, 0.53	
SLB-IL111	Supelco/Sigma Aldrich	WCOT	Proprietary bonded ionic liquid	0.08, 0.20	0.10, 0.25	
TraceGOLD GC column with SafeGuard	Thermo Fisher Scientific	WCOT	Not applicable	Not applicable	Not applicable	
TraceGOLD TG MT Metal	Thermo Fisher Scientific	WCOT	See comments	0.10–3.00	0.25, 0.53	

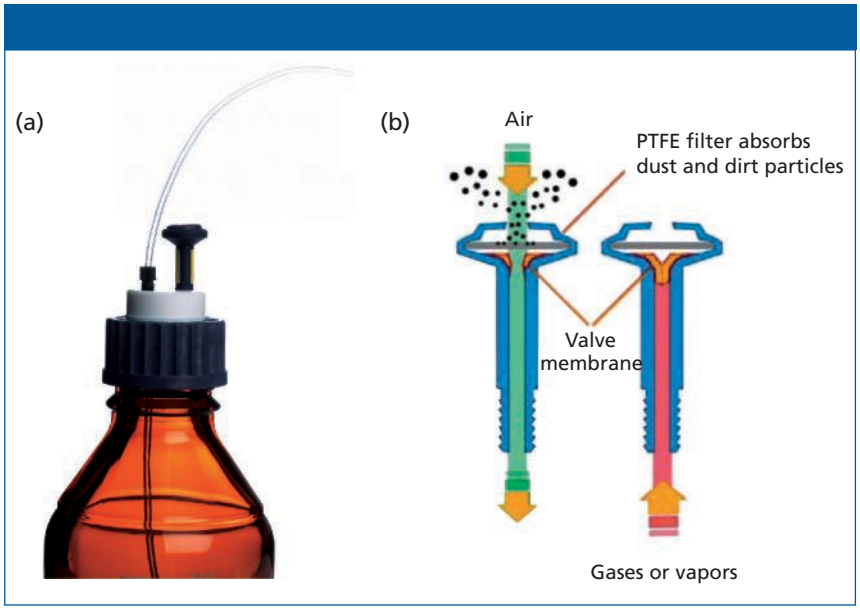

Figure 2: Solvent safety caps for HPLC reservoir bottles: (a) solvent safety cap, (b) schematic showing operation of safety cap. (Courtesy of Agilent Technologies.)

plates. The plates have an indicator of manganese-activated zinc silicate with green fluorescence of shortwave UV (254 nm). Special plates are available with a concentrating zone.

Sample Preparation Products

As indicated in Table II, Pittcon 2012 was another productive year with 19 new sample preparation products or families of products displayed. Not included in that count were sample preparation accessories (Table III), which will be covered later. The most popular application areas include various pollutants in environmental samples, drugs and drug metabolites in biological fluids, and toxins in food. Formats included cartridges, 96-well plates, and spin columns for use with a centrifuge as the driving force.

Many of the products were for solid-phase extraction (SPE) and just about every mode (reversed phase, strong or weak anion and cation exchange, adsorption and normal phase, partition, and mixed mode) was represented. A wide variety of media was introduced at Pittcon including polymer-based, graphitized carbon black, silica-based bonded phases, bare silica, alumina, titania, and diatomaceous earth.

The majority of the SPE products introduced at Pittcon 2012 were polymer-based.

Polymeric SPE products are particularly attractive because they can dry out

	Column Length (m)	Comments
	30–60	Improved temperature stability compared to conventional PEG phases; temperature limits: 260–270 °C; chemically bonded and solvent-rinsable; aqueous samples are tolerated.
	Not specified	General purpose column for GC–MS; upper temperature limits: 330–350 °C; phase ratio: 250; optimized silphenylene content compares to the company's range of 5% phenyl capillary columns; low bleed, increased inertness.
	Various	Wide variety of packed GC phases packed into stainless steel, UltiMetal, nickel, glass, copper, and PTFE; typical phases include Carbosieve, Carbowax, Chromosorb, HayeSep, MolSieve, Porapaks, and silica gel; fits most gas chromatographs, both standard and custom columns available.
	Not specified	Nano stationary phase has an upper temperature limit of 360 °C; shows low bleed at the retention times of heavier compounds; shorter columns can be used; columns show increased selectivity compared to conventional 5% phenyl.
	30, 50	Columns are made specifically for the analysis of petrochemicals and downstream products such as ethylene, propylene, butylenes, and butadiene; recommended for trace levels of polar hydrocarbons; available in fused-silica or metal configuration; temperature limit: 250 °C; columns show high capacity; specially tested.
	30	Recommended for blood alcohol analysis; optimized column selectivity; 2-min analysis; also available with two new resolution control standards depending on which internal standard an analyst works with. BIO
	15, 30, 60, 100	Very high polarity column with 270 °C upper temperature limit; phase less susceptible to damage from moisture or oxygen in extracts and carrier gas; suggested applications: benzene and other aromatics in gasoline, FAME profile of blended biodiesel, *cis–trans* FAME isomers, edible oil FAME fingerprinting, and GC×GC.
	Not applicable	SafeGuard is an integrated guard column used to extend the life of a GC column; can be trimmed as needed with little to no impact on performance.
	15, 30, 60	TG-1MT = 100% dimethylpolysiloxane; TG-5MT = 5% phenyl–95% methylpolysiloxane; TG-WAX MT = polyethyleneglycol; upper temperature limit: 430 °C for TG-1MT and TG-5M and 260 °C for TG-WAX MT; columns are made of stainless steel construction.

during the conditioning steps without affecting the recovery and reproducibility of the SPE experiment. Also, they can be treated with more aggressive washing solvents than silica-based sorbents because their pH range is greater. Generic methods are often used, which makes method development simpler. Another advantage of the polymeric SPE sorbents is that they usually display mixed mechanisms and multiple interactions can be used to selectively retain analytes or matrix compounds. Because the surface area of polymers is higher than that of silica-based bonded phases, a smaller amount is used in cartridges and well plates. Thus, smaller amounts of sample and solvents are required and extraction times and evaporation times are faster.

For the third year in a row, additional products for QuEChERS were introduced. Pronounced "catchers," QuEChERS is an acronym for quick, easy, cheap, effective, rugged, and safe sample preparation. This method is an emerging technique that has become increasingly popular in the area of multiresidue pesticide analysis in food and agricultural products. In QuEChERS, food and agricultural samples are first extracted with an aqueous miscible solvent (for example, acetonitrile) in the presence of high amounts of salts (such as sodium chloride and magnesium sulfate) or buffering agents (such as citrate) to induce liquid-phase separation and stabilize acid- and base-labile pesticides, respectively. Upon shaking and centrifugation, an aliquot of the organic phase is subjected to further cleanup using SPE. Unlike traditional methods using SPE tubes, dispersive SPE (d-SPE) cleanup is conducted by mixing bulk amounts of SPE sorbent and additional $MgSO_4$ with the extract. After sample cleanup, the mixture is centrifuged and the resulting supernatant can either be analyzed directly or subjected to minor further treatment before analysis. Two new QuEChERS product offerings were submitted for inclusion in this article (see Table II). For those interested, a brief introduction to the technique was published in earlier installments of "Sample Prep Perspectives" (4,5).

The technique of dried blood spotting (DBS) has been drawing interest in the pharmaceutical industry for the sampling, storage, and shipping of blood samples for the analysis of drugs and metabolites (6). Basically, small volumes (~15 µL) of blood are spotted on a dried matrix card, the liquid is allowed to evaporate, spots are punched out of the media, and analytes of interest are eluted with solvent and either treated further or directly analyzed by liquid chromatography–tandem mass spectrometry (LC–MS-MS). Because more biofluids (for example, plasma, urine, and cerebrospinal fluid) than blood can be handled with DBS cards, it has been suggested that the technique be referred to as *dried matrix spotting* (DMS). The cards are deemed safe as not being a biological hazard when

Table II: Sample preparation products

Product Name	Supplier	Product Type	Mode	Base Material	Functional Group	
Asset Dry Sampler	Supelco/Sigma Aldrich	SPE	Chemical reaction	DBA-coated glass fiber	Not specified	
Bond Elut Dried Matrix Spotting	Agilent Technologies	Biofluid collection and transport	Sorption	Noncellulose paper	Not applicable	
Bond Elut Plexa PAX	Agilent Technologies	SPE	Anion exchange	Hydrophilic polystyrene–divinylbenzene (PS-DVB)	Strong anion exchange	
Celerity Deluxe	Orochem Technologies	SPE 96-well plate	Reversed phase	Polymeric	Not specified	
Clean Screen FASt	UCT	SPE	Unspecified	Proprietary polymeric resin	Not specified	
Cleanert FAT	Bonna-Agela Technologies	SPE	Adsorption	Silica and alumina	Silica and alumina	
Cleanert PEP-2	Bonna-Agela Technologies	SPE	Hydrophobic-lipophilic, weak anion exchange	PS-DVB–pyrrolidone	PS-DVB–pyrrolidone	
Cleanert SLE	Bonna-Agela Technologies	SLE	Partition	Diatomaceous earth	Aluminosilicate	
Enviro-Clean 523	UCT	SPE	Adsorption	Graphitized carbon black (GCB)	Carbon	
Enviro-Clean PAH 50-mL centrifuge tube	UCT	QuEChERS	Adsorption or partition	Polypropylene	Not applicable	
Indicating FTA DMPK cards	GE Healthcare Life Sciences	Biofluid collection & storage	Dried biofluid collection	Cellulose	Not applicable	
InnovSep-Bulk 250, SAM-C18 SPE	Separation Methods Technologiess	SPE/flash chromatography	Reversed-phase	Silica	C18	
Phosphopeptide enrichment and cleanup Kit	Thermo Fisher Scientific	SPE columns	Affinity, adsoprtion	Titania, graphitic carbon	TiO_2, C	
Sagacity HL PDVB	Orochem Technologies	SPE	Reversed phase	Hydrophilic polydivinylbenzene (PDVB)	Not specified	
SiliaPrep HLB Polymeric	SiliCycle	SPE	Reversed phase	DVB-proprietary hydrophilic copolymer	Hydrophobic-Lipophilic	
SiliaPrep Ion Exchange Polymeric	SiliCycle	SPE	Cation and anion	PS-DVB	Strong cation and anion exchange, weak cation and anion exchange	

	Dimensions	Comments
	3-mL denuder + 13-mm cassette	A cartridge containing a di-*n*-butylamine (DBA)-impregnated glass fiber filter is used to collect and measure total aliphatic isocyanates in air. DBA reacts with aromatic and aliphatic isocyanates to form a stable urea derivative that is eluted and analyzed by LC–MS-MS or LC–MS. Attomole levels can be measured.
	Standard DBS card format	A dried matrix (blood) spotting card for collection and transport of biological fluids. Dried blood samples are considered nonhazardous. After drying, spots can be punched out and drugs, drug metabolites, or other compounds can be analyzed by LC–MS-MS or GC–MS-MS. The cards can be shipped and more easily handled than blood samples and also can be used for storage. The easily punched, noncellulose paper reduces nonspecific binding and improves MS analyte response. BIO
	Cartridges: 30, 60, 200, and 500 mg/1, 3, and 6 mL; 96-well plates: 10 or 30 mg/1- or 2-mL wells	A mixed-mode polymeric amide-free phase containing nonpolar and anion-exchange functionality. Recommended for extraction of acidic compounds in the pharmaceutical, clinical or toxicology, forensic, and environmental laboratories. It excludes endogenous interferences to minimize ion suppression and sample cleanliness; particle size: 45 µm; surface area: 550 m^2/g; both round and square well in 96-well plate configuration.
	96-well plate: 2 mg/0.3-mL well	Sample loading volume less than 100 µL; uses only 100–200 µL of conditioning and wash solvents and 100 µL of elution solvent. Recommended for preclinical screening and blood testing in which small volumes of biological fluids are required; particle size: 25 µm; pore size: 100 Å.
	Cartridge and 96-well plate	Recommended for "dilute-and-shoot" rapid sample preparation by removing particulates and matrix from urine samples for LC–MS analysis and for the isolation of drugs (acidic, basic, or neutral), opiates, and benzodiazepines. Sample can be collected directly into an autosampler.
	Cartridge: 3 g/6 mL	Dual adsorbents designed for elimination of lipids from samples. Small-pore silica (30-Å pore size) eliminates lipids by size and alumina (60-Å pore size) retains smaller molecules with stronger polarity than lipids. Avoids complicated manipulation of saponification and gel permeation chromatography for elimination of lipids.
	Cartridge: 60 mg/3 mL; 96-well plate	Recommended for extraction of polar compounds in aqueous samples; better than C18 or PS-DVB alone; particle size of sorbent: 40–60 µm; pore size: 60 Å.
	Cartridge; 96-well plate	Supported liquid extraction (SLE); replacement technique for liquid–liquid extraction. Recommended for extraction of drugs or metabolites from plasma. Aqueous sample is deposited over diatomaceous earth surface and organic solvent percoaled through for on-line extraction; little adsorption of analyte on diatomaceuous earth; particle size: 80–150 mesh; pore size: >1000 Å. BIO
	Cartridge: 250 mg/6 mL	SPE GCB cartridge for EPA Method 523 (Determination of Triazine Pesticides and Their Degradates in Drinking Water).
	50-mL centrifuge tube	A centrifuge tube with no reportable PAHs; prevents contamination with PAHs when using tube for QuEChERS; has plug seal cap to prevent leakage.
	Standard DBS card format	Effective collection of biofluids such as plasma, urine, and cerebrospinal fluid; enables room temperature storage of 15–20 µL samples; also used for storage; no need for dry ice and freeze-thaw cycle; more cost effective than traditional methods. BIO
	Not specified	Packing material (45–74 µm) that has C18 group immobilized on the silica base material using the technique of self-assembled monolayer (SAM) chemistry resulting in high stability. The phase is stable from pH 1 to 12 and shows high capacity for bulk purifications. It will separate 1–15 g per run depending on column geometry; phase coverage: 16% carbon; pore size: 60 Å.
	Not specified	Designed for improving MS analysis of phosphoproteins; consists of TiO_2 resin that has high affinity for phosphorylated compounds; kits will process 24 samples; kit also contains graphite spin columns for further cleanup of recovered phosphopeptides. BIO
	Cartridges: 30 mg/1 mL; 60 mg/3 mL; 96-well plate: 30 mg/3 mL	Although a wide range of chemicals can be extracted, the product is especially recommended for hydrophobic drugs and hydrophilic metabolites in plasma samples and environmental samples; particle size: 25 µm; pore size: 100 Å. BIO
	Cartridge: 30–500 mg/1–6 mL; 96-well plate	Water-wettable copolymer material with hydrophilic–lipophilic balance (HLB) that permits strong retention of neutral, acidic, and basic drugs; high stability in organic solvents; 40-µm particles with a 110-Å pore size; custom formats available. Recommended for drugs or metabolites in biofluids and active pharmaceutical ingredients in tablets or creams. Also can be used for a wide variety of applications in environmental and food and beverage analysis. BIO
	Cartridge: 30–500 mg/1–6 mL; 96-well plates: 10–60 mg/2-mL wells	Functionalized copolymer with strong anion exchanger, strong cation exchanger, weak anion exchanger, and weak cation exchanger. Recommended for acids (pK_a 2–8), bases (pK_a 2–10), strong acids ($pK_a < 2$), and strong bases ($pK_a > 10$), respectively. All resins are highly stable in organic solvents; particle size: 85 µm; pore size: 60 Å. Sample types include biofluids and tissues, food and beverages, and environmental. BIO

Table II: Sample preparation products (continued)

Product Name	Supplier	Product Type	Mode(s)	Base Material	Functional Group	
SOLA	Thermo Fisher Scientific	SPE	Neutral and cation exchange or anion exchange	Polymer	Unspecified	
Strata X Drug N	Phenomenex	SPE	Reversed phase	Proprietary polymer	Proprietary hydrophobic	
Supel QuE Z-Sep/C18	Supelco/Sigma Aldrich	QuEChERS	Adsorption and partition	Silica	C18 and zirconia	

Figure 3: eVol-MEPS setup for microvolume solid-phase extraction. (Courtesy of SGE Analytical.)

blood and body fluids are dried. In addition, analytes are stabilized on the card when dried. The technique saves all the safety and logistics problems of storing and shipping whole blood samples. Products for DBS or DMS were shown at Pittcon, this time including new card formats (Table II) and an instrument that will desorb analytes on-line (Table III).

The supported liquid extraction (SLE) technique uses small particles of diatomaceous earth packed into a cartridge. An aqueous sample (usually buffered) is applied to the packed cartridge and it distributes itself by capillary action over the sorbent. The high surface area of the sorbent ensures that the sample is finely dispersed. Next, an immiscible organic liquid is percolated through the cartridge and comes in intimate contact with the aqueous sample. A liquid–liquid extraction is performed, just as might occur in a separatory funnel using the two immiscible phases. Because vigorous shaking is not required, there is no chance of an emulsion forming. The organic liquid containing the analyte or analytes of interest is recovered from the cartridge outlet. The analytes can be obtained by evaporation. The entire process is very reminiscent of conventional liquid–liquid extraction, but is much simpler and is performed entirely on-line. New formats of SLE were introduced at the exhibition.

Chromatography and Sample Preparation Accessories

Table III lists 42 new hardware products and accessories for HPLC, GC, capillary electrophoresis (CE), and sample preparation that were introduced at Pittcon 2012. This year, I have extended the columns and sample preparation accessories to include small, benchtop dedicated instruments that are designated to automate sample preparation techniques. All of the products introduced were practical devices designed to make a chromatographer's life easier. I will cover each area individually.

General chromatography products: Several products that are useful for both HPLC or ultrahigh-pressure liquid chromatography (UHPLC) and GC (coupled to MS and MS-MS) were shown this year. Syringe filter configurations are used mostly in LC and occasionally in GC applications and three companies introduced new families of these devices. Thermo Fisher Scientific's Titan3, Target2, and Sterlin syringe filters have various membranes, porosities, and diameters to meet most needs. The Pall syringe filters are ultraclean and are recommended for LC–MS applications. The SGE Analytical syringes are also ultraclean for LC–MS and GC–MS applications. An interesting twist is that they have application-specific needles to cut down on analyte interactions, reduce possible sample loss, and minimize carryover.

In this category, 96-well filtration plates are mostly used for HPLC or UHPLC applications but serve the same purpose as the syringe filters by removing particulates from samples. Filtration plates and spin columns now have established a place in the sample preparation laboratory. Many pharmaceutical companies now use protein crashing rather than SPE for the analysis of drugs and their metabolites in biological fluids, mostly using LC–MS-MS. Acetonitrile or methanol is added to diluted plasma and protein is precipitated, usually in a bead form. The supernatant is filtered and analyzed by LC–MS-MS. Because this type of testing is usually performed on many samples, 96-well filtration plates are the preferred format. Spin columns also perform rapid mechanical filtration, chemical filtration (by the use of adsorbents or molecular weight cut-off membranes), and desalting for small volumes of sample and are convenient when only a few samples need to be prepared. Several companies displayed new spin column formats and 96-well filtration plates.

