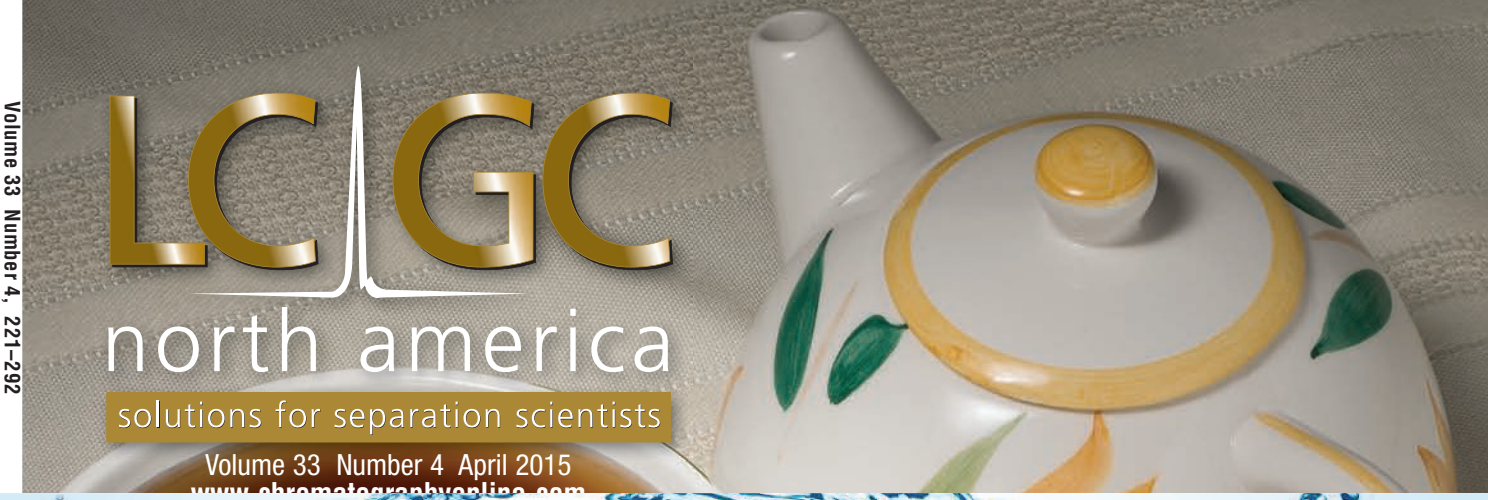
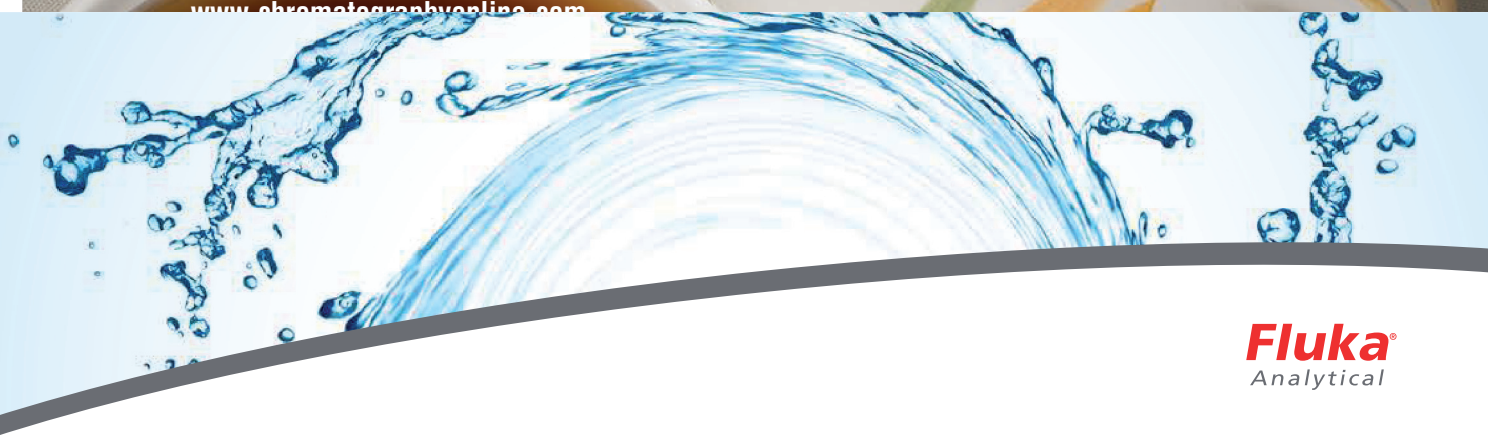
Volume 33 Number 4, 221–292





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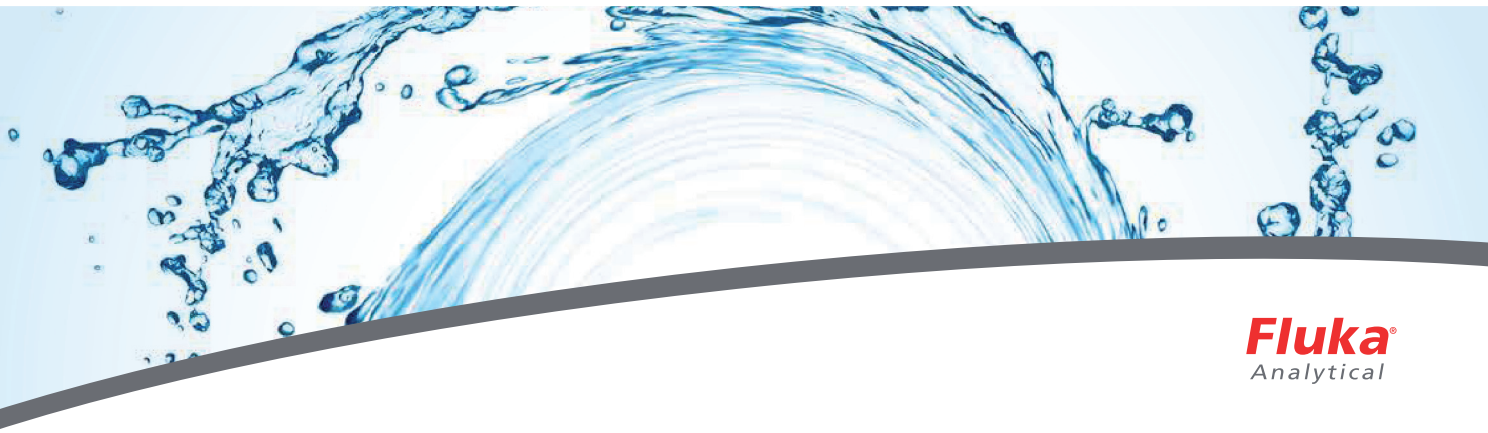

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34671	Methanol with 0.1% formic acid LC-MS CHROMASOLV
34675	Water with 0.1% acetic acid LC-MS CHROMASOLV
34674	Water with 0.1% ammonium acetate LC-MS CHROMASOLV
34673	Water with 0.1% formic acid LC-MS CHROMASOLV

Specifications

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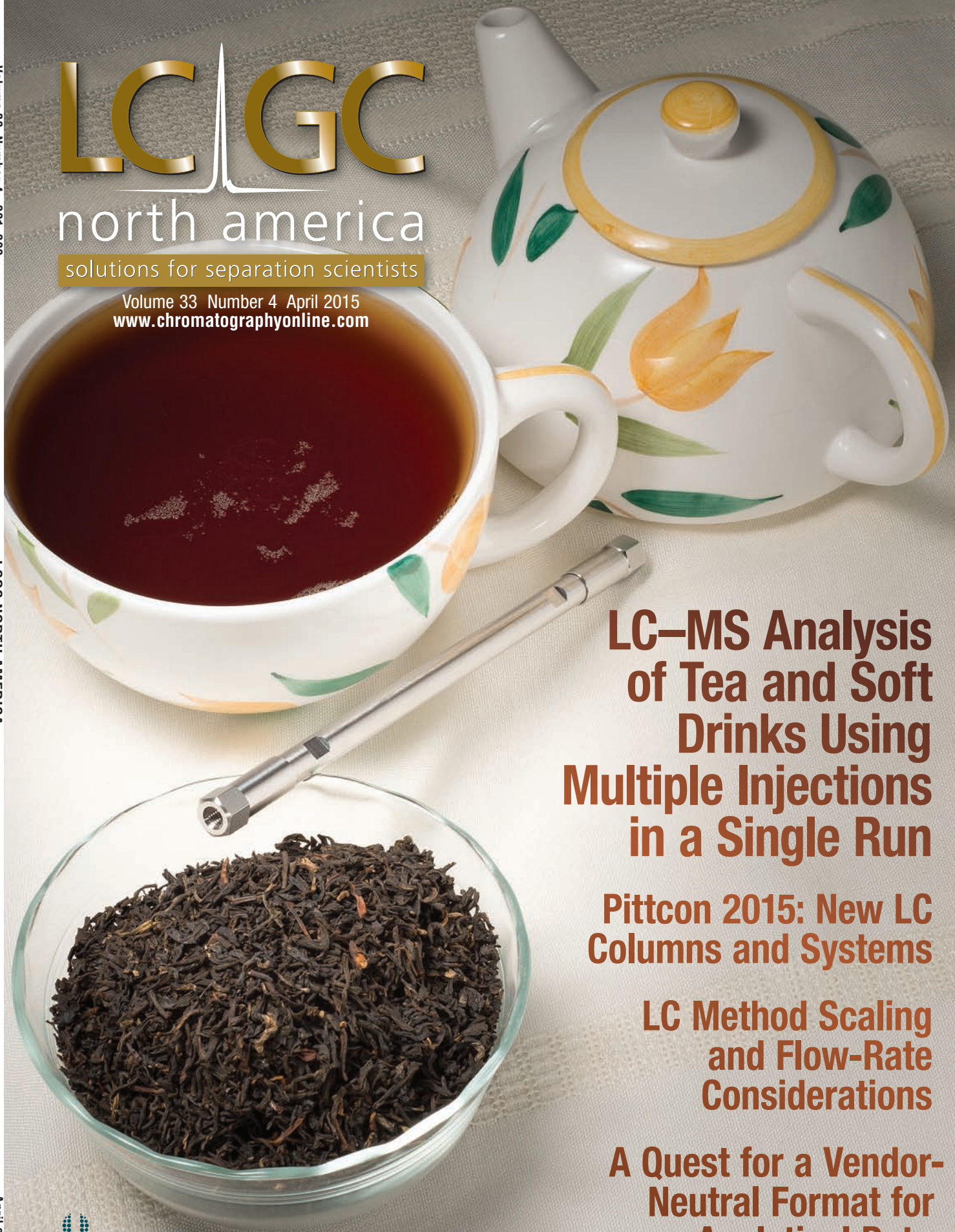

LC–MS Analysis of Tea and Soft Drinks Using Multiple Injections in a Single Run

Pittcon 2015: New LC Columns and Systems

LC Method Scaling and Flow-Rate Considerations

A Quest for a Vendor-Neutral Format for Analytical Data

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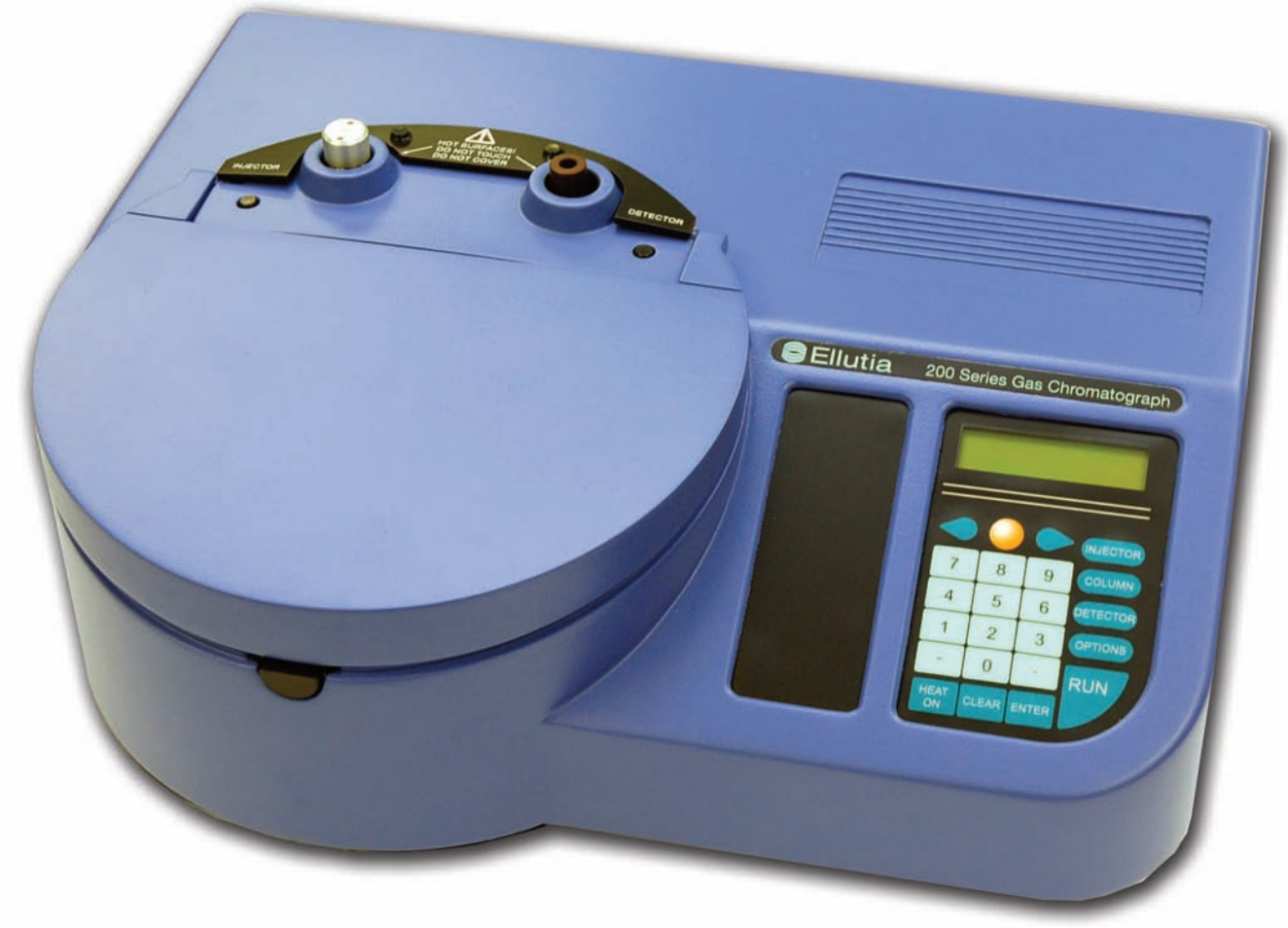

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1.82	19.0	19.0
±3,4,5	3,4,5,6	-1,2,4,6,7

34	91	88	22	8	7	16
78.96	231.036	2326.025	47.867	15.999	14.007	32.07
685	1600	1536	3289	-182.82	-195.65	444.75
221		700	1670	-222.65	-209.86	115.36
2.5	1.1		1.6	3.5	3.1	2.4
Se	**Pa**	**Ra**	**Ti**	**O**	**N**	**S**
$[Ar]3d^{10} 4s^2 4p^4$	$[Rn]5f^2 6d^7 f^2$	$[Rn]7s^2$	$[Ar]3d^2 4s^2$	$[He]2s^2 2p^4$	$[He]2s^2 2p^3$	$[Ne]3s^2 3p^4$
4.79	4.79	5.0	5.0	1.43	1.25	2.07
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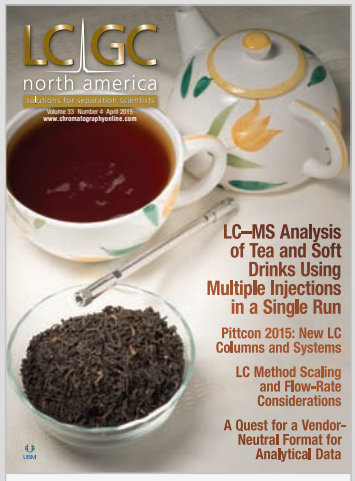

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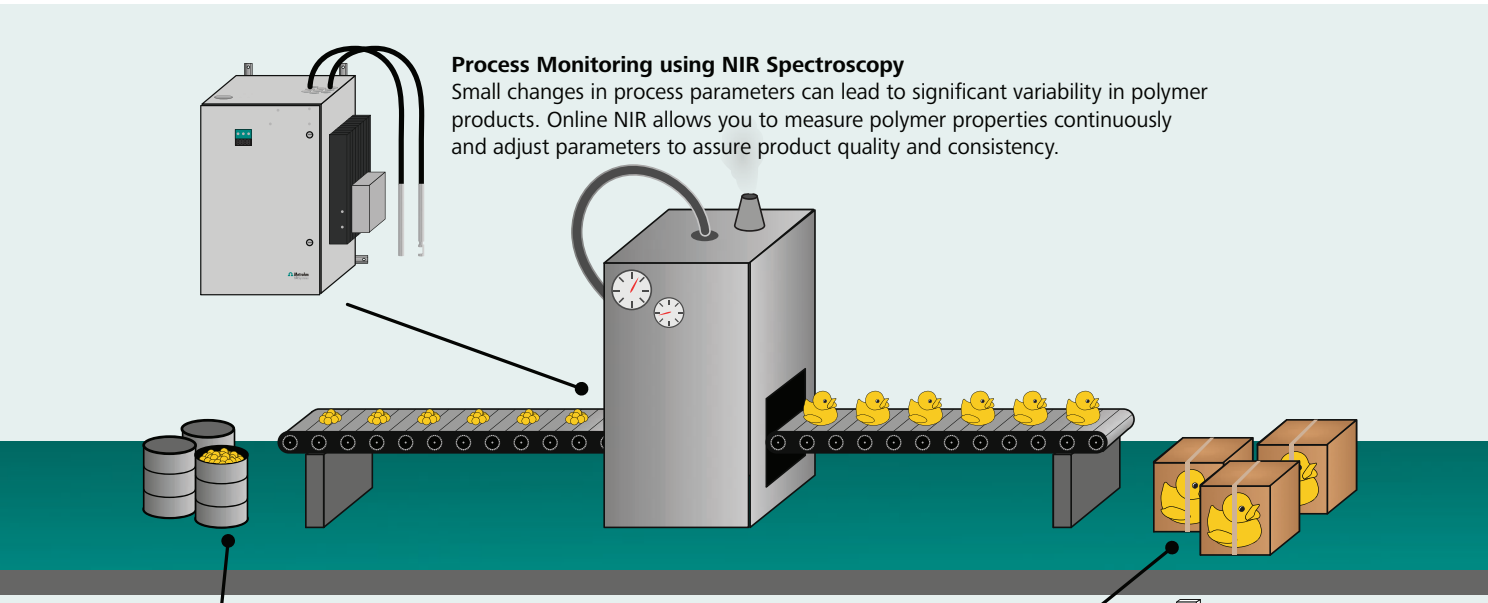

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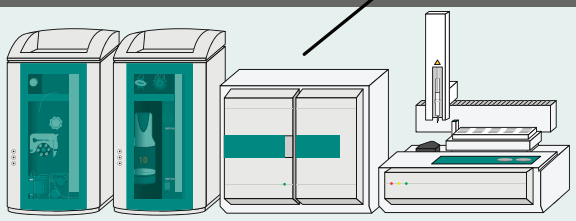

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PEAKS of Interest

Caroline West Joins LCGC's *Editorial Advisory Board*

LCGC magazine is pleased to announce the addition of Caroline West to its editorial advisory board. West is an associate professor of analytical chemistry at the University of Orléans (France), in the Institute of Organic and Analytical Chemistry.

She is the winner of *LCGC*'s 2015 Emerging Leader in Chromatography award, presented at Pittcon in March. The award recognizes the achievements and aspirations of a talented young separation scientist who has made strides early in his or her career toward the advancement of chromatographic techniques and applications. Other awards West has won include the Best Young Scientist award (Chemometrics in Analytical Chemistry 2012, Budapest, Hungary), Best Poster (SFC 2012, Brussels, Belgium), Second Best Poster (Conferentia Chemometrica 2011, Sümeg, Hungary), and Outstanding Poster (HPLC 2010, Boston, Massachusetts).

West's research interests are in supercritical fluid chromatography (SFC), chromatographic columns, hydrophilic-interaction chromatography (HILIC), enantioselective chromatography, and chemometrics.

She is the author of approximately 50 papers in peer-reviewed journals, including an influential review on graphitic carbon stationary phases, a significant paper that unraveled retention mechanisms in the HILIC mode, and an article on the development of a useful classification of stationary phases devoted to SFC.

The original unified classification of stationary phases that West developed is based on a large set of results she accumulated, and can be applied to either high performance liquid chromatography (HPLC) or SFC. Her classification is a five-dimensional one, based on the five coefficients of the linear solvation energy relationship model that she calculated for 100 solutes and over 70 stationary phases, divided into three groups: nonpolar, polar, and aromatic. This nearly exhaustive classification helps analysts choose the stationary phase best suited to perform a new separation.

Before her career in academia, West worked in industrial and governmental laboratories. She spent one year at Kodak Limited R&D in Harrow, England, and later worked with the Laboratory of the Central Police in Paris, in the explosives and fires division. In 2002, she began working in the Laboratory for the Study of Techniques and Instruments of Molecular Analysis with the analytical chemistry group of University of Paris-Sud at the University Institute of Technology.

She received her degree as an Engineer in Chemistry from the National Superior School of Chemistry of Mulhouse in 2002, a master's degree in analytical chemistry from the University of Paris VI in 2002, and a doctoral degree in chemistry from the University of Paris-Sud in 2005. ■

LCGC*tv* New videos from *LCGC*

GIORGIA GRECO ON REVERSED-PHASE LC COUPLED TO HILIC

Greco, of the Technische Universität Munchen, Germany, discusses the different options available for combining HILIC to reversed-phase LC, and how HILIC can be hyphenated with atmospheric pressure chemical ionization MS.