	Dimensions	Comments
	Cartridge: 10 mg/1 mL; 96-well plate: 10 mg/2 mL	Based on a polymeric phase and mixed-mode cation and anion exchange; first fritless SPE products; cleaner extracts with reduced solvent requirements.
	Cartridge: 10–100 mg/1-, 3-, 6-, and 12-mL tubes; 96-well plates	Product is designed for extraction of neutral drugs of abuse (for example, benzodiazepines and barbiturates) in biological fluids. Eliminates the conditioning or equilibration step. Quality control tested on actual drugs of abuse from urine. BIO
	70 mg total packing	Recommended for extraction of pesticides from fruits and vegetables. Consists of 50 mg of Discovery DSC-18 and 20 mg of Z-Sep (zirconia coated on silica gel) placed in centrifuge tube. Improves sample cleanup of complex matrices by removing 30% more fat or hydrophobic compounds and color or green pigments than traditional sorbents for QuEChERS LC–MS methods. Replaces C18 and PSA phases in current methods.

Figure 4: Dried blood spot pneumatic card punch. (Courtesy of Analytical Sales and Services.)

HPLC, UHPLC, LC–MS, and ion chromatography products: The advent of UHPLC has spurred a number of ultrahigh pressure products. This year, two companies introduced reusable high-pressure fittings that will go as high as 27,000 psi. A high-pressure injection valve designed for pressures as high as 20,000 psi was shown. Guard columns are always a good idea and two companies introduced high-pressure guard columns, both rated to 20,000 psi, one of which can be vented using a switching valve. The popular sub-2-µm UHPLC columns are particularly susceptible to plugging because of the small porosity frits, so guard columns or in-line filters are almost a must. A safety bottle cap that prevents loss of volatile solvents from solvent reservoir bottles, yet allows air to enter to prevent pump cavitation is a simple but practical device (Figure 2). For anybody who has used trifluoroacetic acid in his or her mobile phase only to find retention times changing because of trifluoroacetic acid evaporation will appreciate such devices.

LC–MS-MS systems often use a lot of nitrogen in the 10 L/min range and higher. It is highly impractical to use nitrogen cylinders to provide these high flow rates. Two companies have come out with nitrogen generator systems for LC–MS-MS that generate flows as high as 60 L/min at pressures required for operation.

GC products: This year, only three product offerings strictly for GC were provided. An improved gas-tight syringe for headspace sampling was shown. The syringe features a spring-in plunger that made a tight seal against the glass barrel. Gas-tight connections are always a concern in GC flow systems. A new inert microconnector with low thermal mass for rapid temperature response was introduced. A line of ultra-inert inlet liners was extended by the introduction of a dimpled liner (for cold splitless injection) and a double-taper liner (vapor confined to inside of liner) as indicated in Table III.

Sample preparation products: Sample preparation usually is the most popular area for new accessories, and this year proved to be no exception, with some clever products presented at Pittcon 2012. Almost three-fourths of all new products covered in Table III fit into this area. Small benchtop instruments that automate some of the sample preparation steps can save time, eliminate tedium, and reduce errors. Here, I cover 11 of these benchtop instruments that range from workbench designs that perform multiple sample preparation procedures on small sample volumes to a single task of solvent evaporation. Several of the instruments are capable of automating at least part of the SPE experiment. Two instruments are capable of assisting in pressurized solvent extraction. Shaking centrifuge tubes in QuEChERS is a tedious job, and one instrument automates this process.

The combination of a precision delivery pipetting device and a miniaturized SPE needle is an interesting twist to the SPE experiment of small volumes. As depicted in Figure 3, the eVol-MEPS system from SGE combines the two devices to make a semiautomatic liquid handling–SPE device that performs all steps in the SPE experiment: conditioning, sample loading, washing, and elution. Because

Table III: Chromatography and sample preparation accessories

Product Name	Supplier	Application Area	Product Type	Suggested Applications	
7696A Sample Prep Workbench	Agilent Technologies	Sample preparation	Automated sample preparation instrument	General automated sample preparation	
Acrodisc MS syringe Filters	Pall	Sample preparation	Filtration	High sensitivity applications	
Airion-3 Portable Dynamic air sampler	Torion Technologies	Sample collection	Air sampler	Collect gas samples in field	
Captiva ND non-drip plate	Agilent Technologies	96-well filtration plate	Protein precipitation or filtration	Automated filtration of biofluids	
Centrifan PE personal evaporator/condenser	Modular SFC	Sample preparation	Centrifugal evaporator	Solvent evaporation	
Centurion RSK50	EST Analytical	Sample preparation	Sample handling or headspace	Sample preparation for GC and GC–MS	
Cheminert C72 UHPLC injector	Valco Instruments	HPLC or UHPLC	Injection valve	High-pressure liquid injection	
DBS-MS 500 system	Camag Scientific	Sample preparation	DBS card handling automation	Interface to MS system	
Diamond MS syringes	SGE Analytical Science	LC–MS and GC–MS	Syringe	Trace-level liquid sampling	
Digital pulse mixer for QuEChERS	Glas-Col	Sample preparation	Mixer for centrifuge tubes	QuEChERS mixing	
Dried blood spot pneumatic card punch	Analytical Sales and Service	Sample preparation	DBS card spot punch	Removes dried spot from DBS card	
e-Vol MEPS	SGE Analytical Science	Sample preparation	Liquid handling plus SPE	Semiautomated sample cleanup	
eVol XR	SGE Analytical Science	Sample preparation	Liquid handling	Liquid dispensing or sample preparation	
EXP Nano Vented Trap HR	Optimize Technologies	HPLC or UHPLC	Packed bed trap or guard column	In-line trapping and guard column	
EZ-2 Elite	Genevac	Sample preparation	Solvent evaporator	Concentration of solvent extracts	
FastPrep 96	MP Bio	Sample preparation	Sample preparation instrument	Automation of certain functions	
Flip-Flop system	Genevac	Sample preparation	Pressurized liquid extraction accessory	Collection or evaporation of ASE samples	
Freestyle	Pickering Laboratories	Sample preparation	Automated GPC or SPE System	Size-exclusion or SPE sample cleanup	
High Dynamic Plunger headspace syringe	Hamilton	GC	Headspace autosampler syringe	Headspace sampling	

	Comments
	Stand-alone instrument provides liquid handling (for example, dilution, aliquoting, reconstitution, reagent addtion, small volume dispensing, and preparation of calibration standards), spin-vortex mixing, liquid–liquid extraction, integrated bar-code reader, automated sample tracking, sample tray heating, and Peltier cooling. The system is based on standard 2-mL autosampler vials and features an intuitive "drag-and-drop" software interface and color-coded resource management screen.
	Certified for low extractables for LC–MS. The product tubes are packaged individually so those not in use remain sealed; new WWPTFE membrane in high density polypropylene housing.
	Designed to collect samples on solid-phase microextraction (SPME) syringes. It is a portable, battery-powered system featuring variable flow rates of 5–5000 mL/h and a diaphragm air pump. It is programmable and has an RS-232 communication port.
	Dual filter design plate is used for automated, high-throughput in-well filtration of precipitated plasma (crash). The non-drip membrane allows for solvent-first protein precipitation using methanol or acetonitrile. It resists flow until vacuum is applied. The filtrate is collected in a collection plate and all accessories are provided. BIO
	Self-generating blow-down technology without the use of a vacuum. Recommended for LC fractions, synthesis reactions, and toxic compounds. The temperature is programmable to 55 °C; programmable from minutes to hours; uses 20-, 30-, or 40-mL scintillation vials, Eppendorf tubes, 4-mL vials, or test tubes; captured gas recirculates in closed system; cold trap for recovering all evaporated solvent; toxic materials contained.
	Performs sample preparation and headspace analysis for RSK175 and other headspace applications. The entire process including removing sample from one vial, adding it to an empty vial, heating, and stirring in preparation for analysis via headpace extraction for delivery to the GC system an be completely automated for as many as 50 samples.
	Designed for pressures as high as 15,000 or 20,000 psi; new stator coating; manual or automatic control; optional microelectric actuator determines accurate positioning; computer control via RS-232 or RS-485 interface.
	Fully automated handling of as many as 500 dried blood spotting (DBS) cards; optical card positioning including bar-code reader; wash station to eliminate carryover, optional internal standard application on card before extraction; on-line solvent extraction with easy integration to LC–MS. BIO
	Ultraclean syringes and needles recommended for high-sensitivity applications; improved cleanliness compared to standard syringes. Application-specific needles include: low surface active needle, a hydrophobic-coated needle, and a hydrophilic-coated needle for reducing analyte interaction and sample carryover; both autosampler and manual syringes are available.
	Can be used for high- or low-speed pulse vortexing or mixing. It accommodates the popular 50-mL and 8-mL centrifuge tubes used in QuEChERS steps 1 and 2, respectively. Microprocessor-based control of DC brushless motors with speeds of 150–1500 rpm; built-in digital timer; user-selectable pulse profile; can be used for other applications such as volumetric flask, 96-well plates, test tube racks, combinatorial reaction blocks, microreactors, cryogenic tubes, and most any type of mixing application.
	This foot-controlled power punch will reportedly eliminate the fatigue from using a handheld manual card punch and increase the speed and efficiency when punching multispots on multiple cards into 96-well plates. Features include direct insertion of punched disk into plate or tube and two punch sizes: 3 and 6 mm. BIO
	eVol is a precision liquid handler (digitally-controlled) and MEPS is a miniaturized SPE packed (2–3 mg) syringe-needle–based device that interfaces to e-Vol for sample-limited cleanup. Overall, combination provides speed and volume control of each SPE step; SPE phases: C18, C8, C4, hydrophobic PS-DVB; particle sizes: 40–60 µm for silica-based and 50–100 µm for polymer-based packings.
	eVol XR is an expanded range liquid dispenser using a 1-mL syringe that allows volumes dispensed from 200 nL to 1 mL and covers the range typical of five different syringe capacities. It is useful for internal standard addition, serial dilution, microtitrations, and TLC spotting. It reportedly provides high precision and can work with organic, aqueous, and viscous liquids.
	An almost zero-dead-volume in-line device (between injection valve and column) for removing particulates, desalting, detergent removal, and other chemical contaminants. The holder includes auto-adjusting EXP nano fittings for plumbing into a system as well as a "flush kit" for removing especially dirty sample particulates from the inlet frit and protecting expensive capillary columns. Other features include the ability to be hand-tightened to 20,000 psi and vented trap and guard column in one package. Packings are available in cation-exchange, anion-exchange, normal-phase, and reversed-phase modes; and custom packings are also available. Bed volumes of 0.13–4 µL; cartridges can be changed without breaking fluid connections; also can be used in a switching valve.
	An automated benchtop unit designed for concentration, drying, and lyophilization of liquid extracts or HPLC collected fractions where organic solvent can be removed and small amounts of water lyophilized. It can handle solvents with boiling points up to 220 °C, including trifluoroacetic acid and dimethyl sulfoxide; cold trap for solvent recovery with controlled defrost and draining; compatible with acids including model for hydrochloric acid.
	Designed for high-throughput sample homogenization, grinding, and lysis, this product can process as many as 192 samples simultaneously in 2 × 96 deep well-plates. Also, it can accommodate various size tubes and flasks. Homogenization is performed at room or cryogenic temperature to preserve temperature-sensitive componds. FastPrep-24 system can handle as many as 48 samples.
	Interfaces to Thermo Fisher Scientific's Accelerated Solvent Extraction (ASE) system; samples are automatically collected directly from ASE into GC vial in a Genevac Rocket evaporator in which it is concentrated by evaporation; evaporator handles as many as 18 vials simultaneously.
	A fully automated system designed for gel permeation chromatography (GPC) sample cleanup but extendable to SPE; depending on racks, sample tubes and collection devices, as many as 180 samples can be handled; intuitive software navigation system; in SPE mode, 1-, 3-, and 6-mL cartridges can be accommodated; bar-code reader available; switching valve for multiple GPC columns.
	Designed to work with PAL Combi-xt GC headspace autosamplers. Features a spring-in plunger desiged for increased tightness and leak-free operation; spring keeps PTFE plunger tip against glass barrel, preventing gaps that can occur with GC temperature fluctuations; reported longer life over range of temperatures and temperature gradients.

Table III: Chromatography and sample preparation accessories (continued)

Product Name	Supplier	Application Area	Product Type	Suggested Applications	
High Performance Filter Plates	Porvair Sciences	Filtration plates	Protein precipitation or filtration	Automated filtration of biofluids	
Interlocked R.A.M. vial	J.G. Finneran	Sample preparation	Autosampler vial	General autosampler usage	
LC certified vials	Grace Davison Discovery Sciences	Sample preparation	Autosampler vial	High-sensitivity analysis	
Nitroflow 60 membrane nitrogen generator	Parker Balston	HPLC or UHPLC	Nitrogen generator	LC–MS nitrogen generation	
Nitrogen generator NM-3G	Peak Scientific	HPLC or UHPLC	Gas generator	LC–MS nitrogen generation	
Pierce Concentrators PES	Thermo Fisher Scientific	Sample preparation	Ultrafiltration spin columns	Purify biological samples	
PLE	Fluid Management Systems	Sample preparation	Pressurized liquid extractor	Extraction of solid materials	
Pulversette 19	Fritsch	Sample preparation	Universal cutting mill	Size reduction of solid samples	
RapidTrace Plus	Biotage	Sample preparation	Automated SPE workstation	Sample cleanup	
RSA autosampler vials	MicroSolv	Sample preparation	Reduced surface activity vial	High-sensitivity applications	
SecurityGuard ULTRA	Phenomenex	HPLC or UHPLC	Guard column	Protection of analytical column	
SilTite µ-Union	SGE Analytical Science	GC	Microconnector for capillary GC	Column coupling	
SmartPrep extractor	Horizon Technology	Sample preparation	Automated SPE system	Automation of cartridge-based SPE	
Solvent safety caps	Agilent Technologies	HPLC or UHPLC	Solvent bottle cap	HPLC solvent delivery	
SPE-Xpress	Environmental Express	Sample preparation	Automated SPE	Oil and grease analysis	
SpinColumns	Harvard Apparatus	Sample preparation	Chemical filtration	Purification of biosamples	
Sterilin syringe filters	Thermo Fisher Scientific	Sample preparation	Filtration	Sample filtration	
Titan3 and Target2 syringe filters	Thermo Fisher Scientific	General chromatography	Syringe filter	Sample filtration	
TruView certified vials	Waters	Sample preparation	Autosampler vial	High-sensitivity analysis	
UF spin columns	Thermo Fisher Scientific	Sample preparation	Ultrafiltration spin columns	Purify biological samples	
Ultra high pressure fitting	Analytical Sales and Service	HPLC or UHPLC	Fingertight fitting	Liquid line connections	
Ultra Inert (UI) liners	Agilent Technologies	Gas chromatography	Inlet liner	High-sensitivity GC applications	
VHP-320 fitting for UHPLC	Upchurch Scientific/IDEX	HPLC or UHPLC	High-pressure fitting	UHPLC connections	

	Comments
	Available in a wide range of formats (48-, 96-, and 384-well plates), well volumes (350–5000 µL), and filtration materials (glass fiber, nylon, PVDF, and polyethylene); optimized filter plates available for various applications. BIO
	Robotic arm machine (R.A.M.) featuring a 300-µL threaded vial (clear or amber) in which the vial and its insert are fused together, keeping the insert permanently in place. Reportedly eliminates contamination risk around the seam or space between the neck of the vial and the insert. It is designed for easy robotic arm grasping of vials and includes marking spot.
	Each vial is traceable during manufacturing in contaminant-free environment; designed to high tolerances; clear and amber, high-recovery version, vial inserts all available; HPLC batch-tested.
	Self-contained generator that products up to 60 L/min LC–MS grade nitrogen at pressures as high as 110 psig (equivalent to one cylinder of compressed gas every 2 h); integrated scroll compressor and N_2 membrane technologies; mobile unit with small footprint.
	Flow rates: 30 L/min at 6.9 bar pressure; temperature operating range: 5–30 °C; designed with backup compressor capacity; insulated compressor compartment for quiet operation; advanced alarms and self diagnostics.
	Designed for concentrating, desalting, or exchanging buffers of small-volume biological samples including proteins and nucleic acids; uses polyethersulfone (PES) membrane with molecular weight cutoffs of 3000, 10,000, 30,000, and 100,000; recommended for volumes 100–500 µL; can achieve 10–30-fold sample concentration in 10 min; BIO
	Instrument for automated pressurized solvent extraction of solid or liquid samples; extraction cell size: 5–250 mL; simultaneous extraction of as many as six samples; modular design; disposable extraction cartridges as cleanup columns.
	Recommended for comminution of soft-to-medium hard, tough-elastic, brittle, and fibrous materials; high cutting rotor speed (2800–3400 rpm); different cutting rotors available; easily opened and cleaned after use; minimum sample quantity: 20–30 mL; final fineness: 0.20–6 mm.
	Enhanced performance automated SPE tabletop instrument with larger volume samples (five position rack for 40-mL scintillation vials), delay start feature, modular design, positive pressure syringe displacement, eight solvent lines, segregated waste lines, compliance validation capabities, compatible with 1-, 3-, and 6-mL syringe-type SPE cartridges.
	Glass has no acidic surface silanols that can cause adsorption of basic compounds, change pH of sample solution, or hydrolyze some compounds; not coated or silanized; screw top, snap top, clear and amber, write-on, inserts are all available.
	Recommended for any sub-2-µm, UHPLC, or superficially porous particle columns; fits to any manufacturers' 2.1–4.6 mm i.d. columns; pressure rated to 20,000 psi; stationary phases: C18, C8, PFP, HILIC, and phenyl.
	Recommended to connect guard columns or GC×GC columns; connector is treated to ensure inertness and has low thermal mass for rapid tracking of GC temperature changes; low dead volume; fittings can be finger tightened for leak-free connections; constructed of stainless steel.
	Instrument with small footprint that uses standard SPE cartridges (1, 3, or 6 mL); performs all standard SPE steps; can process as many as 12 samples sequentially; automatically rinses sample container; fractionates up to four fractions per sample; PC-controlled (up to eight systems from one PC); can process 96-samples in batch mode; has reagent mixing station, in-line membrane filtration, liquid sensing, solid sample sip tubes, and two 12-port valves; intuitive graphical software interface.
	A safety cap, that fits all solvent bottles, designed to prevent solvent evaporation and composition change and possible chemical spills; provides optimal sealing with an integrated exhaust valve with a PTFE membrane providing pressurization during solvent delivery, allowing proper solvent flow to the LC pump; cap rotates freely, preventing tube twisting during bottle exchange.
	System built around EPA Method 1664 specifically for the hexane extraction and evaporation steps; uses fluid sensor to ensure that sample vessel is empty; runs multiple samples simultaneously on up to six stations; PC controlled.
	Recommended for rapid desalting, removing unbound markers, and the purification of proteins, peptides, and DNA; designed for small volumes of 10–150 µL; single column or 96- and 384-well formats; centrifugal force drives separation process; prefilled with various sorbents with hydrophobic, hydrophilic, ion exchange, adsorption, and size-exclusion media. BIO
	Available with pore sizes of 0.22 and 0.45 µm; membranes: PES, MCE, PVDF, or NY; gamma irradiated and certified as nonpyrogenic; individually wrapped to minimize contamination; filtration unit available with volumes of 150–1000 mL.
	Target2 are general purpose syringe filters with pore sizes of 0.1, 0.20, and 0.45 µm and diameters of 4, 17, and 30 mm; membranes: PTFE, nylon, PVDF, RC, CA, polypropylene, and PES; 30-mm glass microfiber membranes available with different porosities; Titan3 are 30-mm diameter with PTFE membranes only with 0.2- and 0.45-µm pores; all are pressure rated to 100 psi; enhanced design for improved flow-through characteristics.
	Low-adsorption sample vials certified for LC–MS-MS analysis where concentrations are nanograms or picograms per milliliter; manufacturing process limits concentration of free ions on glass surface, which limits analyte adsorption.
	For small volume (<200-µL) disposable and reusable versions to concentrate, separate, or purify samples in the ultrafiltration range using centrifugation as driving force; polysulfone membranes with molecular weight cutoffs of 10,000, 50,000, and 200,000; reusable spin column has separate holder. BIO
	Has floating ferrule that allows fitting to be used multiple times and enables easy removal from tube; designed to connect to 10–32 ports such as a 10-port valve; finger tight to 5000 psi and to 27,000 psi with wrench tightening.
	Extension of UI liner family; helps to ensure an inert GC flow path for higher sensitivity, accuracy, and reproducibility; UI dimpled liner is recommended for cold splitless injection with the multimode inlet, best for heavy matrix samples; the double-taper liner is recommended for those concerned about sample interaction in the inlet itself and confines vaporized sample inside the inlet, minimizing contact with the hot metal inlet weldment.
	Design allows the system to be used for as many as 10 assembly cycles, maintaining pressure rating with no carryover observed; one-piece concept where ferrule is permanently attached to the nut for simplified installation; PEEK polymer-blend ferrule ensures biocompatibility and prevents damage to tubing or ports; tools for achieving optimal torque are available.

the mass of SPE packing is only a few milligrams and the volumes are a few microliters, small-volume cleanup of liquid samples is possible.