Other recent LCGC *TV interviews include:*

- Attila Felinger on how developments in "fast LC" technology have changed the field of bioanalysis
- Anthony Gravell on the advantages of GC×GC for the analysis of passive sampling extracts
- Doug Raynie on trends in sample preparation with respect to both liquid and solid samples

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HIGHLIGHTS OF APPS FOR SEPARATION SCIENCE

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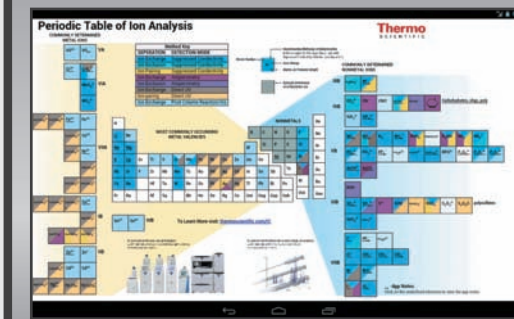
APP NAME: Periodic Table of Ion Analysis

OFFERED BY: Thermo Fisher Scientific

PLATFORMS: Android (requires 2.3 and up). Compatible with iPad (requires iOS 6.0 or later).

WHAT IT DOES: The Periodic Table of Ion Analysis app is designed as a reference of optimal methods for separating and detecting the most commonly determined ions on the periodic table. According to the company, users can click on their element of choice to view application and technical notes to achieve the best chromatographic performance to simplify their analysis.

COST: Free



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COLUMN WATCH

New Chromatography Columns and Accessories for 2015

This is our annual review of new liquid chromatography (LC) columns and accessories introduced at Pittcon and throughout the previous year. This year, Michael Swartz, former author of our "Innovations in HPLC" and "Validation Viewpoint" columns, steps in as a guest columnist to write the review.

M. Swartz, *guest author of Column Watch*

For 32 years, *LCGC* has published an annual Pittcon review article, written by Ron Majors. These reviews started with Majors's second installment of the "Column Watch" column in May 1983. Each year, Majors summarized the new chromatography columns and sample preparation instruments introduced at the conference, while also explaining the significance of these developments and analyzing the trends. Over the years this article has become the standard reference describing the latest developments in the field, and I'm honored to be the guest author of this year's review.

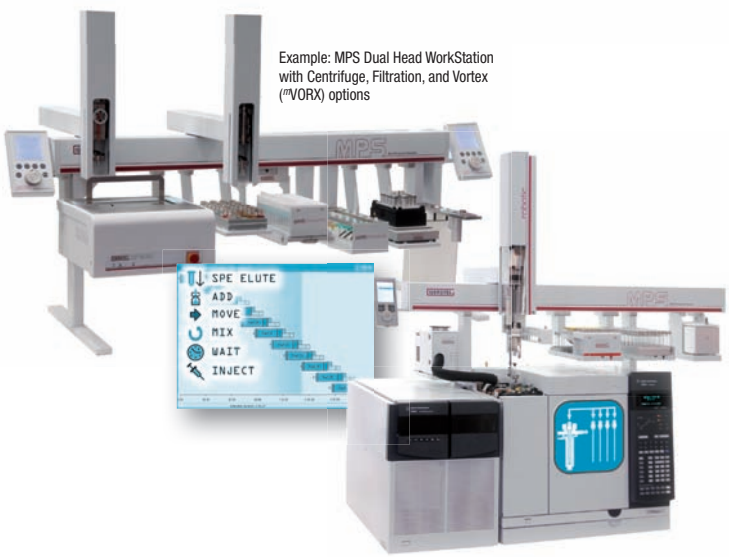
LCGC has decided to change up a few other things too. For years, Pittcon was the focal point for most, if not all vendor new product introductions. However, in recent years, many vendors have turned to other conferences (for example, the International Symposium on High Performance Liquid Phase Separations and Related Techniques [HPLC], Symposium on the Interface of Regulatory and Analytical Sciences for Biotechnology Health Products [WCBP], American Society for Mass Spectrometry Conference on Mass Spectrometry & Allied Topics [ASMS], Analytica, and others), as well as online, website-based, and social media introductions, catering to a more specialized or targeted audience. For this reason, *LCGC* sent out a survey in early 2015 asking vendors to submit products launched at any time during the past year. So, this review will encompass any liquid (high performance liquid chromatography [HPLC], ultrahigh-pressure liquid chromatography [UHPLC], ion chromatography [IC]) and supercritical fluid chromatography (SFC) columns and related devices introduced since Pittcon 2014, with sample preparation and gas chromatography (GC) new product introductions treated similarly, but separately (1–3). The information presented here may not be exhaustive since it is based on the survey, and not all vendors queried responded. However, the vendors that did respond are listed in Table I. Readers are encouraged to consult with the vendors for more-detailed information beyond that included here.

This installment is divided roughly according to column and application types: traditional reversed-phase, core–shell, hydrophilic-interaction chromatography (HILIC), ion-exchange, SFC, biomolecule, and ion chromatography applications, and related devices. The goal of this column installment is to successfully summarize the bulk of the vendor introductions over the last year, and present trends that stimulate ideas for more research from the application niches represented.

Reversed-Phase Chromatography

Several new columns and guard columns were introduced that can be categorized as reversed phase, whether they are totally porous or superficially porous, or alternative reversed-phase (for example, pentafluorophenyl or alkylamide) particle columns, as summarized in Table II. Many, if not all, of these columns were introduced as "families," or columns

Perfect fit!



Example: MPS Dual Head WorkStation with Centrifuge, Filtration, and Vortex (mVORX) options

Example: ALEX-GC-MS/MS-System for QuEChERS, Metabolomics ….

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available over a wide particle size range (1.7 to 5 or 10 µm) to scale or transfer separations from UHPLC to HPLC, to semipreparative or preparative HPLC, and a variety of column dimensions.

YMC, UCT, and SiliCycle all introduced families of C18 particles of varying hydrophobicities. YMC's Triart C18 ExRS and SiliCycle's SiliaChrom ODS, with a 25% and 15% carbon load, respectively, are aimed at more-polar, hydrophobic compound applications. UCT's Selectra Aqueous C18, with modified endcapping and a 10% carbon load, is said to provide different selectivity versus traditional C18 particles for polar compounds.

SiliCycle (SiliaChrom PFP) and Phenomenex (Kinetex F5) both introduced alternative-selectivity reversed-phase columns based on a pentafluorophenyl (PFP) functional group. The PFP functional group, because of the highly electronegative fluorine atoms on the periphery of the phenyl ring, can provide unique selectivity versus C18 columns for aromatic and halogenated compounds. Alternative-selectivity reversed-phase column introductions also included Separation Methods Technology's SMT AquSep (alkylamide functional group), and ES Industries' Epic Diphenyl. The SMT AquSep column, by virtue of having both hydrophobic and hydrophilic spacer ligands, is described as having better retention of polar analytes, and extremely acidic or basic compounds. ES Industries' Epic Diphenyl column takes advantage of improved π-π interactions to provide better selectivity for aromatic compounds.

Solid core–shell or superficially porous particles (SPPs) continued to generate interest during the past year, with both new introductions and product-line extensions. SPPs are attractive because they can provide efficiencies equivalent to a smaller particle, at a much lower back pressure. Consequently, existing HPLC systems can be used, although a case can still be made that lower dispersion systems (such as UHPLC systems), capable of operating at higher back pressures, will result in separations with even higher efficiencies. Waters introduced the Cortecs family of columns in mid-2014. Cortecs columns are available as both 1.6- and 2.7-µm SPPs, in a variety of column dimensions, and in three stationary phases: C18, HILIC (unbonded), and a charged C18+ chemistry. The charged C18+ chemistry features a positively charged surface modification for improved peak shape of basic compounds when they are analyzed using acidic, low-ionic-strength mobile phases such as formic acid. Supelco expanded upon its SPP product line with the introduction of the Ascentis Express 2.0 family of columns. These columns are another good choice for improving the performance of traditional HPLC systems. The Supelco 2.0 µm SPP is available in C18, HILIC, OH5 (pentahydroxy for the fast analysis of polar compounds), and an F5 (PFP) stationary phase, and

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Table I: 2015 *LCGC* new product survey, responding vendors

Company	Product Name
Agilent Technologies	Agilent A-Line Quick Connect fitting AdvanceBio Glycan Mapping (HILIC) columns AdvanceBio RP-mAb Poroshell 120 4 µm
AkzoNobel PPC AB	Kromasil SFC family of columns
BIA Separations	CIMac 0.1-mL analytical columns (QA, DEAE, EDA)
ColumnTek	Enantiocel-C6 chiral column
ES Industries	Epic Diphenyl GreenSep Naphthyl MacroSep HP
Fortis Technologies	SpeedCore pH Plus
Grace	ProVance prepacked disposable protein A columns
MicroSolv Technology	Cogent Diol 2.o Cogent UDA 2.o
Optimize Technologies	UHPLC Nano fittings
Phenomenex	Kinetex EVO C18 Kinetex F5 Luna 10µm-PREP C18(3), C8(3) and Silica(3)
Separation Methods Technologies, Inc.	SMT AquSep reversed-phase columns
Shodex/Showa Denko America, Inc.	Shodex HILICpak VG-50 4E Shodex HILICpak VG-50 4D
SiliCycle Inc.	New SiliaChrom ODS SiliaChrom PFP (pentafluorophenyl) HPLC column SiliaChrom Tosic Acid (SCX)
Supelco	BIOshell Glycan HPLC column Ascentis Express 2.0-µm columns
Thermo Fisher Scientific	Dionex IonPac AS22-Fast-4µm anion-exchange column with the Thermo Scientific Dionex Sodium Carbonate Eluent Generator Cartridge (EGC 500 K_2CO_3) Dionex IonPac AS27 anion-exchange column Dionex Chemically Regenerated Suppressor (CRS 500) (accessory) MAbPac HIC Family: MAbPac HIC-10, HIC-20, HIC-Butyl MAbPac HIC column family
Tosoh Bioscience	CaPure-HA Toyopearl NH2-750F
UCT	Selectra Aqueous C18
Waters	XBridge Protein BEH SEC columns ProteinPak Hi Res HIC VanGuard cartridge columns Acquity UPC^2 Torus 1.7 µm columns iKey Separation Device Cortecs 2.7 µm columns Acquity UPC^2 Trefoil 2.5 µm columns
YMC Co., Ltd.	BioPro SmartSep Q/S Chiral Art Polysaccharide derivatives Triart C18 ExRS

in a variety of column dimensions. Both Fortis and Phenomenex also introduced columns in the SPP category, both aimed at extending the available pH range. Fortis introduced the SpeedCore pH Plus column, in both 2.6- and 5.0-µm particle sizes, and 2.1-, 3.0-, and 4.6-mm internal diameters. Phenomenex introduced Kinetex EVO C18, a 5.0-µm SPP available in analytical (30 mm × 2.1 mm) to preparative (250 mm × 30 mm) dimensions. Both the Fortis and the Phenomenex SPPs can operate over a pH range of 1–12, providing a valuable tool in method development selectivity screening. Agilent Technologies expanded on their SPP product line with the new Poroshell 120 4-µm particle size columns, an addition to the existing 2.7-µm version. The new Poroshell 120 columns are a family of five chemistries consisting of C18, C8, PFP, phenyl-hexyl, and HILIC, and are available in a variety of dimensions with internal diameters ranging from 2.1 to 4.6 mm and lengths ranging from 50 to 250 mm.

The often complex sample matrices encountered in pharmaceutical, natural product, environmental, and industrial chemical analysis can lead to short column lifetimes and degrade chromatographic performance. Guard columns are often useful for removing particulate and chemical contamination that may be present in the mobile-phase or samples, without compromising chromatographic performance. For this reason, Waters introduced the general purpose, easy-to-use VanGuard family of cartridge columns, designed to protect HPLC and UHPLC analytical columns. They are available in 2.1- and 3.9-mm internal diameters, 2.5- and 5.0-µm particle sizes, and a variety of stationary phases (C18, C8, HILIC, amide, phenyl-hexyl, PFP, and cyanopropyl).

Ion Chromatography

In ion chromatography (IC), the stationary phase surface contains ionic functional groups that interact with analyte ions of opposite charge. IC is further subdivided into anion- and cation-exchange chromatography. Three companies provided information about new products introduced for IC applications: MicroSolv Technology Corporation, SiliCycle Inc., and Thermo Fisher Scientific, split evenly between anion and cation exchangers (Table III).

In the anion-exchange category, Thermo Fisher Scientific introduced two new products, the IonPac AS27 and the IonPac AS22-Fast-4µm anion-exchange columns. The IonPac AS27 column is a polymeric 55% divinylbenzene–ethylvinylbenzene

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Table II: Reversed-phase column introductions

Company	Product Name	Phases	Particle Sizes	Dimensions	Comments
Agilent Technologies	Poroshell 120	C18, C8, PFP, phenyl-hexyl, and HILIC	4 µm	50 mm × 2.1 mm through 250 mm × 4.6 mm	Scalable complement to existing 2.7-µm columns. Direct method replacement for typical 5-µm columns, offering higher efficiency. More forgiving for "dirty" samples due to 2-µm frits.
ES Industries	Epic Diphenyl	Diphenyl	1.8, 3, 5, and 10 µm	Capillary to preparative	Diphenyl functional group for increased π-π interaction.
Fortis Technologies	SpeedCore pH Plus	C18	2.6 and 5 µm	2.1, 3.0, and 4.6 mm i.d.	Extended pH range 1–12 SPP.
Phenomenex	Kinetex EVO C18	C18	5 µm	30–250 mm × 2.1–30 mm	100% aqueous stable over a pH range of 1–12.
	Kinetex F5	Pentafluoro-phenyl (PFP) with propyl linker	1.7 and 2.6 µm	30, 50, 100, and 150 mm × 2.1, 3.0, 4.6 mm	Reversed-phase, 100% aqueous, 2D-LC, HILIC, and SFC separation modes, with orthogonal separation to C18 of polar and nonpolar analyte mixtures; 100% aqueous stable.
Separation Methods Technologies, Inc.	SMT AquSep	Alkylamide	3 and 5 µm	Analytical to preparative	Stronger retention of polar molecules in aqueous eluent. Reduced back pressure; the hydrophilic hybrid phase enhances the solvation in an aqueous environment.
SiliCycle Inc.	SiliaChrom ODS	C18	3, 5, and 10 µm	Analytical to preparative	Extra pure silica gel, high retention for nonpolar compounds, useful for the purification of pesticides, PCBs, PAHs, drugs, and proteins.
	SiliaChrom PFP	Pentafluoro-phenyl (PFP)	3, 5, and 10 µm	Analytical to preparative	Long lifetime stability; purification of halogenated and aromatic conjugated products, phenols, and isomers.
Supelco	Ascentis Express 2.0	C18, F5, OH5, HILIC	2 µm	2–15 cm × 2.1 and 3.0 mm	Less susceptible to column plugging because of the large 1.0-µm frits on the column inlet. Can be used up to 1000 bar (14,500 psi), producing ~20% lower back pressure than sub-2-µm UHPLC columns.
UCT	Selectra Aqueous C18	C18 with modified endcapping	1.8, 3, and 5 µm	50 mm × 2.1 mm through 250 mm × 4.6 mm	Wide range of mobile phase options; can be used in 100% aqueous mobile phases. Different selectivity for polar compounds from traditional C18 phases.
Waters	Cortecs 2.7 µm	C18, C18+ HILIC	2.7 µm	30, 50, 75, 100, and 150 mm × 2.1, 3.0, and 4.6 mm	High-resolution small-molecule separations at HPLC- and UHPLC-optimized back pressures. Full scalability between 2.7- and 1.6-µm particle sizes; high efficiencies and resolution at lower back pressure. Improved peak shape, MS sensitivity, and loadability with unique, positively charged surface C18+ chemistry.
	VanGuard Cartridge Columns	C18, C8, HILIC, amide, phenyl-hexyl, fluorophenyl, cyanopropyl	2.5 and 5.0 µm	5 mm × 2.1 and 3.9 mm	Extends HPLC and UHPLC column life without negatively impacting the chromatographic separation. Intuitive and easy-to-use cartridge column format.
YMC Co., Ltd.	Triart C18 ExRS	C18	1.9, 3, and 5 µm	Analytical to semipreparative	High hydrophobicity hybrid silica-based particles, with alternative C18 selectivity due to high steric selectivity or planer cognitive interactions. Suitable for separation of hydrophobic compounds and their structurally similar analogs.

cross-linked phase, with an alkanol quaternary ammonium ion functional group, on a 6.5-µm particle. The IonPac AS27 column is aimed at the trace-level analysis of bromate in the presence of parts-per-million levels of ethylenediamine (EDA) in drinking water samples. The IonPac AS22-Fast-4µm anion-exchange column is based on the same chemistry as the AS27, but on a smaller, 4-µm particle, and when combined with the company's EGC-500 K_2CO_3 cartridge provides electrolytic generation of carbonate and carbonate–bicarbonate mixtures at pressures commonly required for columns based on 4-µm particles, up to 5000 psi. Figure 1

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Table III: Ion chromatography columns

Company	Product Name	Phases	Particle Sizes	Dimensions	Comments
MicroSolv Technology Corporation	Cogent UDA 2.o	Undecanoic acid on silica hydride	2.2 µm	20–150 mm × 2.1 mm	Ability to combine weak cation-exchange with aqueous normal-phase chromatography to enhance selectivity for the separation of closely related ionizable compounds.
SiliCycle Inc.	SiliaChrom Tosic Acid (SCX)	Spherical strong cation-exchange	3, 5, and 10 µm	Analytical to preparative	For the purification of complex reaction mixtures containing basic or cationic compounds. High lot-to-lot reproducibility.
Thermo Fisher Scientific	Dionex IonPac AS27 anion-exchange column	Polymeric 55% divinylbenzene–ethylvinylbenzene cross-linked, alkanol quaternary ammonium ion functional group	6.5 µm	Up to 250 mm × 0.4, 2, and 4 mm	Easier, more-reliable quantitation of trace levels of bromate in the presence of 50 ppm ethylenediamine (EDA) in drinking water samples. EDA is used as a sample preservative and can interfere in the analysis.
	Dionex IonPac AS22-Fast-4µm anion-exchange column	Polymeric 55% divinylbenzene–ethylvinylbenzene cross-linked, alkanol quaternary ammonium ion functional group	4 µm	Up to 250 mm × 0.4, 2, and 4 mm	Fast, high-resolution separation of inorganic anions. EGC-500 K_2CO_3 cartridge capable of generating carbonate eluents at pressures up to 5000 psi.

Table IV: Biomolecule columns

Company	Product Name	Phases	Particle Sizes	Dimensions	Comments
Agilent Technologies	AdvanceBio RP-mAb	C4, StableBond C8, diphenyl	3.5 µm	50–150 mm × 2–4.6 mm	450-Å SPP allows full access to porous structure for monoclonal antibodies. SPP reduces diffusion distances, decreasing band broadening for slow diffusing mAbs; compatible with both HPLC and UHPLC systems.
BIA Separations	CIMac	QA, DEAE, EDA, anion exchange	1.5 µm pore	0.1 mL analytical	Analysis of large biomolecules (viruses, IgG, IgM, and other large proteins). Flow rates up to 30 CV/min.
	CIMac Adeno	QA, anion exchange	2 µm pore	5.0 mm × 5.2 mm o.d., 0.106-mL bed volume	For adenovirus separations. Flow rates up to 20 CV/min.
	CIMac pDNA	DEAE, anion exchange	1.5 µm pore	15 mm × 5.2 mm o.d., 0.32-mL bed volume	Plasmid-DNA separations. Flow rates up to 10 CV/min.
	CIMac SO_3	SO_3, cation exchange	1.5 µm pore	5.0 mm × 5.2 mm o.d., 0.106-mL bed volume	Monitoring and quantitation of large proteins, plasmid DNA, viruses, virus-like particles, and phages. Flow rates up to 30 CV/min.
ES Industries	MacroSep HP	C4, C8, C18, PFP, and phenyl	2.1, 3, 5, and 10 µm	Capillary to preparative	Highly deactivated for the separation of proteins, peptides, and other biological compounds.
Grace	ProVance Prepacked Disposable Protein A	Protein A	70 µm	1.2–45 cm diameter	Single-use columns expedite purification steps, improved overall downstream productivity, eliminate the need for clean in place and column packing procedures. Constructed from an incompressible resin for high flow rates.
Thermo Fisher Scientific	MAbPac HIC-10, HIC-20, HIC-Butyl	HIC, polyamide, alkylamide, butyl	5 µm	100 and 250 mm × 4.6 mm	Unique column chemistries engineered to provide high resolution, excellent biocompatibility, and complementary selectivity suitable for a broad range of assays for mAbs and related substances including mAb variants and oxidized mAbs, mAb aggregates, antibody fragments, ADCs, and other proteins.
Tosoh Biosciences	CaPure-HA	Ca_2^+, PO_4, and OH^- (calcium phosphate)	39 µm	Not applicable	Mixed-mode hydroxyapatite (calcium phosphate) for mAb and DNA purification and aggregate removal.
	Toyopearl NH2-750F	Methacrylic polymer, primary amine	45 µm, >100 nm pore	Not applicable	Purification of biomolecules at increased mobile-phase salt concentrations, mechanically stable to 0.3 MPa.
Waters	ProteinPak Hi Res HIC	HIC polymethacrylate butyl	2.5 µm	35 and 100 mm × 4.6 mm	Suited for high-resolution analyses of mAbs and ADC samples. Stable in either acid or caustic cleaning regimes up to 1 N, pH 2–12.
	XBridge Protein BEH SEC	Diol-coated, ethylene-bridged, hybrid	3.5 µm	30 mm × 7.8 mm (guard), 150 and 300 mm × 7.8 mm	Use of ethylene-bridged, hybrid (BEH) particles minimizes nondesired ionic interactions, higher flows (versus silica-based SEC particles) for increased sample throughput. SEC of proteins, peptides, mAb, and ADCs.
YMC Co., Ltd.	BioPro SmartSep Q	Quaternary ammonium, anion exchange	10 and 30 µm	Analytical and bulk	Extremely low nonspecific adsorption, high binding capacity over a wide range of flow rate. Separation of proteins, peptides, and nucleic acids.
	BioPro SmartSep S	Sulfobutyl, cation exchange			

shows an example of the separation of seven common anions on the IonPac AS22-Fast-4µm column, demonstrating good repeatability.