In addition to the DBS cards introduced in Table II, two products for dried blood spotting were shown (Table III). Hand punching of dried blood spots is time consuming and tedious. One product shown at Pittcon was a foot-controlled card punch that will directly insert a punched disk into a 96-well plate (Figure 4); the second product is an automated system that will process as many as 500 DBS cards by providing on-line solvent extraction directly integrated with LC–MS.

Almost anyone who does chromatographic samples uses 2-mL sample vials. Earlier, irregularly shaped vials were often the culprit when shutdown occurred on automated overnight runs or when trace analytes with sensitive compounds were lost. Recently, certified vials that are tested to rigid standards and LC–MS vials with low levels of extractables and inert surfaces have been introduced. At this year's Pittcon, additional high quality and certified vials were introduced by four companies.

Acknowledgment

I would like to thank the manufacturers and distributors that kindly furnished the requested information in advance of Pittcon 2012, thus allowing a timely report on new product introductions. Manufacturers who would like to be considered for inclusion into Pittcon 2013 coverage should send the name of the primary company contact, the mailing address, phone number and e-mail address to Laura Bush, Editor, *LCGC North America*, lbush@advanstar.com, with the subject line, "Pittcon 2013 Column Watch."

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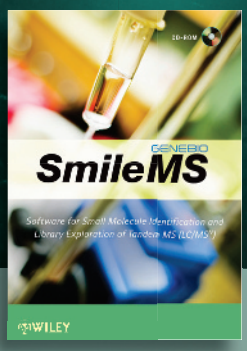

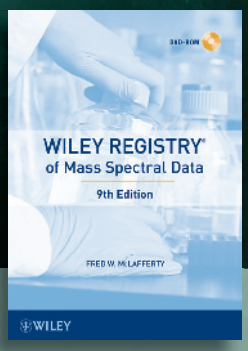

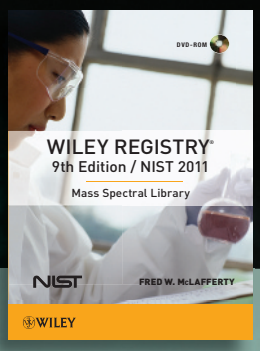

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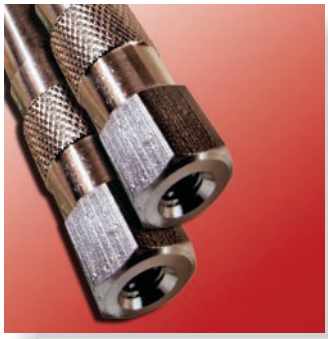

LC TROUBLESHOOTING

Extra Chromatographic Peaks — A Case Study

What do you do when an unexpected peak appears in the chromatogram?

John W. Dolan
LC Troubleshooting Editor

A common liquid chromatography (LC) problem that I get e-mails about from readers of this column relates to unexpected and unwanted peaks that appear in the chromatogram. This month's discussion centers around an e-mail discussion I had with a reader of the electronic version of *LCGC* (available on-line in countries where the paper editions are not available) about a problem they were experiencing. Because this problem relates to a pharmaceutical product and contains proprietary information, I've exercised some obfuscation privileges to hide some of the specifics, but retain the key information.

The problem relates to the analysis of an antibiotic for impurities. It is a fairly simple method, comprising a phosphate–acetonitrile gradient on a reversed-phase column. The method had been validated and worked quite well to determine a mixture of eight antibiotics and impurities. After successful use in the development laboratory, it was transferred to another laboratory for routine analysis. For identification, I'll refer to these as the R&D laboratory and the production laboratory.

Extra Peak

When the method was transferred to the production laboratory, it appeared to work well, with one exception — a broad peak appeared at a retention time that overlapped the elution of antibiotic 2. In Figure 1, I've shown a section of the baseline including peaks for antibiotics 1 (13.4 min) and 2 (15.6 min) from a chromatogram obtained in the R&D laboratory. This was a typical result for the injection of these two compounds. When the same sample was run in the production laboratory, the results of Figure 2a were observed. The peak for antibiotic 1 appears as normal, but there is a broad peak that all but obscures antibiotic 2.

An extra peak, that is not related to the sample itself, in a gradient run typically arises from one of three sources:

- late elution from a previous chromatogram
- carryover from a previous injection
- contamination of the mobile phase (ghost peaks).

Many people call all three kinds of peaks ghost peaks, but I like to distinguish between them because, although they may look similar in the chromatogram, their sources are different, and so the process of eliminating them will be different. Let's look briefly at my definitions of each of these peak types.

Late elution of a peak from a previous chromatogram results when you don't allow enough time for all the peaks to be eluted following sample injection. As a result, the problem peak continues to travel through the column, but shows up in a later chromatogram, instead of the one in which it belongs.

Carryover from a previous injection is caused by sample that is actually injected, but its injection is unintentional. For example, you make an injection and get a normal run, then you make a blank injection with no analyte present, but the peak appears anyway, usually much smaller than the original.

Ghost peaks tend to be unique to gradient elution, whereas late elution and carryover are common for isocratic separations, as well. Ghost peaks appear when a contaminant is present in the mobile phase (usually the weak, A-solvent), is concentrated on the column and then

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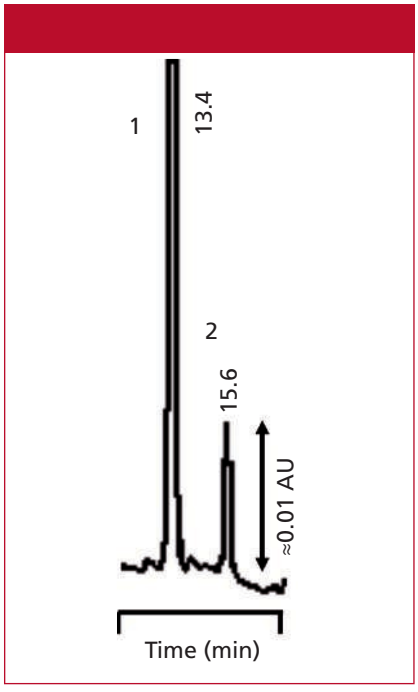

Figure 1: Antibiotics 1 and 2 in a chromatogram obtained from the R&D laboratory.

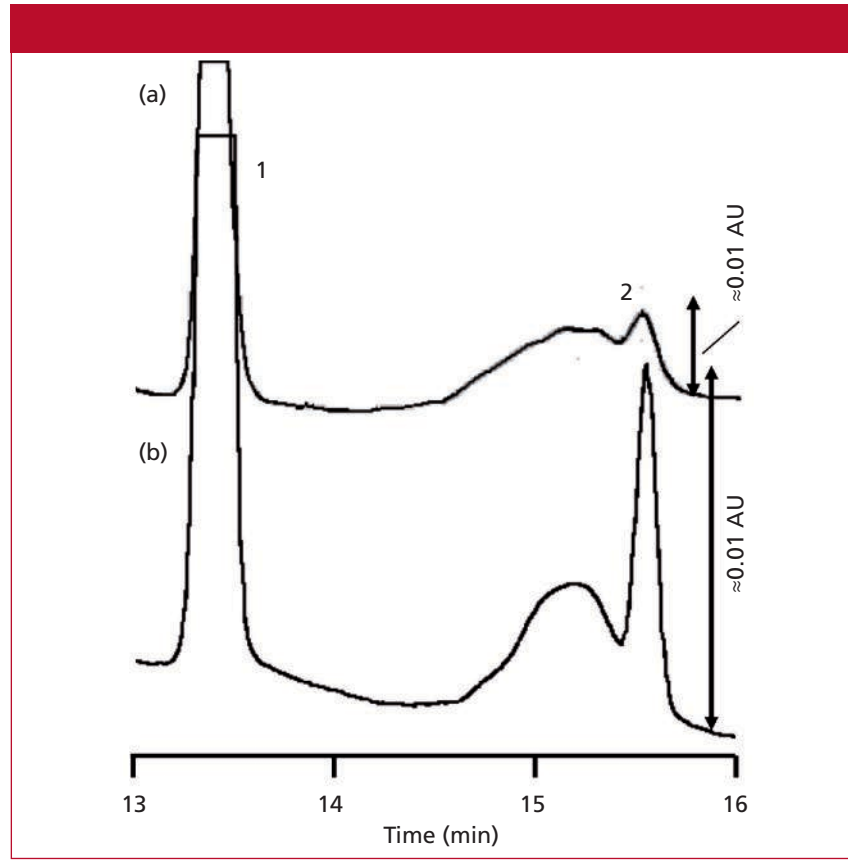

Figure 2: Antibiotics 1 and 2 in the production laboratory: (a) Before system cleaning with broad, extra peak at ≈15.2 min; (b) after acid-cleaning of the system, showing reduced response for extra peak.

is released as the gradient runs. Ghost peaks will appear in sample injections, when a blank is injected, and even when a gradient is run with no injection at all.

Do Your Homework

Troubleshooting can be a time-consuming process and unless you have a well-thought-out plan, a lot of time can be wasted and the problem source may be difficult to isolate. I like to make a list of the most probable causes, in this case the potential differences that exist between the two laboratories. Here are the most likely possibilities:

- mobile phases (both A and B)
- column
- instrument
- how the method is run.

I'm basically lazy, so I usually apply my "easy vs. powerful" rule of thumb at this point. That is, I'll often do an easy check first, even if it isn't as likely to isolate the problem as a more difficult one. Another practice that I take advantage of is module substitution — replacing a suspect part with a known good one. One of the ways we do this almost instinctively is to make up a new batch of mobile phase or replace the column. Both of these changes were made in the production laboratory, one at a time, which is a good idea if you aren't sure of the problem source. Neither a fresh batch of mobile phase nor a new column solved the problem.

My contact in the R&D laboratory developed the method on one brand of high performance liquid chromatography (HPLC) equipment, but the production laboratory was using another brand. Part of the question about instrument differences was answered easily when the R&D laboratory transferred their mobile phase and column to the second brand of equipment in the R&D laboratory — it worked just fine. This indicated that there wasn't an inherent instrument design difference that was causing the problem, although other problems, such as contamination could exist.

Now that the easy things are out of the way, it is time to roll up our sleeves and figure out where the problem lies. Let's look at each of the three peak sources mentioned above and work through them.

Carryover

Carryover has something to do with the equipment, usually the autosampler and not the column. A carryover peak is an actual sample peak, not something left on the column from a prior injection. Usually carryover is associated with insufficient flushing of the autosampler between injections, and generates a much smaller peak than normal. For example, after the injection of one sample, an injection of a blank is made and a peak appears at the same retention time as the sample peak, but much smaller. Another blank injection will usually be proportionally smaller than the second injection. Because carryover under normal circumstances is <0.1%, it is very rare to see the same peak appear after two blank injections (0.1% × 0.1% = 0.01%, probably too small to see). Sometimes a sample component can be adsorbed on surfaces of the autosampler tubing or injection valve and will bleed off slowly, resulting in carryover peaks that decline in size only slowly with successive injections, but this is not common for most samples.

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Once again, we can do some mental work, rather than physical, and eliminate this source of the current problem. The peak we observe has an area that has a different retention time, is much larger than our sample peak, and is much broader (note the peak width of the problem peak at 15.2 min compared to the analyte peak at 15.6 min in Figure 2b). If the problem was carryover, the presence of the carryover peak should make the normal analyte peak larger but should not change its area or retention time. So we can eliminate carryover from the probable causes.

If carryover was the problem, it usually can be corrected by one or both of two fixes. First, use a more thorough autosampler wash routine. Use the strong solvent of the mobile phase (acetonitrile in the present case) as the autosampler wash solvent and increase the number of wash cycles or wash volume between injections. If this does not solve the problem, look for poorly swept passages in the plumbing that might act as tiny reservoirs to trap sample that would then mix with the next injection. Check to be sure that all the tube fittings are seated properly. This is more common when PEEK (polyether ether ketone) fittings are used to make connections. If the system overpressures, the tubing can slip in these connections, but not enough to come completely loose, leaving a small gap at the tip of the tubing that can trap a small amount of sample. If the injection valve is worn, sometimes it will be less-well flushed and carryover can occur. This is fairly rare, because injector valves are made to cycle 100–500,000 times before a rebuild is necessary. However, if you have >50,000 injections on the valve and have eliminated other potential problem sources, replacement of the injector rotor might be a good idea.

Late Elution

Late eluted peaks usually are easy to isolate. As a general rule, whether the separation is isocratic or gradient, all the peaks in a given region of the chromatogram should be about the same peak width. If a peak appears that is much wider than its neighbors, the first thing I suspect is that it comes from a previous injection. An example of this is shown for the simulated chromatograms of Figure 3. In Figure 3a, the peak just after 2 min (arrow) is much wider than the neighboring peaks, so it is suspected as a late-eluted peak from a previous injection. In Figure 3b the run time is extended, and now there are two broad peaks, the early one from the previous run and the one at 7 min where it belongs for that injection.

Late-eluted peaks typically are sample-related — that is, they are sample components that don't have enough time to come off the column. Because the broad peaks were never seen in the R&D laboratory, and the same sample was being analyzed, it is not likely that the problem source is late elution. However, if the problem was isolated to this cause, there are two simple fixes. First, you could extend the run time to allow the peak to be eluted normally. This would work for the example of Figure 3, but would double the run time, which may not be desirable. Another alternative, especially if the problem peak is not of interest, is to flush it off the column with a strong solvent. When a gradient is used, as in the present problem, the column is

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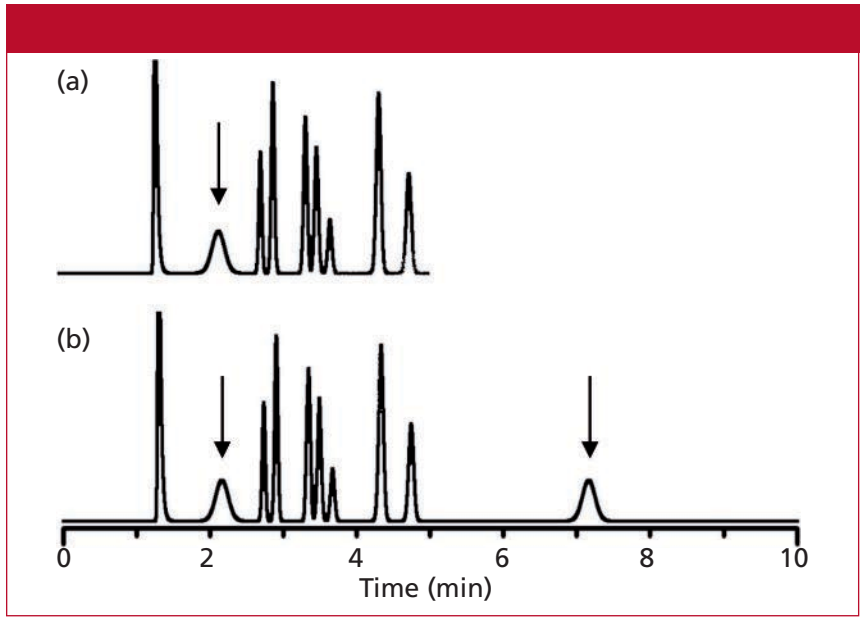

Figure 3: Simulated chromatograms showing problems because of late elution: (a) Broad peak (arrow) near narrow peaks in the chromatogram; (b) same as (a), but run time extended. Adapted from reference 1.

flushed easily by extending the gradient to 95–100% of the B-solvent (acetonitrile in this case). To speed the process, this can be done as a step. For example, if the normal gradient ran 5–70% B in 20 min, it could be followed by a 70–100% step, a 5-min hold to flush strongly retained material from the column, then a step back to 5% to re-equilibrate for the next injection.

Ghost Peaks

When gradients are run, any organic material that does not pass through the column under the starting conditions will build up at the head of the column and will be eluted later in the gradient. This is because at the starting conditions (5% acetonitrile in the present case), most organic compounds will have nearly infinite retention, so they just collect on the column as the mobile phase passes through. This is an easy problem to diagnose. Just perform the following sequence of runs. I'll assume for the moment that the normal equilibration between runs is 10 min.

1. Equilibrate normally (10 min)
2. Run a blank gradient (no injection)
3. Equilibrate 10 min
4. Blank gradient
5. Equilibrate 30 min
6. Blank gradient

After you've run this sequence, examine the chromatograms. Ignore the first gradient, because it rarely is equilibrated exactly the same as a normal run. Look at the size of the ghost peaks in the second run (10-min equilibration) and

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compare this to the same peaks in the third run (30-min equilibration). If the peaks in run 3 grow in the same proportion as the ratio of equilibration times between run 2 and run 3 (threefold in the present example), the source is probably the A-solvent. This is because the longer equilibration allowed three times as much junk to collect before it was eluted from the column.

When the A-solvent is the problem, each of the components should be checked separately by eliminating them in a stepwise fashion. For example, just use water for A instead of phosphate buffer. If the problem disappears, the phosphate is the probable cause, if not, it is the water. If the chromatogram is better without buffer, try a new bottle of phosphate. Some other possible sources of A-solvent contamination include dirty glassware, the pH meter, and contaminated water. Several of these possible sources are discussed in detail in chapter 17 of reference 1.

If the above tests seem to confirm the problem source, but careful elimination of mobile-phase components did not solve the problem, another, less common source is left: system contamination. Usually the HPLC system is exposed only to high-purity solvents and reagents, but contamination can occur if you don't practice good laboratory habits. This can be a particular problem when acetate or phosphate buffer are used, because they are good energy sources for microbial growth. If the mobile-phase reservoirs are not replaced with clean ones on a regular basis or if the system is left unattended with buffers standing in the tubing, contamination can occur when bacteria grow in the aqueous phase. Normally, replacement of the reservoir with each new batch of mobile phase and flushing with nonbuffered water or solvent at the end of each day's work are sufficient to keep the system clean. But if you don't regularly practice these preventive measures, any component that contacts the mobile phase can become contaminated, including the reservoir, inlet frits, tubing, degasser, pump, and autosampler. If you've eliminated the obvious causes, an aggressive cleaning may be in order.

Here's the recommended procedure for aggressive system flushing:

1. Remove the column, and replace it with a piece of tubing routed to waste.
2. Make a batch (for example, 0.5 L) of ≈30% phosphoric acid (just dilute concentrated phosphoric acid — 1 part of acid + 2 parts water) and place in a clean reservoir.
3. Flush all buffers and organic solvents from the system with HPLC-grade water.
4. Place all inlet lines in the 30% acid reservoir and pump 25–50 mL of acid through each solvent inlet line. Flush the autosampler in both the load and inject positions. Use the same solution and perform multiple injector needle and seal washes.
5. Remove the acid reservoir and briefly rinse the inlet tube ends and frits with HPLC-grade water.
6. Fill a fresh reservoir with HPLC-grade water and flush all lines, needle and seal washes, and any other passages exposed to the acid flush. A 25–50 mL water flush should be sufficient, but you can check the pH of the waste line to be sure it is no longer acidic. If the pH is still too low, replace the water and continue to flush.

This acid-washing procedure was implemented in the production laboratory and a significant improvement was observed, as is seen in Figure 2. In Figure 2b, the relative size of the contaminant peak to antibiotic 2 is greatly diminished (note that for the chromatograms of Figures 1 and 2, the same concentration of standard was used, so the peak height for antibiotic 2 should be the same in all cases; the scale is noted in each chromatogram). It does look like system contamination was at least part of the problem.

What's Next?

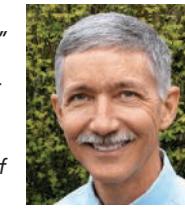
So, where do we go from here? We've eliminated several possible causes of the unwanted peak and reduced the peak size, but we haven't eliminated it completely. We've discovered that the system was contaminated and cleaned it. I still don't know the results from the stepwise isolation of mobile-phase components (I would have done that series of experiments before acid-washing the hardware), and I suspect that this may be the source of the remaining problems. Because of the presence of contamination in the system, I suspect that there may be other problems caused by contaminants in the laboratory. Specifically, I suspect that the water is the problem source, but that is just a gut-level hypothesis. I would replace the water with water that is known to be pure, such as purchased HPLC-grade water or HPLC-grade water obtained from the R&D laboratory's water source. I don't know how physically close the two laboratories are to each other. If they are in close proximity, I would take known good mobile phases and a good column from R&D to the production laboratory and substitute each separately, and at that point the remaining source of the problem should be obvious.

It would be nice if all these case studies were wrapped up neatly with cause and effect, but the truth is that often when good progress is being made on fixing the problem, I don't hear any more about it. In spite of that, I think the present case study gives us a good context for discussion of how to distinguish between the various sources of extra peaks and how to isolate the source so it can be eliminated.

References

(1) L.R. Snyder, J.J. Kirkland, and J.W. Dolan, *Introduction to Modern Liquid Chromatography, 3rd ed.* (Wiley, Hoboken, New Jersey, 2010).

John W. Dolan



"LC Troubleshooting" Editor John Dolan has been writing "LC Troubleshooting" for LCGC for more than 25 years. One of the industry's most respected professionals, John is currently the Vice President of and a principal instructor for LC Resources in Walnut Creek, California. He is also a member of LCGC's editorial advisory board. Direct correspondence about this column via e-mail to John.Dolan@LCResources.com.

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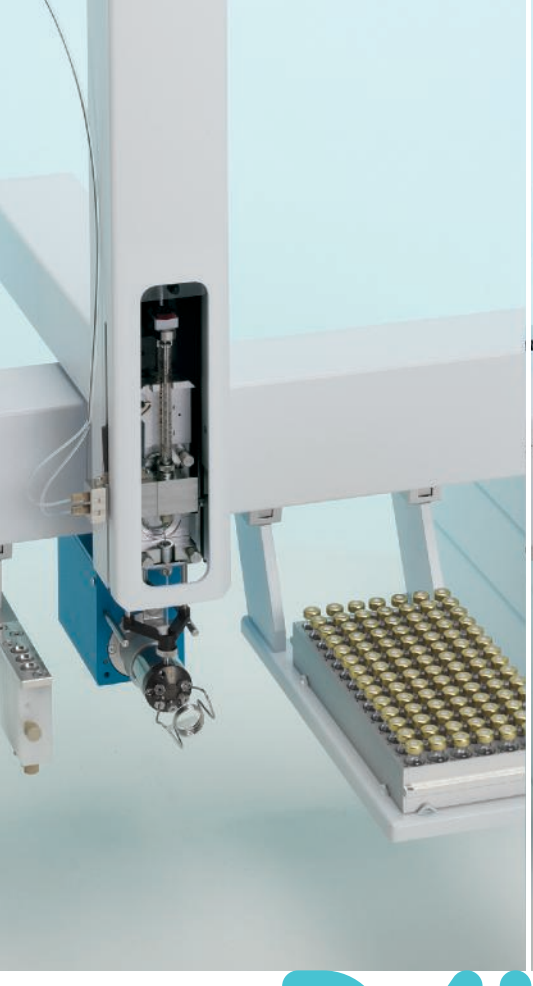

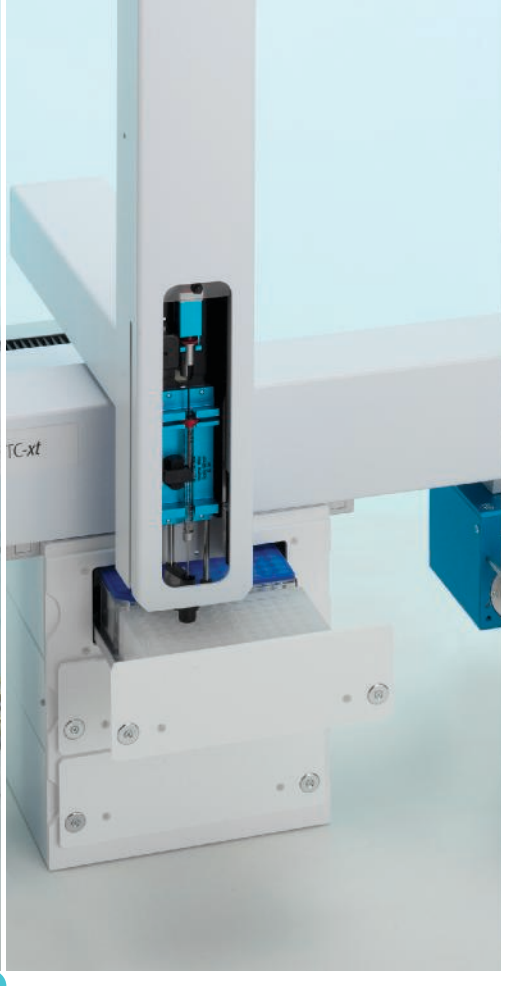

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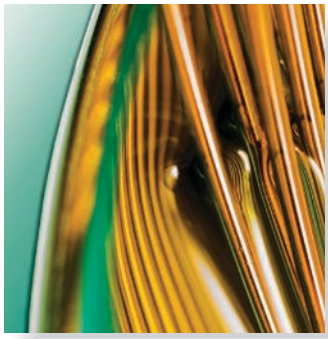

GC CONNECTIONS

New Gas Chromatography Products 2012

In this installment we review gas chromatography (GC) instruments and accessories that were newly presented at Pittcon 2012 or were introduced to the marketplace in the preceding year.

John V. Hinshaw
GC Connections Editor

The 2012 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) was held March 11–15 in Orlando, Florida, at the Orange County Convention Center. The 63rd annual occurrence of this venerable analytical chemists' symposium was only slightly less attended than the conference's 2010 southern sojourn at the same location, with 15,754 registrants. I'm comparing the attendance to that at the previous Orlando venue rather than Atlanta from 2011 because the attendance always has been somewhat lower when the conference heads south. The amount of exhibitor booth space also declined by a similar margin, driven by many companies' decisions to attend with a smaller footprint, although the total number of exhibiting companies only dropped by about 2%.

Significantly absent this year were Agilent and PerkinElmer, which are both engaging in postconference seminar tours and on-line product displays. Both companies exhibited at Analytica in Munich, Germany, April 17–20, 2012. Several companies opted to meet key customers at nearby hotel suites rather than hosting an in-exhibition booth. To an aisle wanderer such as myself, the overall impression was one of reduced acreage accompanied by a larger Technology Park area and, although I did not measure them, perhaps slightly wider corridors. Smaller though it may have been, the exhibit floor experience was almost relaxed, with more time to visit each exhibitor of interest, unlike some earlier years where I often had to choose between visiting a booth or attending a session.

The conference organizers' emphasis on the technical program was evident in the consistent number of poster sessions that were mounted in two large areas of the exhibition floor, as well as in the continuing high count of oral presentations, symposia, and workshops. Short-course attendance was up 16% from last year.

Among the conferees, 36% listed liquid chromatography (LC) and 34% gas chromatography (GC) among their selected scientific specialties; these were the top two categories at the conference. Spectroscopic specialties accounted for the majority of the remaining mentions;

Table I: Companies listed
Bruker Daltonics
Dani
Defiant Technologies
EST Analytical
Gerstel
HTA s.r.l
Markes International
Justice Laboratory Software
PAC (AC Analytical Controls)
PerkinElmer
Shimadzu
Teledyne Tekmar
Thermo Fisher Scientific
Torion

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Example of instant connect module installation by user

With its intelligent features and operational simplicity, Chromeleon 7 is simply intelligent

Thermo Scientific columns and consumables provide quality and productivity to your new GC

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Table II: GC instruments

Product	Company	Description
GCMS-QP2010 SE system	Shimadzu Scientific Instruments	Shimadzu's enhanced version of the GCMS-QP2010S system employs high-performance ion optics with a quadrupole mass filter designed by Shimadzu's proprietary Optdesign simulation program. It features fully automated MS tuning. An optional direct sample injection (DI) probe obtains spectra from nonchromatographable materials. The pumping system accommodates column flows up to 4 mL/min. An ecology mode reduces energy consumption while waiting to start analyses. A front-opening chamber allows maintenance to be performed from the front of the instrument, and maintenance is possible without venting. Multiple system configurations, including headspace, purge-and-trap, sample injection, pyrolysis, and thermal desorption are available. An automatic adjustment of retention time function estimates the retention times of target components from retention index values.
Scion TQ GC–MS-MS system	Bruker Daltonics	The Scion TQ GC–MS-MS system features a dual-filament interchangeable ion source with an active focusing 90° q0 quadrupole, a lens-free ion path, and a 180° collision cell with elliptical design, all in a relatively small footprint. Scan modes include full scan with Q1or Q3; precursor, product, and neutral loss or gain monitoring; selected ion monitoring (SIM); multiple reaction monitoring (MRM); and result-dependent scanning. Electron ionization (EI) is standard; optional ionization modes include chemical ionization (CI) with positive (PCI) and negative (NCI) modes. The source and transfer line temperatures can be set as high as 350 °C. The mass range (*m/z*) is 10 to 1200 Da with scan rates up to 14,000 Da/s. Bruker specifies a 500:1 signal-to-noise ratio (S/N) or higher with 1 pg octafluoronaphthalene (OFN) for *m/z* 272, scanning from *m/z* 50 to 300. A dual-stage air-cooled 310/400-L/s turbomolecular pumping system functions with helium carrier gas flows up to 25 mL/min. The Scion TQ works with the company's model 436 and 451 gas chromatographs.
Tridion-9 GC–TMS system	Torion	Torion's portable GC–toroidal MS (GC–TMS) system is aimed at the rapid screening of chemicals such as environmental volatile organic chemicals (VOCs) and semivolatiles (SVOCs), explosives, chemical threats, and hazardous chemicals such as those found in water and air quality analysis, soil sample screening, chemical and petrochemical, food safety, flavors and fragrances, forensics and drugs of abuse, military, security, and first response applications. The Tridion-9 system integrates a high speed low-thermal-mass (LTM) capillary GC system with a miniaturized toroidal ion trap mass spectrometer for fast, reliable, and easy-to-operate GC–MS. Samples are injected using Torion's Custodion solid-phase microextraction (SPME) fiber syringe. The SPME syringe allows users to collect and concentrate analytes without the need for other extraction equipment or solvents. Total weight including battery and carrier gas is 32 lb. As a fully self-contained field instrument, the T-9 system operates on battery power (rechargeable) for 2.5 h, and the on-board helium carrier gas cartridge is capable of up to 150 sample runs. The system is ready for analysis in about 5 min from a cold start, and typical run-to-run cycle times are only 5 min, allowing for analysis of about 12 samples/h. With an on-board color touch screen, the user interface directs the operator through sample introduction and analysis. The on-board library automates target compound deconvolution and identification. The results are displayed on-screen for quick and easy data interpretation.

Table II: GC instruments *(continued)*

Product	Company	Description
Frog-4000 GC–PID system	Defiant Technologies	This miniaturized field-portable GC system for VOC analysis in water, air, and soil features photoionization detection (PID), which detects common VOCs such as benzene, toluene, and xylene (BTX), trichloroethylene (TCE), and others for applications such as environmental or process monitoring. The analysis cycle time is 5 min. The unit includes a micro preconcentrator, and uses scrubbed air as the carrier gas. It weighs 4.8 lb and runs off a 9-V supply or for up to 6 h on one battery charge.
CNS SIMDIS for Crude Oil	PAC (AC Analytical Controls)	PAC introduced the AC Analytical Controls CNS SIMDIS for Crude Oil analyzer. It simultaneously determines the boiling range distribution of carbon, nitrogen, and sulfur in crude oils and final products, which helps producers optimize the manufactured end product. It is also able to analyze a wide variety of crude products, ranging from heavy gas oils to naphtha. The system is built around recognized simulated distillation (SIMDIS) methods, with the addition of additional chemiluminescence detectors. Comparable to standard methods, such as ASTM D2887, D6352, D7500, and D7169, the CNS SIMDIS system completes assays within 30 min.
Clarus SQ 8 GC–MS system	PerkinElmer	The PerkinElmer Clarus SQ 8 GC–MS system acquires spectra at up to 12,500 amu/s across a mass range of 1–1200 u. It features the company's SMARTsource (simplified maintenance and removal technology) ionization source, which enables tool-free removal or disassembly and operates in EI and CI modes. An ion-filtering, cleanable, prequadrupole section removes nontarget ions before the analytical quadrupole section. The GC–MS system employs the Clarifi detector with a high-voltage conversion dynode, ion deflector, and electron multiplier. With an available 251-L/s pump capacity the Clarus SQ 8T and SQ 8C models deliver an 800:1 S/N for 1 pg octafluoronaphthalene and pump-down times under 3 min. The company states that the system is compatible with hydrogen carrier gas.
Trace 1300 Series GC system	Thermo Fisher Scientific	The Thermo Fisher Scientific Trace 1300 Series GC systems both feature the same unique user-exchangeable, miniaturized plug-in injectors and detectors. The Trace 1310 GC system features an icon-driven touchscreen user interface, and the Trace 1300 GC system is a single-button system for minimal instrument interaction. According to the company, the modular design provides quick accessibility to injectors and detectors and greatly reduces maintenance downtime. It enables operations such as adding a second injector or detector to a single-channel configuration or swapping a flame ionization detector with an electron-capture detector to be performed in 2 min or less by the user, without requiring a service visit. An analysis can be ready to start only 3 min after the instrument is turned on, partly because of the reduced thermal mass of the oven and other heated components. The Trace 1300-series also features newly designed microvolume detectors; an optional injector back-flush option that eliminates heavy or undesired compounds to protect column and detectors; and full control with the scalable Thermo Fisher Scientific Dionex Chromeleon Data System. The Trace 1300-series oven operates from -100 to +450 °C with a maximum heating rate of 125 °/min and a 4-min cool-down time from 450 to 50 °C. Up to two inlets — split–splitless and programmed-temperature vaporizer (PTV) — plus up to two detectors ranging from flame ionization, electron capture, photoionization, nitrogen–phosphorus, and thermal conductivity detectors can be installed. The Trace 1300-series supports seven heated zones, eight valves, and three auxiliary gas modules.

ion chromatography was cited by 11%, and other chromatography techniques were cited by 7%.

Pittcon 2013 will be held in Philadelphia for the first time, March 17–21, at the Pennsylvania Convention Center. The conference is scheduled to return to Chicago (2014, 2017), New Orleans (2015), and Atlanta (2016), before returning to Orlando in 2018.

This installment is our annual review of GC instrumentation and accessories shown at this year's Pittcon or introduced during the previous year. For a review of new GC and LC chromatography columns and related accessories, please see Ron Majors's "Column Watch" in the April and May 2012 issues of *LCGC North America*, which are also available online at www.chromatographyonline.com/ColumnWatch.

Nearly half of the newly introduced instruments are tightly associated with a mass spectrometric detector. Most were more about the MS detector than the GC system itself.

The information presented here is based on manufacturers' replies to questionnaires, as well as on additional information from manufacturers' press releases, websites, and product literature, and not upon my actual use or experience. During Pittcon, I took time to stroll around the convention aisles and see some of the new products firsthand as well as discover a number of items that weren't covered by the questionnaires. Every effort has been made to collect accurate information, but because of the preliminary nature of some of the material, *LCGC North America* cannot be responsible for errors or omissions. This column cannot be considered a complete record of all new GC products introduced this year at Pittcon or elsewhere because not all manufacturers chose to respond to the questionnaire, nor is all of the submitted information necessarily included here because of the limited available space and the editors' judgment as to its suitability.

New GC Instruments

Another new crop of GC instruments appeared in 2012, which demonstrates once again the ongoing resilience of the technique and also emphasizes a continuing shift toward mass-selective detection since what used to be top-end capabilities have become more affordable. Nearly half of the newly introduced instruments are tightly associated with a mass spectrometric (MS) detector. Most were more about the MS detector than the GC system itself. See Table II for the details of each new product.

Shimazdu's GCMS-QP2010 SE system is an upgraded version of

Table III: Sampling systems and accessories

Product	Company	Description
SHS-40 headspace sampler	Bruker Daltonics	The SHS-40, Bruker's headspace sampler offering for its 400-series GC systems, is a fully automated sampler that can be integrated into a Bruker 400-GC Series or Scion series GC–MS system. The SHS-40 provides a 40-vial sample capacity of 20-mL vials, or 10-mL vials with adapters. The 1-mL sample loop and the entire gas module may be separately heated up to 350 °C. The heated incubation oven accommodates up to 12 samples heated at 40–200 °C in 1 °C-steps, plus an integrated sample shaker. The sampler supports multiple headspace extraction (MHE). System control is provided either stand-alone or remotely via Compass CDS software .
CIA Advantage canister sampling system	Markes International	The Markes International CIA Advantage canister sampling system is designed for the analysis of volatile organic compounds (VOC) in air and gas samples that are acquired using canisters. The system allows analytical chemists to analyze a wide range of analyte concentrations without the need for dilution. Built-in sorbent tube functionality provides the capacity to analyze an extended range of compounds. The system is fully compliant with US EPA Method TO-15. The system can handle component concentrations that range from parts-per-trillion (10^{-9}, ppt) to low-percent levels using a combination of loop-sampling, large-volume sampling, and sample flow splitting. The sampler operates cryogen-free and combines heated internal lines and purge steps to minimize sample carryover with less-volatile analytes.
mVAP multiposition evaporation station	Gerstel	The mVAP six-position evaporation station works with the company's MultiPurpose Sampler (MPS) to concentrate samples under user-defined temperatures and vacuum levels. Solvent exchange to a GC- or high performance liquid chromatography (HPLC)–compatible solvent can be performed to match chromatography requirements. The mVAP can be used with sample preparation and clean-up techniques such as solid-phase extraction (SPE), dispersive SPE, liquid–liquid extraction (LLE), or filtration to achieve automated injection of the concentrated sample into a GC–MS or LC–MS system. Each step in the sampling process is controlled by the company's Maestro PrepBuilder software, using a single method and sequence table for the entire process including GC–MS or LC–MS analysis.
mVORX vortex and shaker accessory	Gerstel	The Gerstel mVORX accessory works with the company's MultiPurpose Sampler (MPS) and performs vortexing and agitation at speeds up to 3000 rpm, which speeds up sample preparation steps such as liquid–liquid extraction, dissolution, and homogenization. The accessory performs simultaneous vortex mixing of up to eight samples, depending upon the vial size. The accessory's orbital motion is provided by a precise linear, direct drive motor. Mixing movements are restricted to the horizontal plane, allowing even the most sensitive samples to be mixed efficiently without over-agitation or wetting of the vial cap. The Gerstel MPS system uses the company's Maestro software control for automated sample processing. The Maestro PrepAhead functionality ensures just-in-time sample preparation followed directly by sample introduction to an LC–MS or GC–MS system.