Two cation-exchange offerings were also introduced. MicroSolv Technology introduced the Cogent UDA 2.0 column, which is a 2.2-µm weak cation-exchange phase consisting of an undecanoic acid ligand on a silica hydride surface. The Cogent UDA 2.0 column combines a weak cation-exchange mechanism with an aqueous normal-phase separation mechanism to enhance selectivity for the separation of closely related ionizable compounds. SiliCycle introduced the SiliaChrom Tosic Acid cation-exchange columns, intended for the purification of complex reaction mixtures containing basic or cationic compounds. This family of columns is available in 3-, 5-, and 10-µm particle sizes, and in analytical to preparative column dimensions.

Biomolecule Chromatography

Several manufacturers introduced columns during the past year that fit into what I'll call the "biocolumn" category. Many of these columns could fit into one of the other categories in this review, but I've given them their own category based on their intended application for biomolecules. Columns in this category are ion-exchange, reversed-phase, or "specialty" columns; the latter includes columns for size-exclusion chromatography (SEC), hydrophobic interaction chromatography (HIC), affinity, and HILIC. Table IV summarizes the vendors that responded with new product introductions for the separation of biomolecules.

The largest number of new column introductions in the biocolumn category are columns intended for monoclonal antibody (mAb) separations using HIC, reversed-phase, affinity (protein A), or mixed-mode separation mechanisms. Agilent Technologies, Grace, Thermo Fisher Scientific, Tosoh Bioscience, and Waters all introduced HIC columns aimed at mAb separations. Tosoh Bioscience also premiered a mixed-mode hydroxyapatite (calcium phosphate) column for mAb and DNA purification, and aggregate removal, the CaPure-HA. Grace introduced a family of prepacked disposable protein A affinity columns called ProVance. These are single-use columns available in a range of sizes intended for good manufacturing practice (GMP) purification of monoclonal antibodies. Single-use columns can help to expedite purification steps and improve overall downstream productivity, while eliminating the need for tedious clean-in-place and column packing procedures. ProVance columns are constructed from an incompressible resin that ensures performance is maintained at high flow rates, at a cost savings compared to agarose resins.

Agilent Technologies debuted an SPP family of columns named AdvanceBio RP-mAb. Available in three different chemistries, C4, StableBond C8, and diphenyl, AdvanceBio RP-mAb columns are designed specifically for reversed-phase

monoclonal antibody separations. The column's 450-Å SPP allows full access to the porous structure for monoclonal antibodies. Like a standard SPP, the AdvanceBio particle technology reduces diffusion distances, resulting in decreased band broadening for slow diffusing mAbs, and is compatible with both HPLC and UHPLC systems.

Thermo Fisher Scientific introduced the MAbPac HIC family of columns consisting of three unique column chemistries: MAbPac HIC-10 (proprietary polyamide), HIC-20 (proprietary alkylamide), and HIC-Butyl for the analysis and characterization of a wide range of mAb samples, including mAb variants and oxidized mAbs, mAb aggregates, antibody fragments, antibody–drug conjugates (ADCs), and other proteins. The HIC-10 and HIC-20 columns are porous (1000-Å pore size) and the HIC-Butyl column is a nonporous hydrophilic polymer-based phase. These unique column chemistries were engineered to provide high resolution, biocompatibility, and complementary selectivity suitable for a broad range of assays for mAbs and related substances.

Waters premiered ProteinPak Hi Res HIC columns for the separation of proteins including mAbs and ADCs. Featuring polymethacrylate base material and a 2.5-µm particle, the ProteinPak Hi Res HIC column is stable in either acid or caustic cleaning regimes up to 1 N, or pH 2–12. Another recent Waters offering is the XBridge Protein BEH SEC column. The XBridge column use of diol-coated, ethylene-bridged hybrid (BEH) particles minimizes undesired ionic interactions and can be operated at higher flows (versus silica-based SEC particles) for increased sample throughput. These columns are quality control (QC) tested with relevant protein and peptide standards, also available for purchase direct from the vendor, for SEC applications of proteins, peptides, mAb, and ADC separations. Figure 2 illustrates an example separation using the XBridge Protein BEH SEC column.

ES Industries released a pure reversed-phase column targeted toward biomolecules this year. The MacroSep HP line is a family of C4, C8, C18, PFP, and phenyl phases in a variety of common dimensions, from capillary to preparative dimensions. The MacroSep HP columns are highly deactivated for the separation of proteins, peptides, and other biological compounds.

Several ion-exchange phases were introduced for biomolecule separations. BIA Separations introduced three anion-exchange columns and one cation-exchange column. The CIMac, CIMac Adeno, and CIMac pDNA columns are anion-exchange monolith-based columns designed for the analysis of large biomolecules and viruses, adenovirus, and plasmid DNA, respectively. The CIMac SO_3 column is a cation-exchange monolith-based column used for the monitoring and quantitation of large proteins, plasmid DNA, viruses,

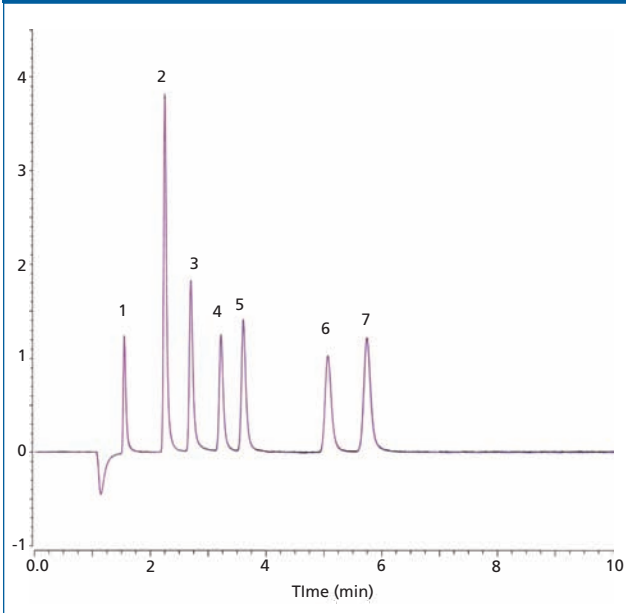


Figure 1: Separation of seven common anions. Column: 150 mm × 4 mm IonPac AS22-Fast-4 µm (Thermo Fisher Scientific); fluent: 4.5 mM potassium carbonate–1.4 mM potassium bicarbonate (EG); flow rate: 1.5 mL/min; temperature: 30 °C; back pressure: 3550 psi; loop: 10 µL. Peaks: 1 = fluoride (1 mg/L), 2 = chloride (5 mg/L), 3 = nitrite (5 mg/L), 4 = bromide (5 mg/L), 5 = nitrate (5 mg/L), 6 = phosphate (10 mg/L), 7 = sulfate (5 mg/L). (Figure courtesy of Thermo Fisher Scientific.)

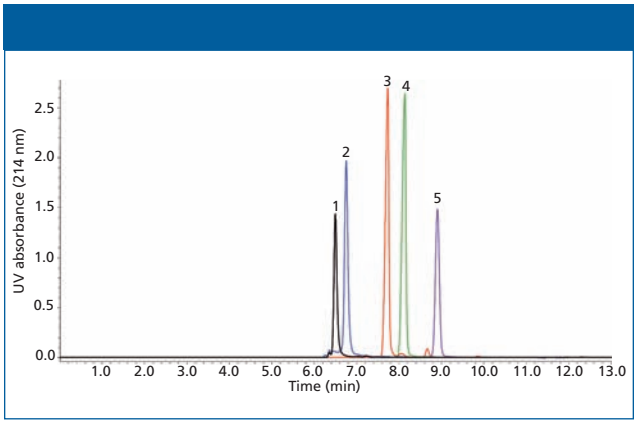


Figure 2: Example separation on a Waters XBridge Protein BEH SEC 125 Å column. Column dimensions: 150 mm × 7.8 mm, 3.5-µm d_p; mobile phase: 30% acetonitrile, 0.1% trifluoroacetic acid; temperature: 30 °C; flow rate: 0.84 mL/min. Peaks: 1 = ubiquitin (MW 8565), 2 = aprotinin (MW 6511), 3 = angiotensin (MW 1296), 4 = bradykinin (MW 1060), 5 = Asp-Leu-Trp-Gln-Lys (MW 618). (Figure courtesy of Waters Corporation.)

virus-like particles, and phages. The monolith-type medium is a convective interaction medium that is not diffusion limited. All of the BIA columns are available in a variety of configurations, and are billed as high-flow, fast, and reliable process analytical technology (PAT) tools.

Tosoh Bioscience also introduced an anion-exchange column, the Toyopearl NH2-750F. It is a semirigid methacrylic

polymer bead phase, stable at pressures as high as 0.3 MPa. Because it is tolerant to increased mobile-phase salt concentrations, it is well suited for aggregate removal, DNA purification, and mAb purification.

YMC introduced a line of ion-exchange columns called BioPro SmartSep Q/S for the separation of proteins, peptides, and nucleic acids. The "Q" is a quaternary ammonium anion-exchange resin, and the "S" is a sulfobutyl cation-exchange resin. The balanced binding capacity and particle rigidness results in high mechanical stability, expanding the usable flow rate range. By optimizing surface modifications, high binding capacity over a wide range of flow rate is obtained, improving throughput for intermediate purification and polishing operations.

Hydrophilic-Interaction Chromatography

Four HILIC columns turned up in this year's survey, all with a diversity of ligands bound to either silica-based totally porous particles (TPPs), or SPPs, in a variety of dimensions. The majority of the HILIC columns featured here are actually for biomolecule separations, but I've broken them out here instead, as summarized in Table V.

Shodex introduced the HILICpak VG-50 4E and 4D columns for reducing sugar and saccharide separations. The tertiary amino phase is usable over a wide pH range of 2–13. Agilent Technologies introduced a wide-ranging family of HILIC columns, on both TPPs and SPPs, in a range of sizes and dimensions. Called AdvanceBio, the columns are aimed at analyzing N-glycans cleaved (in this case) from mAbs to characterize their glycosylation. The AdvanceBio columns can be used in both HPLC and UHPLC modes, offering rapid, high-resolution separations without instrument limitations. The choice of particle size or morphology and column formats allows users to optimize for absolute speed or highest resolution. AdvanceBio columns are QC tested with application-specific glycan samples for more-consistent performance results. Supelco also introduced a line of SPP columns directed at glycan analysis. The BIOshell Glycan columns are intended for glycoprotein analysis, and have a unique stationary phase of a highly polar ligand that possesses five hydroxyl groups tethered to the silica via a novel, proprietary chemical linkage.

Columns for Chiral-Compound Separations

There were three entries in the category of columns for chiral compound separations; all were intended for both HPLC and SFC applications as outlined in Table VI. As a side note, although other SFC columns can be used for chiral compound separations, I've included only columns branded or marketed specifically for chiral compound separations here. For additional SFC introductions, see the section on SFC that appears next.

ColumnTek introduced the Enantiocel-C6 column, a silica-based polysaccharide derivative-coated cellulose tris (phenylcarbamate) phase.