Table III : Sampling systems and accessories *(continued)*

Product	Company	Description
Master SHS static headspace sampling system	Dani	The Dani Master SHS static headspace sampling system features a 120-position sample vial carousel that accommodates 10-, 20-, and 22-mL headspace vials. Any vial position may be designated as priority. The internal vial heating oven holds up to 18 samples simultaneously, with vial agitation. A leak-checking routine detects improperly sealed vials, and a bar-code reader identifies each one. The autosampler uses a heated valve and loop sampling system, and can be interfaced with any standard GC system via a heated transfer line. It supports multiple headspace extraction (MHE), multiple headspace injection (MHI), and method development optimization sequences. The system is controlled by an integrated touch-screen or from the company's Clarity software station.
Centurion RSK50 sample preparation system	EST Analytical	EST Analytical's Centurion RSK50 system is designed specifically for the complete automation of RSK 175 analysis as outlined by the US EPA National Risk Management Research Laboratory as well as the Pennsylvania Department of Environmental Protection (DEP). The system performs sample preparation of a full 40-mL volatile organics analysis (VOA) vial by removing an aliquot of sample and placing it in a helium-filled empty 40-mL vial. The vial is then heated to equilibrium, followed by loop or pressure-balanced headspace injection directly to the GC system. The process is completely automated, does not expose the sample to atmosphere, and yields sample carryover of less than 0.08%.
HT3000A Series autosamplers	HTA s.r.l	The HT3000A autosampler holds 121 2-mL liquid sample vials and has a touch-screen display. The HT3200A autosampler holds up to 209 2-mL vials and features SyringeID radio-frequency identification (RFID) of syringes and a vial bar-code reader. The HT 3100A autosampler can hold up to 15 vials at once and uses a dedicated keypad for control. The autosampler series supports internal standard technique with sandwich injection, multiphase injection, ambient headspace sampling, and priority injection. High-speed injection is accomplished in less than 100 ms.
Versa automated headspace vial sampler	Teledyne Tekmar	The Teledyne Tekmar Versa automated headspace vial sampler is a compact 12-in. (30.48-cm) wide 20-position autosampler with a single position heated-plate oven for static sampling analysis of 22-mL vials. The device features sample heating to 200 °C throughout the gas pathway; built-in pressure control for sample volume control regardless of external conditions; an inert sample pathway including transfer line, sample needle, and loop; automatic leak check and benchmark test for quick troubleshooting and method development using Method Optimization Mode (M.O.M.). The sampler is controlled by the company's TekLink 2G software. It will interface to virtually all commercially available GC instruments.

the company's QP2010 S system that features high-performance ion optics and a quadrupole mass filter along with lab-friendly energy- and time-saving features. While not seen at Pittcon, PerkinElmer earlier this year introduced the Clarus SQ 8 GC/Mass Spectrometer system with high sensitivity, tool-free source maintenance, and a rapid scanning speed. Bruker Daltonics highlighted its high-end Scion TQ GC–MS-MS system with 180° optics that enables multiple ion-selective scan modes for resolution of complex samples and matrices. This triple-quadrupole GC–MS system, introduced in July 2011, won the 2012 Pittcon Editors' Silver Award. All of these new GC–MS systems operate in tandem with the companies' respective existing gas chromatograph offerings.

The new crop of instruments demonstrates the resilience of GC and emphasizes a continuing shift toward MS detection.

From Torion, the Tridion-9 GC-TMS system is a gas chromatograph–toroidal mass spectrometer system that is aimed at the rapid screening of chemicals such as environmental volatile organic compounds (VOCs) and semivolatile organic compounds (SVOCs), explosives, chemical threats, and hazardous chemicals. The unit is billed as a "Person-Portable" GC–MS system that can easily be carried to remote sites. It uses a conventional 100-µm i.d. capillary column bundled with a low-thermal-mass resistive heater and helium carrier gas. Sample injection is accomplished via the company's Custodion solid-phase microextraction (SPME) syringe.

Thermo Fisher Scientific came to Pittcon with the 1300-Series GC

Table III : Sampling systems and accessories *(continued)*

Product	Company	Description
TriPlus RSH autosampler	Thermo Fisher Scientific	The Thermo Fisher Scientific TriPlus RSH autosampler is compatible with the company's GC and GC–MS systems. It features an Automatic Tool Change (ATC) function that automates the exchange of syringes when switching to different sampling tasks — such as standard or stock sample dilutions or internal standard addition and derivatization — in a single, unattended sequence before sample injection. The autosampler has a sample-vial capacity of 648 2-mL vials, with multiple 100-mL wash or waste bottles. A vial bottom-sensing capability enables the autosampler to accurately withdraw microliters or fractions of microliters from a sample. Less than 2% relative standard deviation (RSD) and up to three injections are possible with a 5-µL sample volume.
7200 preconcentrator	Entech	The Entech Model 7200 automated preconcentrator features H_2O and CO_2 management via three-stage trapping procedures. The company's Accu-Sample Technology supports 10–1000 mL preconcentration volumes with reduced carryover and improved accuracy down to 10-mL sample volumes. Sample loops from 0.25 mL to 1 mL are available, in a Silonite-D coated flow path. The system is compatible with the Teledyne Tekmar 7016CA, 7016D, 7032AQ-L rotary-valve autosamplers, and the 7600 Soil Gas/Air Toxics robotic autosampler. The sampler supports matrix spiking and adds internal standard automatically. It performs automated leak-checks and instrument bake-outs, and occupies 9.5 in. of linear bench space. There are four sample inlets, compatible with low cost MiniCan and Bottle-Vac canisters, as well as with canisters, Tedlar bags, and adsorbent traps. The system is compatible with most major GC systems, and it may be interfaced with purge and trap for maximum GC–MS utilization. As many as three autosamplers can be added to maximize throughput.

system, a completely new frame. With a unique system of "instant-connect" modular interchangeable inlet and detector options, the 1300-Series system allows end users to quickly exchange or replace either or both inlets or detectors as pluggable modules. Each module includes pneumatic and electronic connections that are engaged by putting them in place and tightening a couple of screws.

PAC introduced the AC Analytical Controls CNS SIMDIS for Crude Oil analyzer. The system simultaneously determines the boiling range distribution of carbon, nitrogen, and sulfur in crude oils and final products, using simulated distillation and a combination of flame ionization detection (FID) and chemiluminescence detection.

A unique portable GC instrument was on display at the 2012 Pittcon. The Frog-4000 system from Defiant Technologies is a miniaturized GC system with photoionization detection (PID). Based on micromachined preconcentrator and GC designs, the Frog-4000 system targets field analysis of VOCs in water and soil.

Sampling and Accessories

This year saw strong growth in new automated headspace GC (HSGC) samplers. Together with Agilent's 7697A headspace sampler that was introduced at Pittcon 2011, this

burgeoning of HSGC is indicative of the ongoing demand for volatiles analysis in solid, semisolid, and liquid matrices such as for pharmaceutical and food or flavor products. Table III lists the salient details.

The new offerings bring a wide range of sizes and capabilities. The SHS-40 headspace sampler from Bruker Daltonics is a multivial equilibrium headspace autosampler for the company's 400-series GC systems as well as the Scion Series GC–MS system. It accommodates up to 40 vials and supports a range of modes and capabilities. The Master SHS static headspace sampling system from Dani is designed to work with the company's Master GC system, but it also interfaces with most other GC systems. The Master SHS system has a 120-vial capacity and a range of options such as a bar-code reader. The Versa automated headspace vial sampler from Teledyne Tekmar is a compact device that holds up to 20 vials and works with most GC systems as well.

A related application-specific headspace sampler, EST Analytical's Centurion RSK50, performs US EPA RSK 175 analysis for dissolved methane, ethane, and ethylene in water, including sample preparation of the requisite 40-mL volatile organics analysis (VOA) vial and sampling of the evolved headspace gases onto nearly any model of GC system.

Strong growth in automated headspace GC samplers is indicative of demand for volatiles analysis in solid, semisolid, and liquid matrices, such as pharmaceutical and food or flavor products.

Two new canister sampling devices were on-hand. Markes International introduced its CIA Advantage canister sampling system in 2011, after previewing it at last year's Pittcon. The cryogen-free sampler handles multiple canisters and is fully compliant with US EPA TO-15. From Entech, the 7200 Automated Preconcentrater uses a three-stage trapping procedure to preconcentrate gaseous samples from a variety of sources, add internal standards or spikes, and like the Markes unit it works with most GC systems.

Two new liquid GC autosampler systems were shown this year. HTA s.r.l. displayed its model HT3100A autoinjector, HT3000A autosampler, and HT3200A top-class autosampler. Together, the three models deliver a wide range of capabilities to almost any

GC system. From Thermo Fisher Scientific, the TriPlus RSH autosampler is a robotic *x-y-z* axis sample handling system and autoinjector with all of the flexibility and versatility that comes along with this type of device. One TriPlus RSH autosampler can address up to two GC systems with sample preparation, derivatization, and microvolume injection among many sampling options.

Finally, Gerstel introduced two accessories for its robotic MultiPurpose Sampler (MPS). The mVAP Multi-Position Evaporation Station concentrates up to six samples at user-defined temperatures and vacuum levels. Solvent exchange, solid-phase extraction, and filtration are a few of the possible sample preparation techniques. Gerstel's mVORX vortex and shaker accessory for its MPS system agitates as many as eight sample vials in the horizontal plane to eliminate vial-cap contamination and over-mixing of microsized samples.

Acknowledgments

I would like to thank the manufacturers and distributors that kindly furnished the requested information before, during, and after Pittcon 2012, allowing a timely report on new product introductions. For those manufacturers who did not receive a preconference questionnaire this year and would like to receive one and be considered for early inclusion into Pittcon 2013 coverage, please send the name of the primary company contact, the mailing address, fax number, and e-mail address to Laura Bush, Editorial Director, *LCGC* and *Spectroscopy*, Advanstar Communications, 485F US 1 South, Iselin, New Jersey 08830, Attn: 2013 New GC Products.

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John V. Hinshaw

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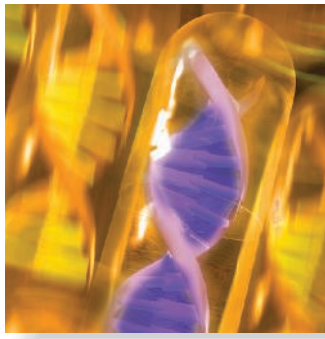

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BIOTECHNOLOGY TODAY

Bioanalytical Tools for the Characterization of Biologics and Biosimilars

One of the fastest growing segments in the biopharmaceutical market is biosimilars. This emerging biotech sector requires novel bioanalytical tools to address the challenging regulatory aspects entailing comprehensive characterization by sensitive and high-resolution bioanalytical methods covering all four structural levels of biotherapeutic proteins. Here is an overview.

András Guttman
is the guest author of this month's column.
Anurag S. Rathore and Ira S. Krull
are the editors of Biotechnology Today.

In spite of the recent slowdown in the approval rate of new biopharmaceutical entities, dozens of new biologicals were introduced in the United States and the European Union during the past 5 years, adding up to more than 200 products already on the market (1). One of the fastest growing segments in this market is biosimilars (also referred to as follow-on biologics or subsequent entry biologics). This emerging biotech sector requires novel bioanalytical tools to address the challenging regulatory aspects (2), which entail comprehensive characterization by sensitive and high resolution bioanalytical methods covering all four structural levels of biotherapeutic proteins (that is, from primary to quaternary). During the development phase of both the novel biologics and biosimilars, full sequence coverage, purity assessment, quantification, and product identification is necessary. In addition, quite a few other critical features should be analyzed, such as glycosylation (including their microheterogeneity and site specificity), mutation, phosphorylation, sulfation, disulfide linkages, oxidation, deamidation, glycation, proteolytic clipping, and several others. Analysis of isomerization, especially isoaspartic acid formation (isoAsp and isoD), is another important requirement for their analysis because it might reveal immunogenic structural changes (3). Identification of host-cell impurities, aggregation, and determination of higher order structures and isoforms are also highly important (4).

The analytical needs depend on the actual application in hand; for example, high throughput is a prerequisite during clone selection and high sensitivity is important during product release. To attain such levels of characterization, orthogonal bioanalytical methods should be applied including liquid chromatography (LC), capillary electrophoresis (CE), mass spectrometry (MS), and their combinations: LC–MS and CE–MS. These methods enable thorough analysis of intact proteins (top down), peptide digests (bottom up), and even in between (middle down or up) (5). The most frequently used proteolytic enzyme for these applications is trypsin that produces the majority of fragments below 3 kDa, a size range that is readily compatible with most present day MS instruments. Higher resolution mass spectrometers such as hybrid MS units enable routine analysis of larger fragments with higher charge states (6).

The Bioanalytical Toolset

Mass spectrometers are the most important workhorses in the characterization of biologics and biosimilars (7,8). Important features include mass resolution and accuracy. In this respect, Table I delineates the capabilities of the top-of-the-line MS systems these days.

However, even for the best mass spectrometers on the market, the use of separation methods (LC or CE) before the mass analysis is highly recommended to decrease the complexity of the sample entering the device (9), as shown in Figure 1. Pre-MS separation also increases the dynamic range and decreases ion suppression in mass spectrometry. One of the preferred separation modes is LC, typically using narrow-bore or capillary columns with packing particle sizes of 3–5 µm or lower (1.7 µm). The porosity of the stationary phase should match the size

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Table I: MS capabilities

System	Capabilities	
Ion trap	• 0.5 Da FWHM independent of *m/z* • UltraZoom scan 0.05 Da • MS^n	
Triple-stage quadrupole (MRM)	• Similar to ion trap • Fast, high dynamic range • MS^2	
Time of flight (TOF), quadrupole TOF	• 10,000–40,000	1 ppm
Orbital trap	• 60,000 at 400 *m/z*	1 ppm
Fourier transform ion cyclotron resonance	• 400,000 at 400 *m/z*	1 ppm

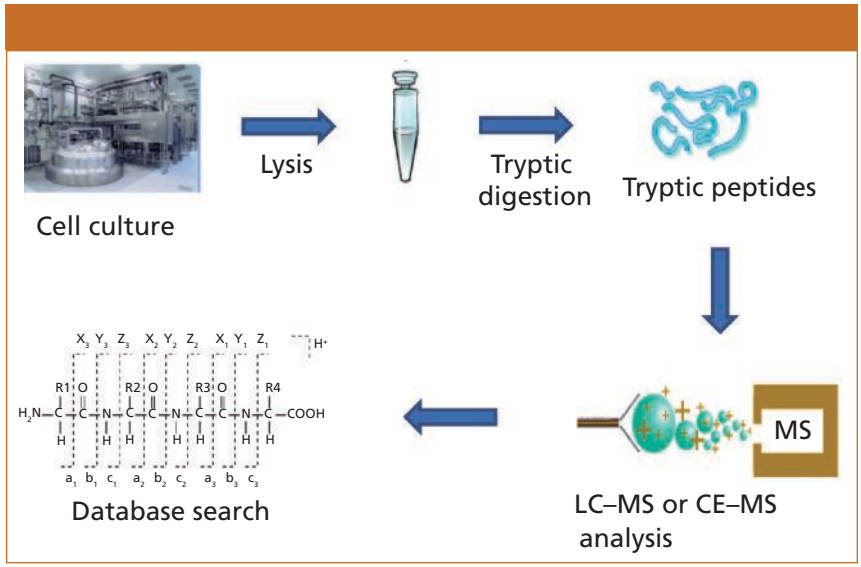

Figure 1: Workflow of the proteomic analysis of biologics or biosimilars.

of the solute molecules; for example, for peptides it should be 20 nm and for proteins 30 nm. The pressure drop on such systems ranges from 6000 to 15,000 psi depending on the particle size and the column length. Ultra-high-pressure liquid chromatography (UHPLC) provides higher resolution and sensitivity for peptide mapping than regular high performance liquid chromatography (HPLC), enabling better sequence coverage and variant characterization (10). Other separation parameters, such as column temperature, are also important for special applications. Because of its higher speed of analysis, UHPLC is a good choice for high-throughput peptide mapping, providing fine details about differences between a biosimilar and its innovator product counterpart. State-of-the-art software tools help with automated searches of LC–MS datasets to assign peptides and annotate peptide variants in minutes even at low levels, a process that manually takes several days per data file. Differential plotting options enable quantitative comparison between different peptide maps of, for example, a biosimilar to an innovator product.

Capillary electrophoresis is another high efficiency separation method frequently used in bioanalysis providing fast separation (minutes to seconds) with predictable selectivity, requiring only small sample volumes (1–10 µL) (11). Features of CE also include quantification in a reasonable dynamic range. The option to use multicapillary devices can significantly increase the throughput of the analysis (for example, DNA sequencing). Coupling CE to a mass spectrometer is an emerging field of research. From the various modes of CE, capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), and capillary isoelectric focusing (cIEF) are most frequently used for the analysis of biologics and biosimilars.

In the analysis of biotherapeutics, with the goal of good recovery, the hydrophobicity and size of the sample molecules should be considered as well as their aggregation tendency. Samples should be treated accordingly with detergents or denaturants. In peptide analysis by LC–MS, the size and hydrophobic or hydrophilic characteristics of the peptides should be considered. If the peptides are too small or hydrophilic then they are not retained. If the peptides are too big or hydrophobic then they are not recovered properly. To avoid these size-related issues, one option is to implement a multienzyme approach that provides suitable sized peptides for the analysis, keeping in mind that peptide sizes between 1000 and 3000 Da work well for most LC–MS systems. On-line LC–MS, for the analysis of larger peptides, may need a combination of collision-induced dissociation (CID) and electron-transfer dissociation (ETD). CID of an isolated charge-reduced species derived from ETD is an effective approach to determine phosphorylation sites and glycosylation modifications as shown in Figure 2 (12). To further analyze glycosylation modifications, hydrophilic interaction liquid chromatography (HILIC) stationary phases have been introduced using amide, diol, amine, aminopropyl, and zwitterionic (ZIC-HILIC) phases (13). However, normal-phase silica-based columns are still being used for glycopeptide or glycan analysis. Capillary electrophoresis (as well as CE–MS) is another good option to analyze glycosylated proteins or peptides (14). Rapid analysis of glycosylation, especially for immunoglobulins, is also possible by a novel microchip-based LC–MS approach that utilizes an integrated microfluidics-based sample preparation system before MS analysis (15). The built-in PNGase F enzyme reactor cuts off the IgG glycans, and the resulting glycosylamines are separated on a porous graphitized carbon column followed by detection in the connected MS unit.