Waters now offers a family of columns specifically for chiral SFC (or what Waters has branded as UPC^2) called

Table V: HILIC columns

Company	Product Name	Phases	Particle Sizes	Dimensions	Comments
Agilent Technologies	AdvanceBio Glycan Mapping	Proprietary glycan-specific	1.8 µm (TPP) 2.7 µm (SPP)	100 and 150 mm × 2.1 mm TPP 100–150 mm × 2.1–4.6 mm SPP	Specifically designed for selectivity of glycans. Excellent resolution power improves accuracy of quantitation for critical quality attribute. Ideal for both UHPLC and HPLC.
Supelco	BIOshell Glycan	Penta-hydroxyl	2.7 µm SPP	150 mm × 2.1–4.6 mm	Fast, high-resolution, reproducible glycan identification.
Shodex/ Showa Denko America, Inc.	HILICpak VG-50 4E	Tertiary amino	5 µm	250 mm × 4.6 mm	For saccharides, especially reducing sugars, pH range 2–13.
	HILICpak VG-50 4D			150 mm × 4.6 mm	

the Acquity UPC^2 Trefoil Columns. Given that chiral compound separations are often referred to as requiring three points of attachment or interaction, the Waters columns are really appropriately named since "trefoil" literally means three-leaved plant. Trefoil columns have a silica-based 2.5-µm particle and are available with three modified polysaccharide-coated chiral phases: amylose tris-(3.5-dimethylphenylcarbamate), cellulose tris-(3,5-dimethylphenylcarbamate), and cellulose tris-(3-chloro-4-methylphenylcarbamate), in 2.1- and 3.0-mm internal diameters, and 50- and 150-mm lengths. Modified polysaccharide-based stationary phases provide broad-spectrum chiral selectivity, and extensive application research of mobile-phase cosolvents and additive blends has demonstrated enhanced chiral recognition for each chiral stationary phase, enabling targeted method development strategies. The Acquity UPC^2 Trefoil columns are uniquely designed for the Waters Acquity UPC^2 system to enable both selectivity and speed in chiral separations and reduce chiral method development time.

A new column for chiral compound separations, also suitable for

Table VI: Columns for chiral compound separations

Company	Product Name	Phases	Particle Sizes	Dimensions	Comments
ColumnTek LLC	Enantiocel-C6	Silica-based polysaccharide derivative-coated cellulose tris (phenylcarbamate)	5 µm	250 mm × 4.6, 10, and 21.2 mm	Complementary selectivity from a new chiral selector.
Waters	Acquity UPC^2 Trefoil	Three modified polysaccharide-coated chiral phases: amylose tris-(3.5-dimethylphenylcarbamate); cellulose tris-(3,5-dimethylphenylcarbamate); and cellulose tris-(3-chloro-4-methylphenylcarbamate)	2.5 µm	50 and 150 mm × 2.1 and 3.0 mm	Broad-spectrum chiral selectivity, targeted method development strategies. Uniquely designed for the Waters Acquity UPC^2 system to enable both selectivity and speed in chiral separations and reduce chiral method development time.
YMC Co., Ltd.	Chiral Art	Silica-based polysaccharide derivatives (amylose and cellulose)	3, 5, 10, and 20 µm	Not applicable	Extremely low column bleeding, identical separation characteristics across particle sizes.

Table VII: Supercritical fluid chromatography columns

Company	Product Name	Phases	Particle Sizes	Dimensions	Comments
AkzoNobel PPC AB	Kromasil	100-2.5-Diol (polar), 100-2.5-CN (π-π-interactions), 100-2.5-Sil (polar) and 100-2.5-2 EP (endcapped/low silanol activity)	2.5 µm	3 and 4.6 mm i.d.	Green SFC technology, alternative selectivities, and decreased tailing for the separation of chiral substances, drugs, and natural products.
ES Industries	GreenSep Naphthyl	Silica-based naphthyl	1.8, 3, 5, and 10 µm	Capillary to preparative	Unique selectivity through strong π-π interactions for the separation of nonpolar compounds.
MicroSolv Technology Corporation	Cogent Diol 2.o	Dihydroxy-butyl diol ligand is on a silica hydride surface	2.2 µm	20–150 mm × 2.1 mm	Intended for several separation modes including SFC, aqueous normal phase, classical normal phase, and reversed phase. Versatility with no adsorbed water layer on the surface to promote precise retention.
Waters	Acquity UPC^2 Torus	2-Picolylamine (2-PIC); diethylamine (DEA); DIOL; 1-Aminoanthracene (1-AA)	1.7 µm	50–150 mm × 2.1 and 3.0 mm	For small-molecule achiral SFC separations. Specifically designed for the Acquity UPC^2 system. Four achiral phases make up the series: 2-picolylamine (2-PIC) for general purpose applications; diethylamine (DEA) for basic compounds; diol for acidic compounds; and 1-aminoanthracene (1-AA) for neutral and hydrophobic compounds such as fat-soluble vitamins and lipids. Offering improved robustness, these columns eliminate or reduce the need for additives, over a wide range of selectivity.

use in both HPLC and SFC, the Chiral Art column from YMC is an amylose and cellulose polysaccharide derivative phase offered in a variety of particle sizes and two coated and four immobilized materials. This expanded product range improves the hit ratio on phase screening. Selectivity is guaranteed to be identical across particle sizes, enabling predictable scaling between particles.

Supercritical Fluid Chromatography

Four companies introduced families of SFC columns: AkzoNobel, ES Industries, Microsolv Technology, and Waters (see Table VII).

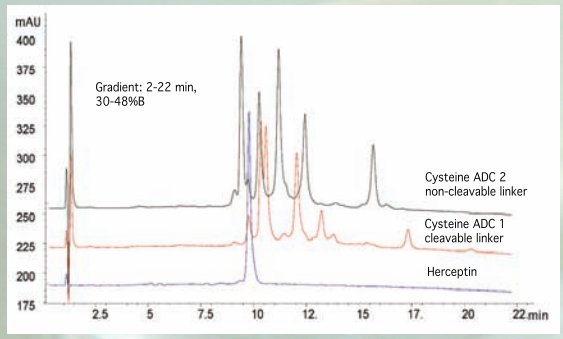
AkzoNobel's Kromasil family of columns for SFC includes four phases: 100-2.5-Diol, 100-2.5-CN, 100-2.5-Sil, and 100-2.5-2EP. Promoting green technology with SFC, the family of 2.5-µm columns provides alternative selectivities and decreased tailing for the separation of chiral substances, drugs, and natural products.

ES Industries checked in with a family of columns for the separation of nonpolar compounds for SFC called GreenSep Naphthyl. This napthyl phase, available in capillary to preparative dimensions and particle sizes of

Complete Protein Characterization

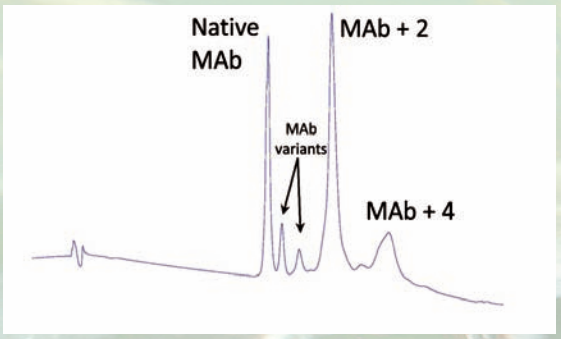
Aggregates - Conjugates - Deamidation - Oxidation

Denatured ADCs by RPC



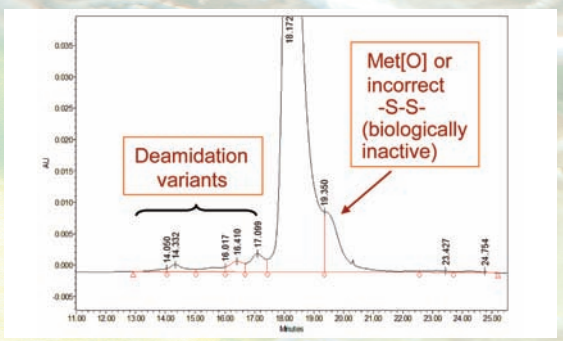
PolyRP™ 1000, 5μm, 1000Å, 4.6 x 100mm
p/n: T 260950-4610
Gradient:
A: 0.1% TFA; B: 0.1% TFA, ACN; 80°C

Native Conjugates by HIC



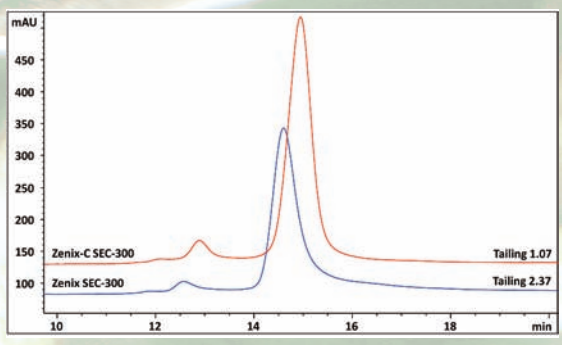
PolyPROPYL A™, 3μm, 1500Å, 4.6 x 100mm
p/n: P104PR0315
Segmented Gradient:
A: 0.9M $NaSO_4$; B: 50mM $NaPO_4$, pH 6

Deamidation by IEX



PolyCAT A®, 3μm, 1500Å, 4.6 x 100mm
p/n: P104CT0315
Gradient:
30-145mM NH_4OAc, pH 4, 40% ACN; 30°C

Aggregates by SEC



Zenix™ SEC 300 & Zenix™-C SEC-300
(7.8 x 300 mm); 0.5mL/min
Sample: Peggylated Exenatide (PEG 23 KDa)
Isocratic: 50mM NH_4OAc : ACN 90:10 (v/v)

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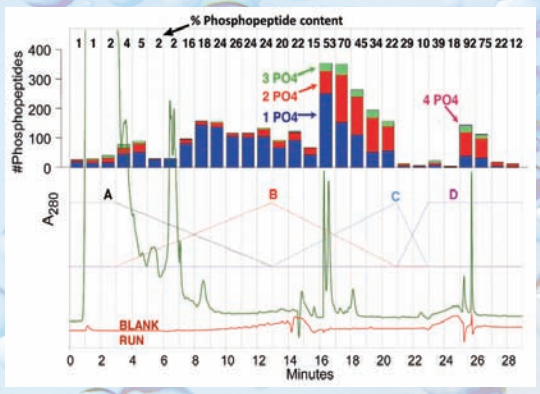

Sample: Tryptic Digest
Column: ERLIC-WAX microSPE p/n: SEM HIL-DE
Load in 85% ACN, 0.1% FA. Elute 10% ACN pH 2, 100mM Na-methylphosphonate
Desalt on MACROspin TARGA® C18 p/n: SMM SS18R
Analytical column: PolyWAX LP™ 4.6 x 100mm, 5µm, 300Å, p/n: P104WX0503

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VS. +3

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EXAMPLE: ERLIC-CAT chromatography