Sample Preparation Issues

Effective sample preparation for the analysis and characterization of biologics and biosimilars is a key issue (16). This includes the collection and handling of the samples as well as the reproducibility of process protocols. Introduction of quality control (QC)

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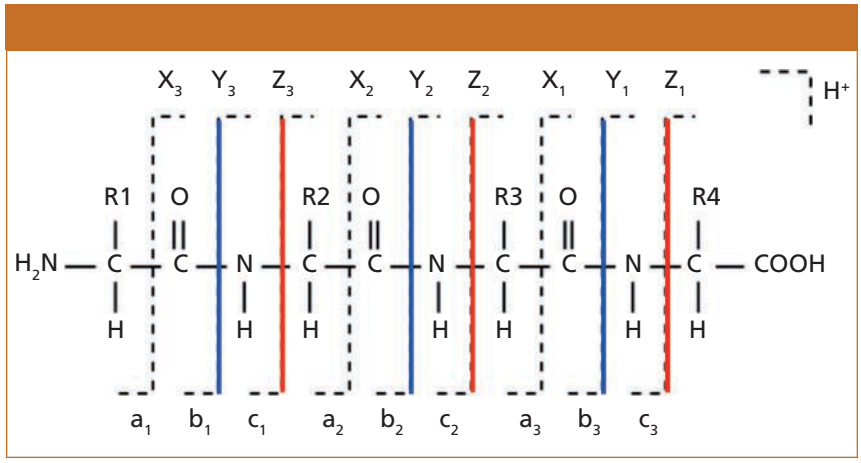

Figure 2: Electron-transfer dissociation (ETD, red) and collision-induced dissociation (CID, blue) fragmentation in mass spectrometry.

points is highly important to monitor the process. Also, reagent components along with their potential effects on the sample during downstream processes (for example, detergents, denaturants, extreme pHs, and protein concentration) should be considered. System suitability tests are necessary to ensure the performance of the analytical approach. Thus, it is important that the sample preparation process is reproducible, including solubilization of all proteins of interest. Protein aggregation should be avoided during sample preparation and the concomitant analytical processes. Also, chemical modifications of the proteins or peptides should be prevented and interfering molecules should be removed. This is important during clone selection and stability studies, in which detection of undesired protein forms are crucial. Obtaining proteins of interest at a detectable level may involve the removal of abundant or nonrelevant classes of proteins. If digestion is among the sample preparation steps, its reproducibility and efficiency is also very important.

Analysis of Critical Features

Among the important critical features, analysis of post translational modifications, especially glycosylation, is becoming more and more important during the characterization of biotherapeutics. The typical goal is to decipher the heterogeneity of the attached glycans and recognize the multiple glycosylation sites per protein. As a matter of fact, each site can hold multiple glycan structures resulting in hundreds of glycoforms, which might be responsible for various biological activities (17). Many factors contribute to alterations in glycan processing during the production of therapeutic glycoproteins, thus their analysis during the process is crucial. With complicated samples such as complex glycan mixtures, the use of orthogonal separation methods is a key to provide full characterization. CE and CE–MS analysis of intact glycoproteins (18) or the released glycans (19) can achieve such a goal. Apparently, reversed-phase LC–based protein separation has limited resolving power for glycoforms. CE, however, in which separation is primarily based on charge and size, provides high resolving power for glycoforms and is helpful in the characterization of multiple glycosylation sites (14).

The comprehensive analysis of intact glycoproteins can be done by first separating them using chromatography or electric field–mediated techniques. Ion-exchange chromatography has limited resolving power, but isoelectric focusing provides high resolution in charge-based separation. Before the separation and analysis, appropriately gentle sample preparation steps such as size-exclusion chromatography or molecular weight cut-off filtration are necessary. Among the MS-friendly separation tools, reversed-phase HPLC has only limited resolution for glycoforms. CE separates analyte molecules based on their charge, mass, and molecular shape; however, it

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requires MS-compatible buffer systems and minimal dead volume at the electrospray ionization (ESI) interface. Typical approaches include using sheath flow, sheathless, or liquid junction methods (20). The orthogonality of the above listed separation methods should be fully utilized in the analysis of biotherapeutics, both for innovative biologics and biosimilars. It is especially important during the development phase as glycosylation processing (microheterogeneity and site specificity) is very host-cell dependent and sensitive to cell culture conditions.

During MS based assignment of glycopeptides, the peptide backbone sequence is obtained by ETD after selecting the best candidates from the CID fragmentation. Confirmation can be accomplished by accurate mass determination or with orthogonal analytical methods such as CE, high-pH anion-exchange chromatography, or reversed-phase LC. Analysis of the released glycans is also possible by MS. However, simple MS can provide information only on glycan composition with respect to the number of hexoses (for example, galactose and mannose), *N*-acetyl-hexosamines (GlcNAc and GalNAc) as well as sialic acids (Neu5Ac and Neu5Gc), and provides no information on their linkages or positions (21). By using MS-MS with appropriate fragmentation modes (CID, post-source decay [PSD], ETD, or photodissociation) some linkage information can be obtained. This, again, emphasizes the necessity of a separation dimension before MS detection and analysis. With the use of MS-friendly high-resolution separation techniques, positional and linkage isomers can be separated and consequently analyzed. The HPLC separation of native glycans can be accomplished by porous graphitized carbon stationary phases, and derivatized glycans (2AB or 2AA) are better separated using HILIC separation mode. The charge-based separation by high performance anion exchange chromatography (HPAEC) is a good tool for determining characteristics such as the number of sialic acids. Another important separation tool before MS analysis is CE of highly charged fluorophore (aminopyrene trisulfonate [APTS] or 8-aminonaphthalene-trisulfonate [ANTS]) labeled glycans. Figure 3 compares the separation of IgG N-glycans by HILIC and CE–laser-induced fluorescence (LIF) methods. An important issue is the localization of the actual glycosylation sites, and because peptides with different glycoforms are eluted closely, MS-based verification is used to obtain such information.

One of the most frequently used applications of CE in the analysis of biotherapeutics and biosimilars is the characterization of protein glycosylation, which requires preseparation derivatization with a charged fluorophore after the release of glycans (both N- and O-linked). This reductive amination-based, rapid, single-step labeling reaction (usually with APTS) features great efficiency (>90%) and provides a triple-charged fluorescent tag supporting excitation at 488 nm with 520-nm emission. Quantification is easy because only one fluorophore is conjugated to one sugar entity (23). Figure 4 delineates a CE-based glycan analysis workflow for therapeutic antibodies, starting with sample purification with protein A cartridges, through APTS labeling, sample cleanup, CE analysis, and database query. Such characterization of monoclonal antibody (mAb) N-linked glycans readily gives comprehensive information on their structural diversities, such as the presence of core fucosylation or immunogenic sugar residues, such as alpha 1→3 galactosylation or *N*-glycolylneuraminic acid. This assessment of glycosylation microheterogeneity is very important during clone selection, process control, and lot release for both the therapeutic recombinant mAbs and their biosimilar counterparts.

Disulfide mapping is another important characterization method, which requires treatment with multiple enzymes and multimode MS to reveal disulfide-linked sites and the sequence information of the linked peptides (24). ETD cleaves the disulfide bonds preferentially over the peptide backbone and CID cleaves the peptide backbone while leaving disulfide bonds intact. The specificity of enzymes used for disulfide mapping should be high enough to avoid miscleavages when multiple disulfide links are in close proximity. Another issue to consider is that enzyme digestion often fails to cleave a desired amino acid right next to a disulfide bond (25). Glycosylation in disulfide-linked peptides further complicates their analysis as the efficiency of peptide N glycanase F (PNGase F) can be poor or the peptide may not be fully recovered after deglycosylation, both leading to decreased sensitivity. Please note that when using ETD fragmentation, the disulfide bonds are preferentially cleaved over glycosidic bonds. Disulfide assignment by ETD or CID uses precursor masses to obtain the most likely disulfide-linked peptides. ETD obtains disulfide-dissociated or partially dissociated peptides, which can be confirmed by further fragmentation with CID.

Other Methods

Even though most of the analyses of biologics and biosimilars focus on the primary structure, the higher order structures of these molecules is also highly relevant, and they are invisible to the above described techniques. Several classical biophysical tools can be used for global structural analysis of biologics and biosimilars, such as analytical ultracentrifugation, size-exclusion chromatography, fluorescence, circular dichroism, isothermal titration calorimetry, and differential scanning calorimetry. These techniques can reveal information about secondary, tertiary, and quaternary structures, but currently there is still a lack of full understanding about the conformations of most therapeutic proteins.

Hydrogen or deuterium exchange using mass spectrometry (HX-MS) is another valuable method for protein conformation analysis because the kinetics of H–D exchange is highly dependent on the protein conformation (26). Advantages of HX-MS include relatively good resolution, picomole-range sample requirement, no restriction in protein size, and a relatively high tolerance of sample impurities. The approach provides data on conformational changes during function and helps identify interactions or surfaces with the aid of known structures. Alternatively, it also provides some useful information when the structure

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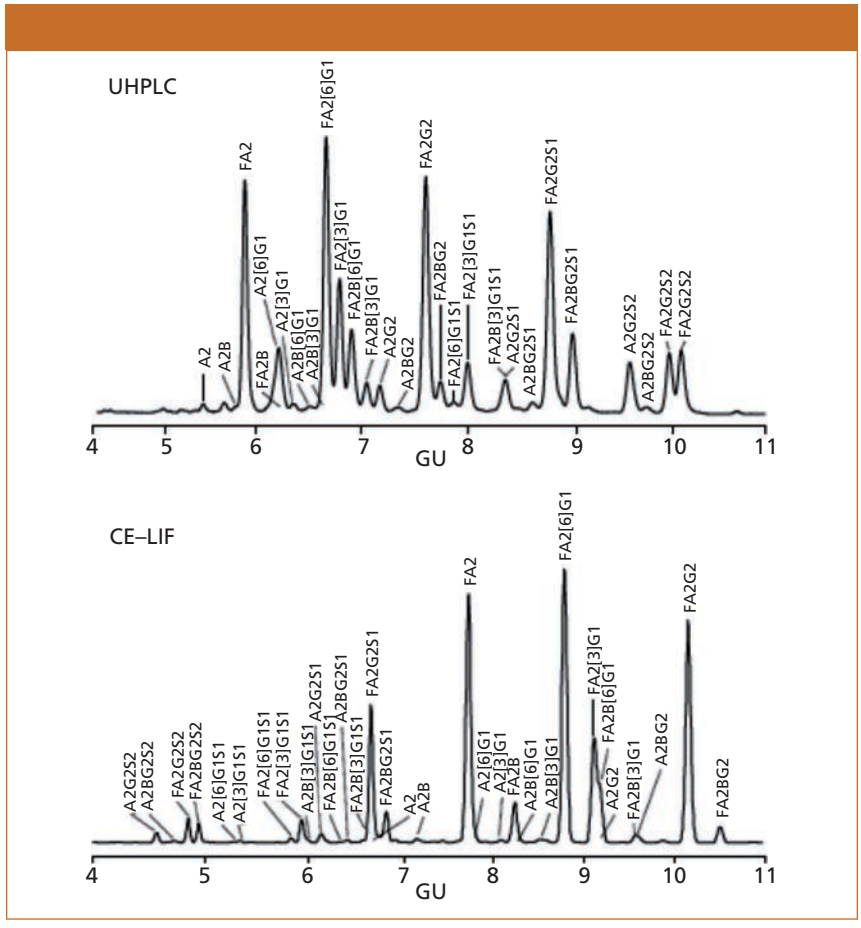

Figure 3: Structural annotation of hydrophilic interaction chromatography (HILIC) ultrahigh-pressure liquid chromatography (UHPLC) and capillary electrophoresis–laser-induced fluorescence (CE–LIF) profiles of IgG N-glycans. Adapted from reference 22.

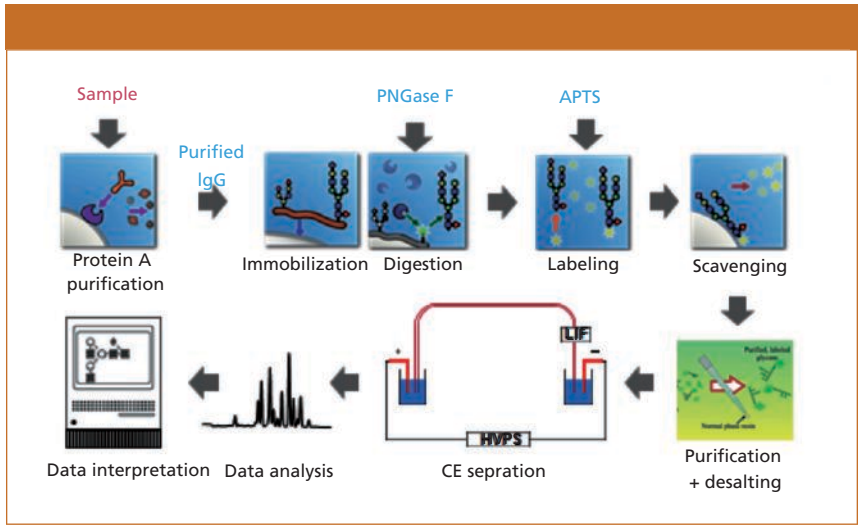

Figure 4: Capillary electrophoresis–based IgG glycan analysis flowchart.

is unknown. Besides being a valuable tool for studying protein function, HX-MS is emerging as a method of quality control in biopharmaceutical development and a diagnostic tool for proper folding.

Closing Remarks

Biotherapeutics are highly complex molecules with complicated higher order structures that are subject to a plethora of post-translational modifications. The biologic substance (the molecule itself) and biologic product (the pharmaceutically formulated final product) also contain process- and product-related impurities such as aggregates, heterogeneous structures, and fragments (27). These factors underline the importance of the subject matter discussed in this column, helping to understand the analytical science accompanying the development of these complex therapeutic molecules and keeping pace with the rapidly developing regulatory framework for these products.

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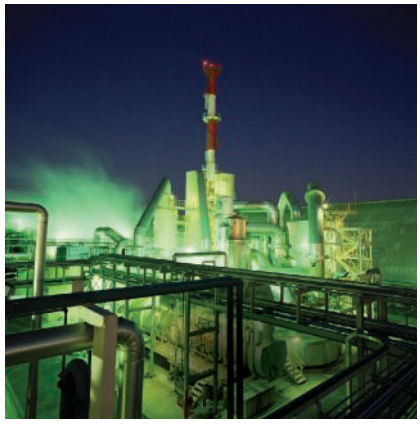
Ultratrace Quantitative Analysis of Catalyst Poisoners Using a Dedicated GC–MS Analyzer



A dedicated gas chromatography–mass spectrometry (GC–MS) analyzer was developed to address the increasing need for more sensitive catalyst poisoner analysis. The system combines the separation power and robustness of a classic backflush configuration with the selectivity and sensitivity of MS.

The use of high-yield metallocene catalysts has dramatically increased both efficiency and selectivity of polymerization processes (1). Unfortunately, these catalysts are extremely prone to poisoning by feedstock impurities, such as arsine (AsH_3), phosphine (PH_3), oxygenates (for example, dimethyl ether), and sulfur-containing compounds (mercaptans, sulfides, and so forth) (2,3). Minute amounts of these compounds are sufficient to impose undesirable effects and induce immediate loss of catalytic activity and reaction yield. At the same time, trace contaminants at parts-per-billion concentration levels can end up in the polymers and alter subsequent polymer properties and characteristics.

For decades, process chemical and petrochemical analysts addressed their analytical challenges mainly by relying on superior chromatography and smart tools such as valve switching, backflush, and Deans heart-cut. In combination with relatively cheap, robust, and selective detectors, they were capable of providing all information necessary to control and tweak petrochemical processes.

Organic catalyst poisoners are usually determined using dedicated chromatographic analyzers. These systems are, typically, equipped with a dual capillary column configuration with backflush and fitted with flame ionization detection (FID). Under these conditions, limits of detection are usually approximately 100 ppb, depending on the compound investigated and the complexity of the matrix that is introduced (4). Unfortunately, this is far from sufficient to protect the latest catalysts, which start to deteriorate as soon as fed with low parts-per-billion amounts (5,6). An overview of some typical specifications for catalyst poisoners in polymer-grade hydrocarbons is given in Table I.

Mass spectrometry (MS) is hardly used in petrochemical QC laboratories, which is primarily because of its apparent complexity and higher cost-of-ownership. Nonetheless, MS detection has several distinct advantages over classic analog detectors. In full-scan acquisition mode, for example, it allows tracking and identification of unknown components using spectral deconvolution and subsequent library matching. In selective ion monitoring (SIM) mode, on the contrary, MS permits trace and ultratrace quantification of target analytes which is often superior to classic selective detectors. Furthermore, MS permits the use of mass-labeled internal standards that behave identically to their native analogs, which has a positive effect on overall method precision and accuracy. It is no surprise that instrument manufacturers have invested substantially in solutions aimed at reducing overall MS system complexity and total cost of ownership in the past couple of years. Easy tune and calibration functionalities, increased sensitivity and speed, new acquisition modes, and elegant solutions that eliminate downtime, such as vacuum lock technology, have contributed largely in this respect.

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**Ghent University, Laboratory for Chemical Technology, Zwijnaarde, Belgium, †IS-X, Louvain-la-Neuve, Belgium.*

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Table I: Typical specifications for catalyst poisoners in polymer grade hydrocarbons (6)

Impurity	Typical Specification
Arsine	Less than 20 ppb
Phosphine	Less than 20 ppb
Ammonia	Less than 100 ppb
Hydrogen sulfide	Less than 20 ppb
Carbonyl sulfide	Less than 20 ppb
Nitrogen dioxide	Less than 50 ppb
HCN	Less than 100 ppb
HCl, HF	Less than 200 ppb
Phosgene	Less than 50 ppb
Sulfur dioxide	Less than 50 ppb
Chlorine	Less than 30 ppb

Table II: Overview of the GC settings

Oven	Setting	Remarks
Initial temp. (°C)	50	–
Initial time (min)	5.00	–
Final temp. (°C)	240	–
Final time (min)	10.00	–
Rate (°C/min)	5	Slow heating to maintain resolution
Inlet	**Setting**	**Remarks**
Type	Direct	–
Mode	Splitless	–
Temp. (°C)	200	–
Carrier	**Setting**	**Remarks**
Gas	Helium	–
Mode	Constant pressure	–
Setting (kPa)	50	–
Detector	**Setting**	**Remarks**
Type	FID	1.2
Temp. (°C)	200	1.2

Table III: Peak identification and typical selective ion monitoring ions

t_R, min	Name	SIM ions
24.52	Diethyl ether	59, 74
25.05	Acetaldehyde	44
26.48	ETBE	59, 87
26.79	MTBE	57, 73
26.92	Di-isopropylether (DIPE)	59, 87
27.93	Propanal	57, 58
28.92	*tert*-Amyl methyl ether (TAME)	73, 87
29.42	Propyl ether	73, 102
30.50	Isobutanal	72
31.78	Butyraldehyde	57, 72
32.82	Methanol	29, 31
33.45	Acetone	58
35.26	Valeraldehyde	57, 58
36.13	MEK	57, 72
36.50	Ethanol	31, 45
39.28	Isopropanol	45
39.45	Propanol	59, 60
40.21	Allyl alcohol	57, 58
41.54	Isobutanol	41, 74
41.64	*tert*-Butanol	57, 59
42.51	*n*-Butanol	55, 56

This article gives an overview of the main characteristics and performance of a new gas chromatography–mass spectrometry (GC–MS) analyzer that has been recently developed. The system combines the chromatographic separation power and backflush–Deans heart-cut capabilities of a classic oxygenate analyzer with the orthogonal separation power, sensitivity, selectivity, and overall robustness of the latest generation single-quadrupole mass spectrometers.

Experimental

Standards: Standard oxygenate reference mixture from Spectrum at 10 ppm in hexane. For more details with respect to the composition of the test mixture, please consult Table III. Calibration standards were prepared by gradual dilution in hexane at 0.01 ppm, 0.05 ppm, 1 ppm, and 5 ppm.

Gas chromatography: The GC analyzer consisted of a Thermo Trace GC system refurbished by Global Analyser Solutions (GAS). The system was fitted with a gas sampling valve (GSV), a liquid sampling valve (LSV), a vaporizer, a standard split–splitless injector, and an FID system. Inside the GC oven, a universal pressure balanced Deans assembly was installed to carry out heart-cut and backflush. Auxiliary pressure for balancing was provided and controlled by a separate Trace GC DCC unit.

The first-dimension column was a 15 m × 530 µm, 5-µm d_f Restek Rtx-1 column. The second-dimension column was a 10 m × 530 µm, 10-µm d_f Agilent CP-Lowox column. Restrictions were 250-µm i.d. uncoated Siltek-deactivated fused-silica capillary tubing (Restek) cut to the appropriate length. All connections were made using micro Siltite unions (SGE Analytical Science). Other relevant parameters are summarized in Table II.

Mass spectrometry: The GC analyzer was hyphenated to a Thermo ISQ single-quadrupole mass spectrometer. The system was applied in both full scan (range: 15–250 amu) and SIM (dwell time: 0.2 s) as full scan–SIM mode. All relevant MS settings are summarized in Table III.

All data were acquired using Thermo QuanLab Forms software. The MS system

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A Roadmap for Rapid HPLC Method Development: A Practical Three-Pronged Approach

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EVENT OVERVIEW:

HPLC method development is a challenging task, requiring the ability to optimize a large number of dependent and independent variables for complex samples. The traditional method development approach, while well accepted, is difficult to implement in practice, particularly for those with little experience. In this seminar, we describe the concept of a practical, three -pronged approach for rapid method development. The template consists of three prongs or types of methods:

1. Fast LC sub-2-minute methods for potency assays or simple mixtures.
2. Generic broad gradient or in-process control (IPC) methods for simple molecules or samples.
3. Multi-segment gradient methods for stability-indicating assays or ICH-compliant impurity methods for complex molecules or samples.

This template approach is highly applicable to conventional HPLC or ultra-high-pressure LC and to all sample types. It is particularly attractive for rapid HPLC method development for new drug candidates in early pharmaceutical development. A case study of a complex molecule with multiple chiral centers will be used to illustrate the utility of this approach.

Who Should Attend:

- Method development scientists
- Practitioners performing or wanting to learn more about HPLC method development (particularly those in early-stage pharmaceutical development)

Key Learning Objectives

Learn a template approach for rapid and efficient HPLC method development:

1. Fast LC for potency assays
2. Generic broad-gradient methods for purity assays
3. Multi-segment gradient methods for ICH-compliant stability-indicating assays of complex molecules or products

Presenter:

Dr. Michael W. Dong,
Senior Scientist,
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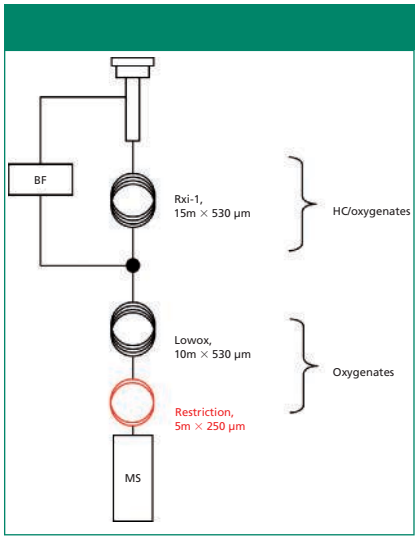

Figure 1: Schematic representation of the GC–MS analyzer. BF = backflush; HCs = hydrocarbons.

was used after running a full electron ionization (EI) tune. System performance was verified using a daily tune check.

Results and Discussion

System setup: The capillary column set comprises the true core of any classic catalyst poisoner analyzer. The second-dimension column is particularly important. Ultimately, it is here that separation of the analytes, from each other as well as from the aliphatic matrix in which they reside, occurs. A CP-Lowox column (Agilent) was used for this purpose. This column, which is based on a multilayer porous layer open tubular (PLOT) concept, is very polar and characterized by a high maximum allowable operating temperature with virtually no bleed at temperatures as high as 350 °C (7). In combination with a backflushed apolar-coated capillary column in the first dimension, matrix separations up to C_{12} hydrocarbons are well within range.

Unfortunately, the CP-Lowox column is not available in MS-friendly narrow-bore dimensions. To avoid the MS vacuum from protruding the system, it is necessary to incorporate an adequate restriction at the back of the column. A schematic representation of system setup for Lowox/MS applications is depicted in Figure 1.

True backflush, as well as Deans heart-cut, is achieved by increasing the auxiliary pressure at the column joint just above the first-dimension residual head pressure at a certain moment in time. Debalancing induces full flow reversal, while maintaining a small flow over the Lowox column for chromatography. It is crucial to know the exact pressure at the column joint for this approach to be successful. When the pressure is set too low, standard flow direction will be maintained and the first column is not backflushed. Conversely, when it is set too high, none of the target analytes will be able to reach the second-dimension column and the detector. The easiest way to determine the pressure at the column joint involves setting the head pressure at regular and then reading the residual pressure at the auxiliary digital carrier gas control, which is kept off at this stage. Pressure differences of 5 kPa are sufficient to induce flow reversal. Although less straightforward because of the vacuum conditions, a similar approach is applied in combination with MS. It also permits users to determine the minimal length of the restriction capillary (Figure 1).

System suitability: System suitability was evaluated by direct injection of the 10 ppm oxygenate standard. To compare with a classic analyzer set-up, experiments were performed using both FID

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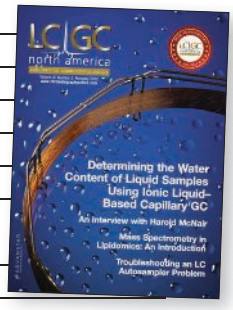

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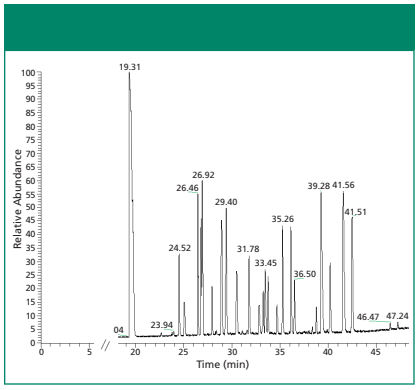

Figure 2: Chromatogram of the oxygenates standard at 10 ppm. The MS system was applied in full-scan mode. Peak identification is referred to in Table III.

and MS for detection. The MS system was applied in both full-scan and SIM modes. A typical chromatogram with the MS in full-scan mode is depicted in Figure 2. Peak identification is referred to in Table III.

A comparative overview of the results is given in Table IV. For each peak, the signal-to-noise ratio (S/N) was calculated (root mean square [RMS]) in full scan, extracted ion, and SIM mode. These results were subsequently expressed relative to the S/N with FID.

The results in Table IV clearly illustrate the significant gains in sensitivity that can be reached when using MS compared to FID. Minimal gain is 3.7 for ethanol. Unsurprisingly, sensitivity gains are particularly significant when the MS was used in SIM mode. Straight full-scan mode proved to be less appropriate for target analysis, which is predominately due to the low molecular weight of the target compounds, and means having to include highly interfering masses such as m/z =18 (water), 28 (nitrogen) and 32 (oxygen) in the scan range. More convenient in this respect is the combined full-scan–SIM mode, which is available on all major instrument brands nowadays.

Afterward, calibration curves were constructed for each of the oxygenates in the standard mixture from 0.05 ppm to 5 ppm. Correlation coefficients were ≥0.995. Some typical SIM traces at the 0.10 ppm level are depicted in Figure 3.

Method repeatability was determined as well. Results at the 10 ppm level are included in Table IV (six consecutive analyses).

Applications

Naptha feed: Naphtha is a complex mixture of hydrocarbons (C_5–C_{12}) in petroleum boiling between 30 °C and 200 °C. Oxygenates are routinely determined in these samples according to reference procedures such as ASTM D7423 (4) as their cracking product causes problems in the downstream separation processes (8). Naphthas are very complex and fully require the chromatographic separation power of the Lowox column. A typical chromatogram of a naphtha sample in SIM mode is depicted in Figure 4. Individual samples were introduced using the LSV of the GC–MS analyzer. The insert shows the methanol trace (ion 29, 0.18 ppm); concentrations of acetaldehyde and TAME were 8.3 and 4.9 ppm, respectively.

When idle, the GC oven was kept at 200 °C with the backflush activated. This was necessary to prevent the accumulation of siloxane bleed from the precolumn.

Conclusions

A GC–MS analyzer is described that substantially expands the workable application range of a classic catalyst contaminants

Table IV: Average sensitivity gains in different MS detection modes. FS = full scan; EIC = extracted ion chromatogram; SIM = selected ion monitoring. %RSD at the 10 ppm level (six analyses).

Name	MS (FS), S/N	MS (EIC), S/N	MS (SIM), S/N	%RSD
Diethyl ether	1.2	2.6	81	9.27
Acetaldehyde	0.2	4.1	10	18.9
ETBE	0.6	9.9	66	7.56
MTBE	0.6	3.1	53	6.84
Diisopropylether	0.7	6.5	70	10.1
Propanal	0.3	6.6	27	11.9
tert-Amyl ether	1.1	4.4	64	4.60
Propyl ether	1.3	17	70	6.56
Isobutanal	0.4	5.0	38	5.35
Butyraldehyde	0.8	4.4	23	2.31
Methanol	1.5	1.8	6.5	12.2
Acetone	0.8	18	100	7.21
Valeraldehyde	1.5	8.4	177	5.09
MEK	1.3	2.0	4.4	10.9
Ethanol	0.3	1.2	3.7	13.4
Isopropanol	0.6	2.9	5.6	18.7
Propanol	0.5	1.6	5.5	12.4
Allyl alcohol	0.3	1.1	14	11.0
Isobutanol	1.1	8.1	45	11.7
tert-Butanol	0.9	2.1	49	9.34
n-Butanol	0.7	1.9	25	11.7

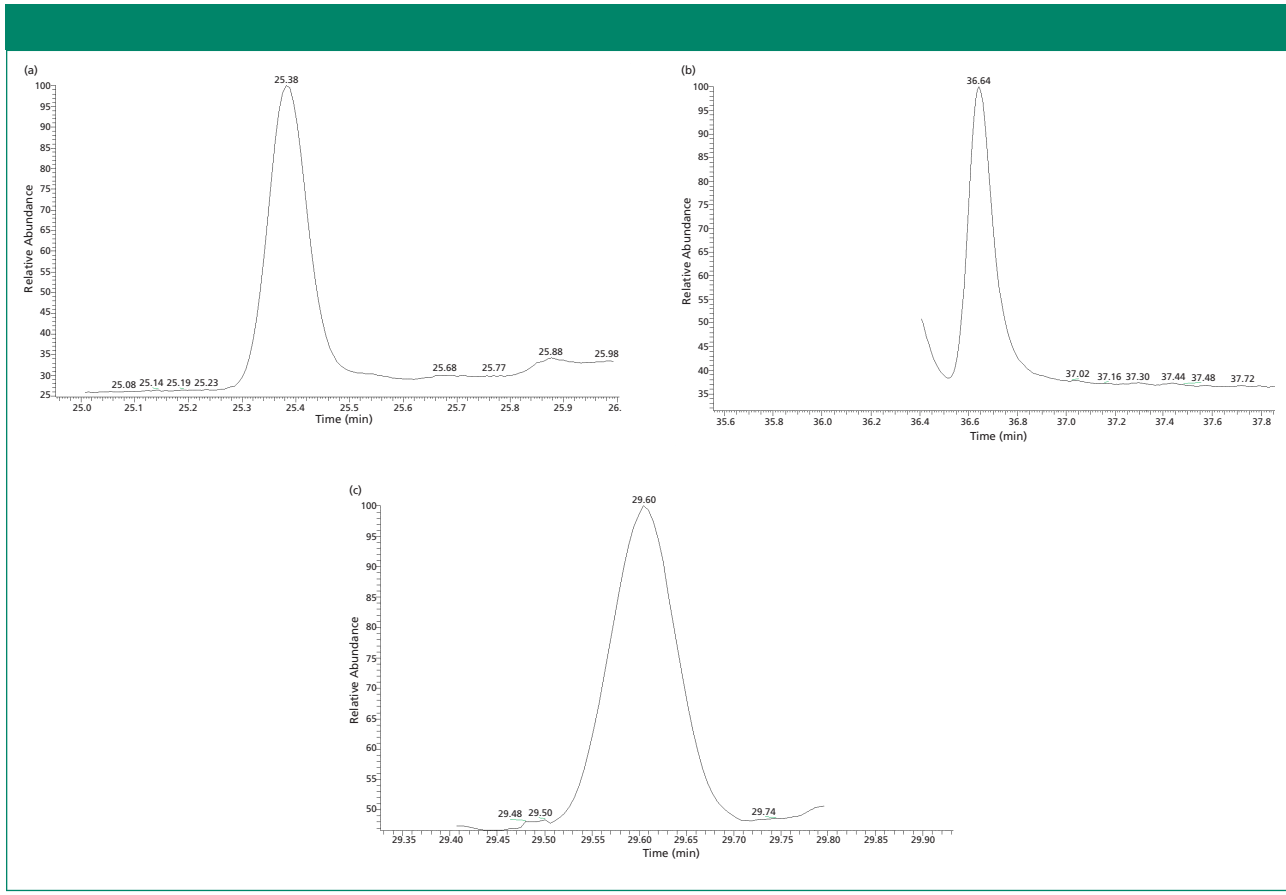

Figure 3: SIM traces at 0.01 ppm: (a) acetaldehyde, (b) ethanol, and (c) propyl ether.

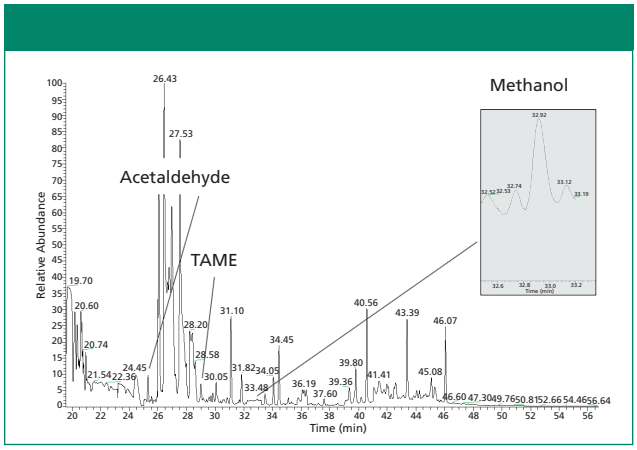

Figure 4: Typical SIM trace of a naphtha feed.

analyzer. The use of MS in full-scan–SIM mode permits identification of unknown contaminants in combination with reliable quantification at trace and ultratrace amounts.

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Jean-Louis Brix *is an analytical support engineer at IS-X. He specializes in instrument design and method development for petrochemical applications.*

Joeri Vercammen *is managing expert of IS-X. He specializes in method development and rationalization from prep-to-rep, method validation, and quality assurance.*

Guy B. Marin *is chair of the department of chemical engineering and technical chemistry at Ghent University, Belgium. Chemical reaction engineering, catalysis in general, and reaction kinetics are the main topics in his research program.* ■

PRODUCT RESOURCES

Analytical SFC system

The Acquity UPC^2 is designed as a laboratory system for analyzing compounds that include hydrophobic and chiral analytes, lipids, thermally labile samples, and polymers. According to the company, compressed carbon dioxide is the primary mobile phase.
Waters Corporation,
Milford, MA.
www.waters.com

Chromatography software

DataApex has translated its Clarity chromatography software to the German language. According to the company, the German version was developed in cooperation with Techlab GmbH and will be distributed to all German-speaking countries. The software reportedly enables users to control more than 300 different instruments.
DataApex, Prague, The Czech Republic.
www.dataapex.com

Vacuum pumps

The nXDS dry scroll vacuum pumps from Edwards are designed as oil-free dry pumps with no need for regular oil changes. According to the company, tip seals may last up to five years, and can be replaced in less than 10 min in the field using basic workshop tools. **Edwards,**
Sanborn, NY.
www.edwardsvacuum.com

Hybrid SFC–UHPLC system

The 1260 Infinity Hybrid SFC/UHPLC system is designed to perform both supercritical fluid chromatography and ultrahigh-pressure liquid chromatography. According to the company, in SFC mode, the instrument uses standard-grade gaseous CO_2 for cost savings over SFC-grade CO_2.
Agilent Technologies,
Santa Barbara, CA.
www.agilent.com

MS sample introduction system

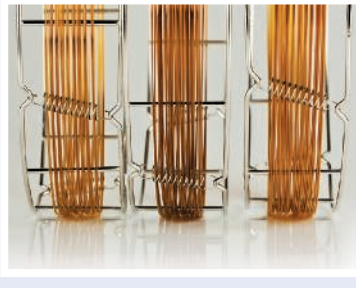
The AxION DSA direct sample analysis system from PerkinElmer is designed to eliminate sample preparation steps and the need for front-end gas or liquid chromatography separation. According to the company, the system enables users to introduce samples directly into the company's AxION time-of-flight mass spectrometer. **PerkinElmer,**
Waltham, MA.
www.perkinelmer.com

Microchannel plates

The L3N microchannel plates from Photonis are designed to provide a 100-fold reduction in background noise when compared to traditional microchannel plates. According to the company, at 0.01 counts/s/cm^2, the microchannel plates' dark count level approaches the background level of cosmic rays.
Photonis,
Sturbridge, MA.
www.photonis.com

GC columns

GC capillary columns from Quadrex are manufactured in standard internal diameters and lengths with a wide range of film thicknesses. According to the company, specialty columns for environmental, petrochemical, triglyceride (biodiesel), pharmaceutical, and high temperature applications are available.
Quadrex Corporation, Woodbridge, CT.
www.quadrexcorp.com



HPLC fluorescence detectors

Shimadzu's fluorescence detectors are designed to provide sensitivity and validation support functions in a range of applications for conventional and ultrafast LC analysis. According to the company, the model RF-20A and RF-20Axs detectors have water Raman S/N ratios of at least 1200 and 2000, respectively.
Shimadzu Scientific Instruments, Inc.,
Columbia, MD.
www.ssi.shimadzu.com

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Reversed-phase protein column

Tosoh's TSKgel Protein C4-300 reversed-phase LC columns are designed for high resolution and recovery of proteins. According to the company, ligand density and alkyl length in the stationary phase is optimized for reduced adsorption of protein and minimized peak tailing.

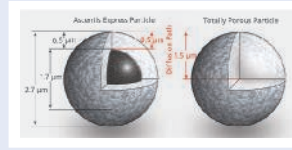
Tosoh Bioscience, LLC, King of Prussia, PA.
www.tosohbioscience.com

Light-scattering instrument

Wyatt's Möbiuζ light-scattering instrument is designed to make reproducible, nondestructive zeta potential measurements of macromolecules as small as 1 nm, under dilute solution conditions. According to the company, the instrument can be connected to an HPLC pump and autosampler for automating the measurements.