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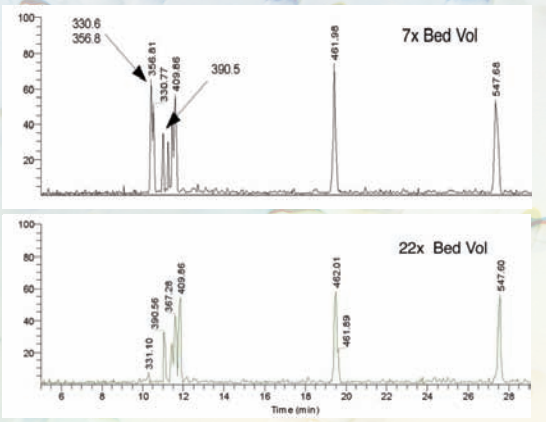
Desorption Increases From Excessive Sample & Wash Volumes



Column: TARGA® C18, 300µm x 30mm Trap
Sample Solvent: 0.1% FA water (volumes as shown). Trap Column in 0.1% FA water
Gradient: 98% water, 0.1% FA, 2% ACN to ACN, 0.1% FA

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UltraMicroSpin™ Column	3-30µg	25µL	12µL	2-25µL
MicroSpin™/TIP Column	5-60µg	50µL	25µL	5-50µL
MacroSpin™ Column	30-300µg	180µL	90µL	25-180µL
96-Well Spin Plate	10-100µg	75µL	35µL	7-75µL
96-Well MACROspin Plate	25-250µg	200µL	100µL	25-200µL
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Waters added to their SFC product line with Acquity UPC^2 Torus 1.7-µm columns for small-molecule achiral SFC separations. Like the Trefoil columns, the Torus family is specifically designed for the company's Acquity UPC^2 system. Four achiral phases make up the series: 2-picolylamine (2-PIC) for general purpose applications; diethylamine (DEA) for basic compounds; diol for acidic compounds; and 1-aminoanthracene (1-AA) for neutral and hydrophobic compounds such as fat-soluble vitamins and lipids. Offering improved robustness, these columns eliminate or reduce the need for additives, over a wide range of selectivity.

Chromatography Accessories and Devices

Several new products were introduced that fit into the category of either chromatography accessories, new devices, or a combination of both.

Thermo Fisher Scientific premiered the Dionex CRS 500 Chemically Regenerated Suppressor, for ion chromatographs with conductivity detectors. The CRS 500 uses a planar packed bed suppression zone for high peak efficiency and suppression capacity, for improved peak efficiency and better compatibility with columns packed with 4-µm particle size columns.

Two companies introduced fittings specifically for UHPLC applications. The new A-Line Quick Connect fittings from Agilent Technologies are the only truly finger-tight reusable fittings available for UHPLC — no tools are needed. By simply closing a lever, users can reconnect the A-Line Quick Connect fittings multiple times without any loss of performance; the fittings are stable to 1300 bar. For harder to reach areas on any HPLC instrument, the Agilent A-Line Quick Turn fitting produces either a finger-tight connection, stable to 600 bar, or a premium UHPLC connection, stable to 1300 bar with a quick turn of a wrench.

Optimize Technologies' EXP2 line of UHPLC Nano fittings feature titanium hybrid Ti-Lok ferrules for 1/16-in. and 1/32-in. PEEKsil tubing. The EXP2 male fittings feature a 3/16-in. hex head with a removable slotted knurled wrench for hand tightening to over 20,000 psi, depending on the tubing internal diameter. EXP2 Ti-Lok Kits are available with EXP2 nuts, Ti-Lok ferrules, and PEEKsil tubing. The 3/16-in. hex-head nuts fit tight spaces such as sample injection valves or loops, column connections, and column ovens.

In what is a unique combination of accessory or device and column, the Waters iKey Separation Device is one of the enabling technologies of the Waters ionKey/MS system, delivers increased sensitivity for sample-limited applications, and combines the advantages of microflow LC with a greatly simplified device. The innovative iKey eliminates the need for traditional fittings and greatly simplifies the microflow-LC user experience. Chromatographers turn to microscale LC when looking for higher sensitivity and lower limits of detection. However, this technique presents unique challenges such as the need for specialized equipment along with having to pay close attention to tubing connections and capillary lengths. The true plug-and-play design of the iKey eliminates these connection and variability challenges. The iKey Separation Device contains the fluidic channel, electronics, electrospray ionization interface, column heater, eCord, and column chemistry for performing UHPLC separations directly in the source of Waters mass spectrometers. The internal column is a high-strength silica-based 1.8-µm C18 TPP available in either 50 mm × 150 µm or 100 mm × 150 µm column dimensions. The high-strength silica phase enables separations at UHPLC pressures, with applications that include high retentivity for polar organic compounds and metabolites and a balanced retention for hydrophobic analytes.

Acknowledgment

I would like to thank the manufacturers and distributors that responded to the *LCGC* survey for inclusion in this review. Although I (and others at *LCGC*) have made every attempt to include all new chromatography column and related products introduced during the past year, I'm sure that this review is not exhaustive so I apologize in advance for any products that were omitted. However, if you did miss out, and would like to be included next year, please contact Laura Bush, Editorial Director, *LCGC North America*, lbush@advanstar.com, with the subject line "2016 Column Review."

References

(1) M.W. Dong, *LCGC North Am.* **33**(4), 254–261 (2015).

(2) J.V. Hinshaw, *LCGC North Am.* **33**(5), in press (2015).

(3) D.E. Raynie, *LCGC North Am.* **33**(5), in press (2015).

This month's guest author:

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is with Boston Analytical in Salem, New Hampshire and is a member of LCGC*'s editorial advisory board. Swartz was also a column editor for* LCGC*'s "Innovations in HPLC" and "Validation Viewpoints" columns.*

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Ronald E. Majors

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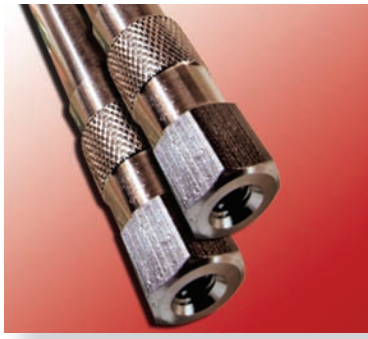
For more information on this topic, please visit www.chromatographyonline.com/column-column-watch



LC TROUBLESHOOTING

Be Careful of the Flow Rate

When converting methods from liquid chromatography (LC) to ultrahigh-pressure liquid chromatography (UHPLC), don't get confused by flow-rate settings.

John W. Dolan
LC Troubleshooting Editor

As more and more workers are transferring liquid chromatography (LC) methods to ultrahigh-pressure LC (UHPLC), I have received an increasing number of email questions that demonstrate a poor understanding of the role of the mobile-phase flow rate in method scaling. For this month's "LC Troubleshooting" I'd like to discuss situations when changes in flow rate are fairly innocuous and when they can get you into trouble.

First, Select the Column

Let's assume that you want to convert an isocratic LC method to a UHPLC one and maintain the same resolution, R_s. To help us understand the important variables, consider the fundamental resolution equation:

$$R_s = ¼N^{0.5}(\alpha - 1)\ (k/(k + 1) \quad [1]$$

where N is the column plate number, α is the separation factor, and k is the retention factor. Definitions of k and α are:

$$k = (t_R - t_0)/t_0 \quad [2]$$

$$\alpha = k_2/k_1 \quad [3]$$

where t_R is the retention time, t_0 is the column dead time (also abbreviated t_m), and k_1 and k_2 are the retention factors of two adjacent peaks. To keep R_s constant, we need to keep N, α, and k constant, too. If we assume that we will not change the chemistry of the system (primarily the mobile phase, column stationary phase, and temperature), k and α will remain constant. This means that when we change from a conventional LC column to a UHPLC column, the column chemistry must be the same, so only the column length, L (in millimeters), internal diameter, d_c (also in millimeters), and particle size, d_p (in micrometers) can change. A simple method to keep N constant is to make sure that the ratio L/d_p is constant. Because of the limited availability of column lengths and particle sizes available, it will be difficult to keep L/d_p exactly the same, so we'll adopt the United States Pharmacopeia's (USP) guidelines (1) to keep this ratio between +50% and -25%.

As an example, let's start with a method on a conventional 150 mm × 4.6 mm LC column packed with 5-µm d_p particles, operated at 1.0 mL/min. If we want to switch to a UHPLC packing with d_p = 1.7, you can determine that (150 mm/5 µm) = 30 ≈ (50 mm/1.7 µm) = 29.4, well within our +50% to -25% limits. Usually UHPLC uses narrower columns than conventional LC, so let's substitute a 50 mm × 2.1 mm, 1.7-µm column for the 150-mm one.

Next, Adjust the Flow Rate

Although it isn't a requirement, it is a good idea to start the method conversion process by setting up the method with the new column and the flow rate adjusted to keep the same linear velocity of mobile phase through the column. To keep the linear velocity constant, the flow rate should be adjusted by the change in cross-sectional area of the column, which is proportional to the square of the ratio of column diameters, or $(2.1\ mm/4.6\ mm)^2 = 0.208 \approx 0.2$. So the initial flow rate of 1.0 mL/min should be reduced to 0.2 mL/min to obtain the same linear velocity.

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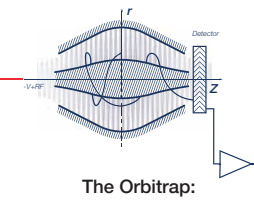

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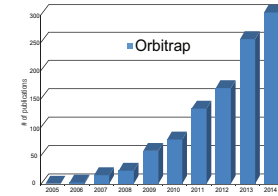

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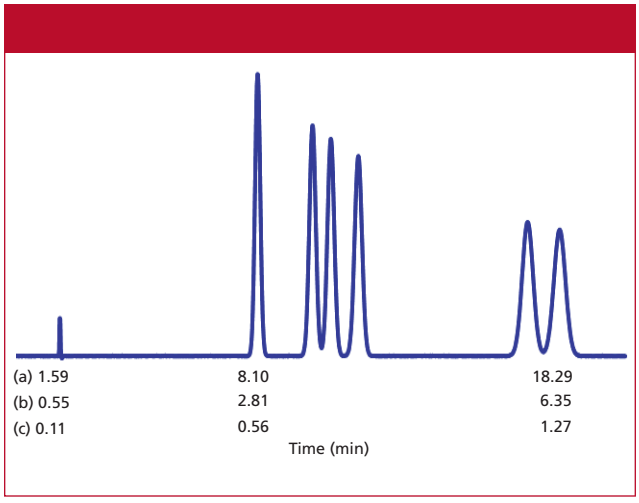

Figure 1: Simulated isocratic reversed-phase runs for a set of nitroaromatic compounds at 55% methanol–buffer and 35 °C: (a) 150 mm × 4.6 mm, 5-µm column operated at 1.0 mL/min; (b) 50 mm × 2.1 mm, 1.7-µm column operated at 0.2 mL/min; (c) same as (b), but at 1.0 mL/min.

These two runs are compared in Figures 1a and 1b, respectively. You can see that the chromatogram looks identical, with the exception of the retention times (see retention times compared below the chromatogram). We would expect the retention times for the UHPLC run to be one-third of those generated by the LC run, because the column is one-third of the length. In fact the retention times differ by 35%, because of the rounding of the ideal flow rate of 0.208 mL/min to 0.2 mL/min. You can check this by comparing the ratios of the t_0 values as well as the ratios of the retention times of the first and last peaks for the UHPLC run (Figure 1b), which are all 35% of the LC run (Figure 1a).