Wyatt Technology Corporation,
Santa Barbara, CA.
www.wyatt.com

HPLC column



The Ascentis Express ES-Cyano HPLC column from Supelco/Sigma-Aldrich is based on 90-Å particles. According to the company, the column's stationary phase is a sterically protected, encapped diisopropyl-cyanopropylsilane reversed-phase packing that can be used for basic, acidic, and neutral compounds. The column's particles reportedly consist of a thin porous shell of high-purity silica surrounding a solid silica core with an overall particle size of 2 µm.

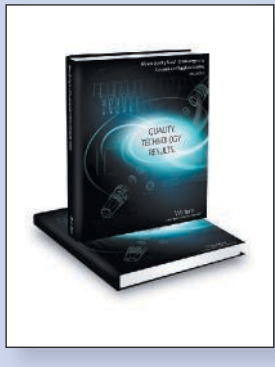
Supleco/Sigma-Aldrich,
Bellefonte, PA.
www.sigma-aldrich.com/express

Ion chromatography system

The Dionex ICS-4000 HPIC system from Thermo Fisher Scientific is designed for high-pressure ion chromatography. The system reportedly integrates multiple detectors and short columns with smaller particles for high-throughput capillary ion chromatography separations. According to the company, the system permits the use of electrochemical detection for carbohydrate analysis. Other applications include environmental, food safety, pharmaceutical, biopharmaceutical, life science, and chemical analyses.

Thermo Fisher Scientific,
Waltham, MA.
www.thermoscientific.com/IC

Literature



Parts and supplies catalog

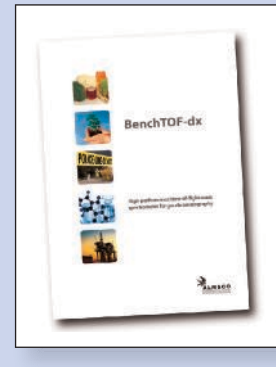
The 2012–2013 *Waters Quality Parts, Chromatography Columns and Supplies Catalog* contains the company's line of parts, branded LC columns, vials, filters, sample preparation products, and supplies for LC, LC–MS, UPLC, UPC^2, GPC, and SFC separations.

Waters Corporation,
Milford, MA.
www.waters.com

Water analysis application note

An application note from OI Analytical describes the use of a purge-and-trap gas chromatography system to analyze methane, ethane, ethene, and propane hydrocarbons (C_1–C_3) in drinking water samples.

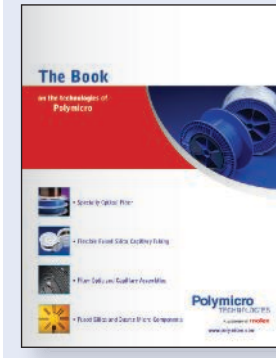
OI Analytical,
College Station, TX.
www.oico.com



Mass spectrometer brochure

Almsco International's brochure for the company's BenchTOF-dx time-of-flight mass spectrometer for GC contains information about the product's ability to provide full-range spectra at the sensitivity levels of quadrupole instruments running in selected ion monitoring mode. According to the brochure, the instrument provides sub-unit mass resolution.

Almsco International,
Llanstrisant, UK. www.almsco.com



Product handbook

Polymicro's *The Book on the Technologies of Polymicro* is a guide to the company's optical fibers, capillary tubing, and related assemblies and microcomponents for chromatography, spectroscopy, and general laboratory applications. The publication also includes specification sheets.

Polymicro Technologies, a Subsidiary of Molex,
Phoenix, AZ.
www.polymicro.com

EDITORS' SERIES

Optimizing GC Methods for Speed: More than Just Columns

LIVE WEBCAST: Wednesday, May 23, 2012 at 2:00 PM EDT

Register Free at www.chromatographyonline.com/speed

EVENT OVERVIEW:

Most discussions of "fast GC" focus on developments related to columns: shorter, smaller inside diameters and thinner films. While the column is the "heart" of the method, the other components and steps in the method also have strong effects on analysis speed and time. In this web seminar, a brief overview of column-related strategies for improving GC method speed will be presented, followed by a discussion of implications on the other components of the method. Faster separations on the column impact all facets of the overall method: sampling, sample preparation, injection, detection and data analysis. Thinking about all of these areas enables chromatographers to more carefully consider how changes to the separation itself to increase speed should be approached. It is possible that the most important gains in the speed of the overall method may not come from the separation itself.

Key Learning Objectives:

- A brief overview of column-related strategies for improving GC method speed
- The implications on the other components of the method such as sampling, sample preparation, injection, detection and data analysis
- How to approach changes to all of those components, when seeking to increases the speed of GC separations

PRESENTER:

Nicholas H. Snow,
Department of Chemistry and Biochemistry,
Center for Academic Industry Partnership,
Seton Hall University, South Orange, New Jersey

MODERATOR:

Meg Evans,
Managing Editor,
LCGC North America

WHO SHOULD ATTEND:

- Bench chemists involved in GC separations
- Method development experts who wish to optimize their GC separations
- Other scientists interested in learning more about fast GC separations

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For questions contact Jamie Carpenter at Jcarpenter@advanstar.com

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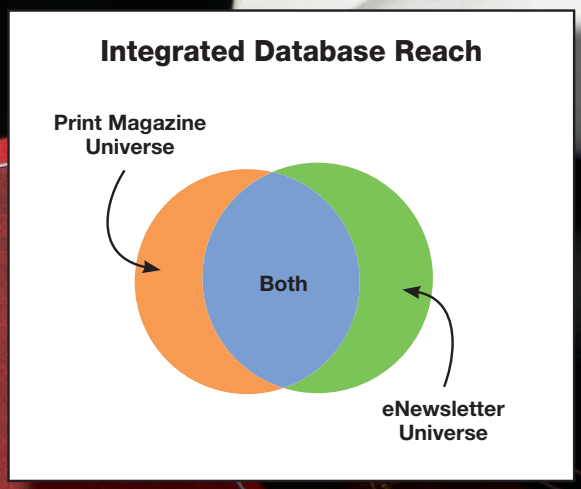

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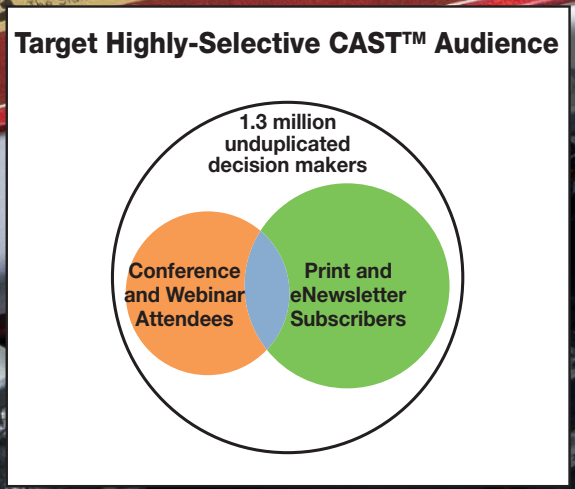
Pharmaceutical Science Webinar Attendees	41,581
Total Pharmaceutical Science Conference and Webinar Attendees	72,988*
Total Unduplicated Print Magazine, eNewsletter, Conference and Webinar	180,609**



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www.chromatographyonline.com

A New Class of Photoresponsive Polymers that Undergo Controlled & Complete Polymer Chain Scission upon Irradiation at 300nm

LIVE WEBCAST: Wednesday, May 16, 2012 at 11:00AM EDT

Register free at www.chromatographyonline.com/300nm

EVENT OVERVIEW:

Stimuli responsive materials are of interest in a number of applications. Of these, photoresponsive materials have a unique advantage since their response can be temporally controlled. We have developed a series of photodegradable phenacyl based polycarbonates. Hydroxyphenacyl based phototriggers, developed by Givens and coworkers, have been demonstrated to be a very efficient photoactivated protecting group for the release of esters, phosphates and thiols. We have incorporated this photoactive unit in the polymer backbone and irradiation of this polymer at 300 nm leads to polymer chain scission that results in substantial decrease in molecular weight. These polymers undergo photoinitiated degradation in both solution and the solid state. Well defined 2D patterns can be formed from films of these polymers by the use of simple masks. These polymers also have the potential for controlled release applications. As a model, the release of dyes such as rhodamine 6G or therapeutics such as indomethacin indicate controlled release upon irradiation of polymer films encapsulated with these molecules.

Key Learning Objectives:

- Learn about a new class of photoresponsive materials that undergo controlled chain scission upon irradiation at 300nm and become familiar with other work in this field.
- Learn about the advantages of such polymers over traditional biomaterials and how they could be used in applications such as in controlled drug delivery systems and in creating micro-patterned surfaces.

Who Should Attend:

- Scientists interested in the synthesis and characterization of photoresponsive polymers
- Scientists looking for alternative approaches for controlled delivery of therapeutics and additives
- Scientists interested in creating micro-patterned surfaces

Speaker:

Abraham Joy
Assistant Professor
Department of Polymer Science
The University of Akron

Moderator:

Laura Bush
Editorial Director
LCGC North America

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For questions contact Jamie Carpenter at jcarpenter@advanstar.com

Increased Throughput for Trace Impurities and Residual Solvent Determination with Unattended Liquid/Headspace Switching

ON-DEMAND WEBCAST

Register free at www.chromatographyonline.com/traceimpurities

EVENT OVERVIEW

US Pharmacopeia (USP) method <467> details the procedures for the identification, control and quantification of Class 1 and Class 2 residual solvents through the use of headspace gas chromatography. Some pharmaceutical laboratories must also analyze solvent impurities as part of the incoming raw material testing process according to the various solvents' monographs. For this purpose, liquid GC injection is normally chosen.

Because of the limitations of the GC instrumentation currently available, most of these laboratories dedicate one instrument to headspace analyses and another one to liquid injection analyses even if their injector/detector configuration is the same, which places limitations on lab productivity and spending.

In this webinar, we will present the use of an innovative robotic autosampling platform which possesses the ability to automatically switch from liquid to headspace mode and use different syringe volumes in the same run or sequence onto a single GC.

Test results of residual solvents quantification, where sample and standard preparation steps according to the USP method <467> procedures are carried out by the autosampler prior to the headspace analysis, will be shown. The lack of carryover achieved by the use of a headspace gas-tight syringe that is heated and flushed between injections will also be discussed.

Key Learning Objectives:

- Identify the sample throughput benefits of a new robotic autosampling platform that is able to switch automatically from liquid to headspace mode
- Discuss the results of a time-saving automated sample preparation procedure executed by the autosampler prior the headspace analysis
- Demonstrate how a GC robotic platform can increase overall laboratory productivity

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Who Should Attend:

- Anyone who uses GC equipment and wants to benefit from the use of a robotic platform that is able to automatically prepare and inject samples during the same sequence
- Laboratories performing VOC analysis by gas chromatography that need to extend their sample throughput, for example, by reducing the time spent on sample/standard preparation by lab personnel
- Quality control laboratories running USP <467> method
- Laboratories analyzing various samples by liquid, headspace and solid phase micro extraction (SPME) injections that may benefit from unattended syringe-switching operations

Presenters:

Silvia Gemme
Gas Chromatography
Product Specialist
Thermo Fisher Scientific

Massimo Santoro
Gas Chromatography
Product Manager
Thermo Fisher Scientific

Moderator:

Laura Bush
Editorial Director
LCGC North America

For questions, contact Jamie Carpenter at jcarpenter@advanstar.com

AD INDEX

CHROMATOGRAPHY SERVICES

H P L C INSTRUMENTS & SUPPLIES

THE ESSENTIALS

Column Characterization Databases

There are many factors influencing the efficiency of a high performance liquid chromatography (HPLC) stationary phase. Of these factors, chemical nature of the bonded phase ligand is important, but by no means all encompassing in determining the important phase characteristics.

In 2005, there were about 220 C18 (L1) phases available (1). One can only speculate on the number available today, and each will be subtly different in separating the analytes that we are interested in.

As users and developers of HPLC methods, we need to improve our understanding of the factors affecting separations so that we can better understand problems when they occur and learn to exploit key stationary-phase characteristics to our advantage during method development or improvement.

This might be achieved by testing each (and every!) stationary phase using a standard set of chemical probes that we know will react in a predictable way, depending upon the phase characteristics. In this way, we can produce comparative data that will allow us to select phases that we suspect might be best at exploiting important chemical and physicochemical differences between our analytes. We can then map column characteristics and group columns (even those of the same "nominal" bonded phase) into those that are "similar" or "different" (sometimes called "orthogonal" in this context), allowing us to manipulate our analyte retention and separation selectivity accordingly.

Several attempts have been made to produce a definitive set of chemical probes and tests to best characterize the huge number of stationary phases available (currently estimated to be well over 1000 distinct chemistries). As yet, a harmonized set of test probes and methodologies has not come to the forefront; however, there are some notable groups of scientists working in this field.

An early attempt at producing a generic set of probes for testing HPLC column characteristics was made by Tanaka and coworkers (2). Since then, work by the USP Working Group on HPLC Columns, the Impurities Working Group of the PQRI Drug Substance Technical Committee in collaboration with Lloyd Snyder (3), and Euerby and Petersson (4) has expanded the original probes designed by Tanaka. These groups have all attempted to identify a definitive set of probes that will allow the various important physicochemical phase characteristics to be specified. Most of these researchers also have combined their data with various chemometric and statistical approaches to produce quantitative databases based on principal component analysis (PCA) or other statistical methods to visualize the relative groupings of commercially available columns according to their key descriptors as well as computing a single numerical "similarity" factor, for simple comparison of orthogonality.

Even though the chemical probes used differ between the research groups, there are some common themes in the attributes of a stationary phase that are considered of primary importance in characterizing the stationary phase behavior:

- retention based on a hydrophobic probe
- ability to discriminate between probes of similar hydrophobicity (hydrophobic selectivity)
- ability to discriminate between analytes of different shape or hydrodynamic volume (shape or steric selectivity)
- extent of hydrogen bonding with acids or bases (typically via the silanol surface, polar end capping reagents, or functional groups within the bonded ligand)
- extent of ion-exchange interactions at low and mid pH (pH 2.8 and 7.0 is typical to differentiate between situations in which surface silanol species will be potentially ionized or ion suppressed).

Figure 1 shows how one might simply represent column characteristics for comparison.

The various databases developed by these three groups can be used to identify similar or different phases and, with some increased understanding of the various characteristics described, make some rudimentary predictions about which column (type) might be best suited for a particular analysis.

The three main databases are all publicly available and can be found at the following locations:

- USP and PQRI databases: www.usp.org/USPNF/columnsDB.html
- ACD Labs Column Selector — based on the work of Euerby and Petersson: www.acdlabs.com/products/adh/chrom/chromproc/index.php#colsel

References

(1) *Pharmacop. Forum* **31**(2), (Mar.–Apr. 2005).

(2) K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, and N. Tanaka, *J. Chromatogr. Sci.* **27,** 721 (1989).

(3) N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, R.G. Wolcott, and P.W. Carr, *J. Chromatogr. A* **961,** 171–193 (2002).

(4) M.R. Euerby and P. Petersson, *J. Chromatogr. A* **994,** 13–36 (2003). ■

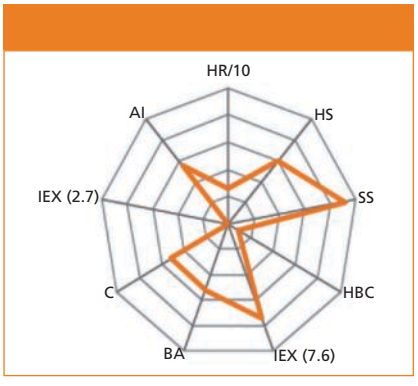

Figure 1: Spider diagram representing the various characteristics of a stationary phase (Accucore PF, Thermo Fisher Scientific). HR = hydrophobic retention; HS = hydrophobic selectivity; SS = steric selectivity; HBC = hydrogen bonding capacity; BA = base activity; C = chelation; IEX = ion-exchange capacity at pH 2.6 and 7.6; AI = acid interaction. (Reproduced with permission from Thermo Fisher Scientific.)

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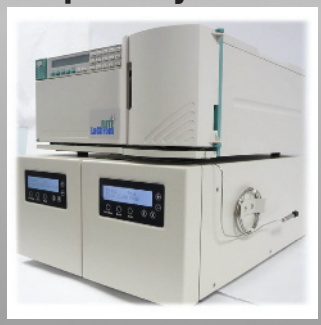

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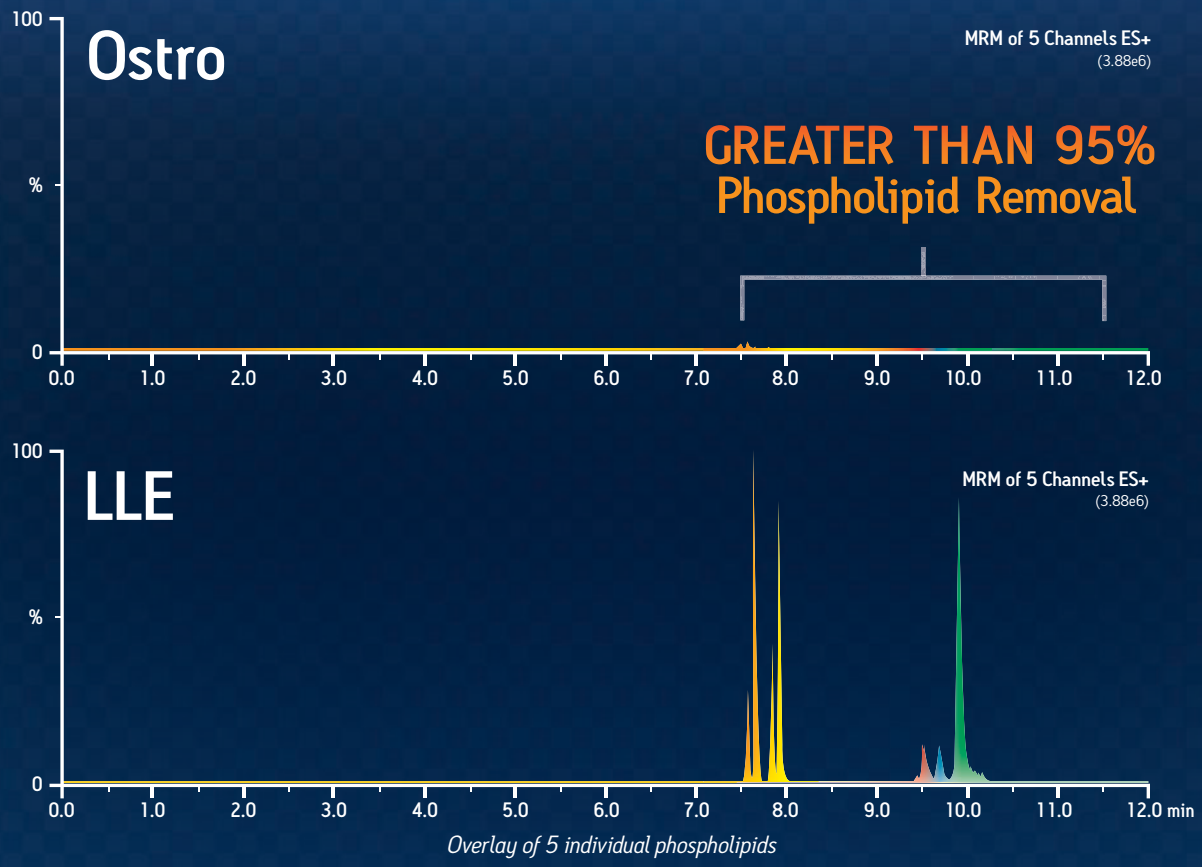

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