The column back pressure (not shown) for Figure 1a is 950 psi (~65 bar), whereas for Figure 1b it is 2635 psi (~180 bar). For UHPLC conditions, we can tolerate much higher pressures, and if we increase the flow rate to 1.0 mL/min, we get the chromatogram of Figure 1c. Because the column efficiency, *N*, does not change with flow rate for the sub-2-µm particles used in UHPLC, we expect to see the same chromatogram in Figure 1c as we did for Figure 1a and 1b, but with shorter retention times. As expected, the retention times for Figure 1c are one-fifth of those of Figure 1b, because we increased the flow rate fivefold from 0.2 mL/min to 1.0 mL/min. The pressure (not shown) also increased fivefold to 13,170 psi (~910 bar).

What About Gradients?

So far, everything is going as we expected. The change from LC to UHPLC is straightforward. Simply keep *N* constant and don't change the chemistry of the system, and we can change the flow rate to obtain an acceptable pressure and short run time. The run of Figure 1c is ~14 times faster than that of Figure 1a, just the kind of improvements we expect when moving from LC to UHPLC. Let's see what happens when we apply the same procedures to a gradient separation.

The sample for the runs of Figure 2 is a set of 12 polyaromatic hydrocarbons, starting with conditions that separate

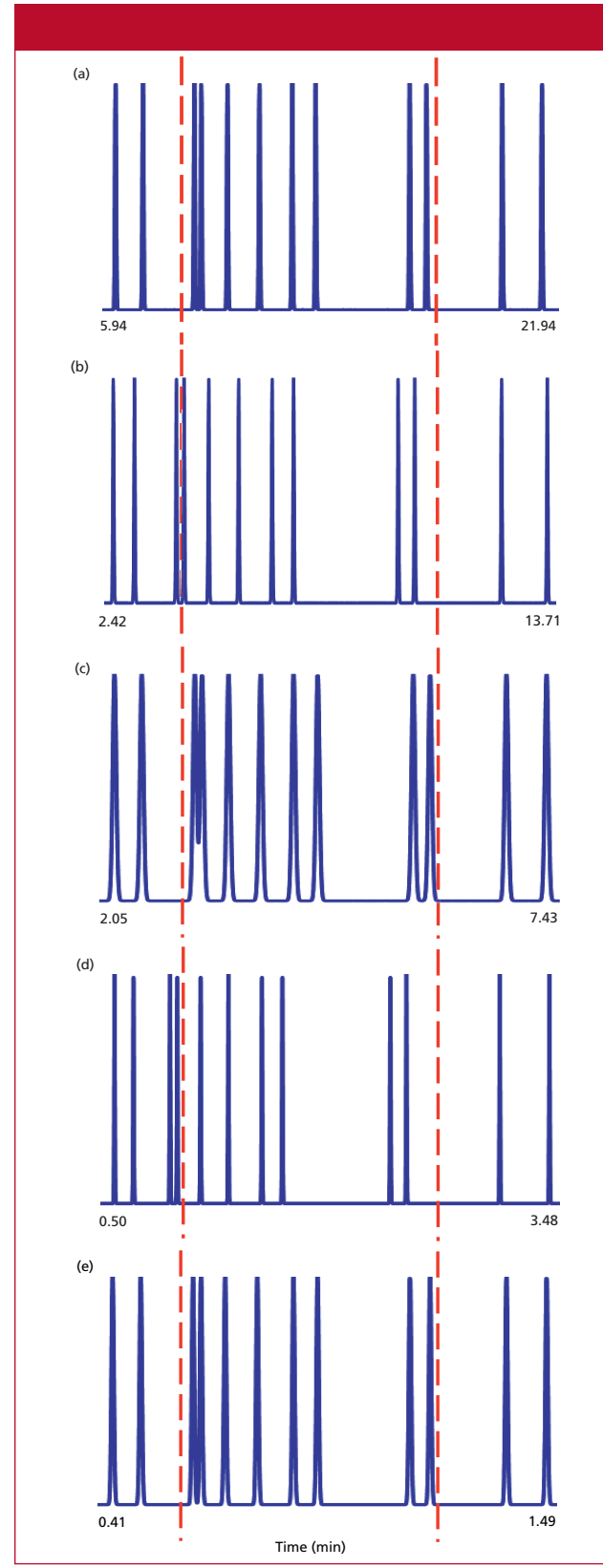

Figure 2: Simulated gradient reversed-phase runs for a set of polynuclear aromatic hydrocarbons for a gradient of 45–90% acetonitrile–buffer and 40 °C: (a) 150 mm × 4.6 mm, 5-µm column operated at 1.0 mL/min with a 30-min gradient; (b) 50 mm × 2.1 mm, 1.7-µm column operated at 0.2 mL/min with a 30-min gradient; (c) same as (b), but F = 0.2 mL/min and t_G = 10 min; (d) same as (b), but F = 1.0 mL/min and t_G = 10 min; (e) same as (b), but F = 1.0 mL/min, t_G = 2 min.

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them on a 150 mm × 4.6 mm, 5-µm column at 1 mL/min; the gradient runs from 45% to 90% acetonitrile over 30 min. In each chromatogram, I've normalized the plots by stretching or compressing the *x*-axis so that the first and last peaks line up vertically. The least-resolved, or "critical" peak pair comprises peaks 3 and 4, which are separated almost to baseline in the initial run of Figure 2a. I've added a couple of vertical dashed red lines to serve as reference markers so we can track the position of this peak pair, as well as for peaks 9 and 10.

Let's apply the same logic we used for the isocratic case and run the method on a 50 mm × 2.1 mm, 1.7-µm column at 0.2 mL/min to obtain the same linear velocity. The results are seen in Figure 2b. Immediately we see that the chromatograms are not the same. Both peak pairs have shifted to (relatively) shorter retention times. Also, peaks 3 and 4 are baseline separated — an obvious improvement over Figure 2a. This cannot be attributed to an increase in *N*, because *N* for the 1.7-µm column is actually ~2% lower than for the 5-µm column. What can be going wrong? Then we remember that the smaller-particle column is one-third the length of the larger-particle one, so t_0 should be one-third also (compare t_0 for Figures 1a and 1b). So what happens if we reduce the gradient time, t_G, to one-third — a change from 30 min to 10 min? This is shown in Figure 2c. Now the peaks all line up again and the resolution values for the various peak pairs compare favorably between Figure 2a and 2c, as we had hoped. The retention times for the shorter column also are reduced to approximately one-third of the original times.

The pressure for the run of Figure 2a is 465 psi (~30 bar) and 1285 psi (~90 bar) for Figures 2b and 2c. Both of these are well below the >6000 psi (>400 bar) expectations of a UHPLC method. This means we should be able to make further gains in throughput by increasing the flow rate, just as we did when changing from the conditions of Figure 1b to those of 1c in the isocratic case. When we change the flow rate from 0.2 mL/min (Figure 2c) to 1.0 mL/min, we get the results of Figure 2d. The pressure increases the expected fivefold to 6420 psi (~445 bar), but now we're back to a separation more like that of Figure 2b than 2a or 2c. Something isn't right!

Gradient Retention Factors Are Different

As we've noticed with the various changes in the gradient method, the results we obtain don't seem to track with the same changes under isocratic conditions. The reason for this is that gradient retention factors are not calculated in the same way as those for isocratic. The gradient retention factor, k^*, can be estimated as follows:

$$k^* \approx (t_G F)/(\Delta\Phi V_m S) \quad [4]$$

where *F* is the flow rate (in milliliters per minute), $\Delta\Phi$ is the gradient range (equal to 0.45 for the current 45–90% gradient), V_m is the column volume (in milliliters), and *S* is a constant for a given analyte. As was the case for the isocratic *k* value in equation 1, we need to keep both *N* and k^* constant if we want to keep resolution constant in gradient elution. We shouldn't change the gradient range, because this will change the chemistry of the system, and we are not changing the sample, so *S* will be unchanged. This allows us to simplify equation 4 as follows:

$$\text{constant} = (t_G F)/V_m \quad [5]$$

That is, when we change the gradient time, flow rate, or column size, we need to make compensating changes to keep equation 5 constant. We need one last equation to estimate V_m:

$$V_m \approx 0.5 L d_c^2/1000 \quad [6]$$

Thus, our 150 mm × 4.6 mm column has $V_m \approx 1.6$ mL and the 50 mm × 2.1 mm column has $V_m \approx 0.11$ mL.

Let's see how the runs of Figures 2a–2d compare when using equation 5. For Figure 2a, (30 × 1)/1.6 = 18.9; for Figure 2b, (30 × 0.2)/0.11 = 54.4; for Figure 2c, (10 × 0.2)/0.11 = 18.1; and for Figure 2d, (10 × 1)/0.11 = 90.7. (My usual warning applies here: If you try to repeat my calculations, the results are likely to vary slightly because of

rounding.) So it is easy to see why Figure 2a and 2c look the same (18.9 ≈ 18.1) and Figures 2b and 2d look quite different (18.9 ≠ 54.4 ≠ 90.7).

We also can use equation 5 to understand how to adjust the conditions of Figure 2d to get the same separation as Figure 2c, but at 1 mL/min. If we reduce the gradient time to 2 min, we get (2 × 1)/0.11 = 18.1, which is the same as for Figure 2c. The results shown in Figure 2e confirm this prediction. We could tweak this further to get the same separation as in Figure 2a (for example, a gradient time of 2.1 min would give [2.1 × 1]/0.11 = 19.0 ≈ 18.9 for Figure 2a), but this is probably not worth the trouble.

With the correct adjustments, we've reduced the retention time for the last peak by ~15-fold by using the smaller UHPLC column at 1 mL/min. This is approximately the same savings we made with the isocratic changes of Figure 1.

Conclusions

We have seen that transferring an isocratic method from LC conditions to UHPLC conditions is pretty simple. Just find a column that has the same chemistry and approximately the same plate number as the original LC column. Then adjust the flow rate for an acceptable back pressure. I recommend the intermediate step of transferring the method to conditions with the same linear velocity (such as going from Figure 1a to 1b), but that isn't essential.

When transferring gradient methods from LC to UHPLC, however, more care needs to be taken. Even with the same column chemistry and plate number, flow rate can be changed only if other compensating changes are made to keep the results of equation 5 constant. For the present example, there were small changes in relative retention and resolution when poor choices were made, but we were lucky because the sample comprises similar compounds, which are expected to respond similarly when conditions are changed. If the sample contained analytes with different functional groups (such as acids, bases, and neutrals) or were more complex (such as a natural product sample or protein digest), complete loss of resolution or retention reversals are possible for some peak pairs when gradient conditions are not changed properly.

As a final caution, the data I used for the generation of the examples of Figure 2 were modified so that the dwell volume was zero. In real systems, the dwell volume typically is in the range of 1.5–3.5 mL for a conventional LC system and 0.3–1.5 mL for a UHPLC system. When methods are transferred between systems, theoretically the dwell volume should be scaled by the same factor as the column volume, V_m. This adds a little more complexity to the method transfer and tends to affect the relative retention of early eluted peaks more than later ones.

The bottom line is that transferring isocratic or gradient methods from LC to UHPLC usually will give a large reduction in sample retention, but it can be much more challenging than simply installing a UHPLC column and changing the flow rate. So, take care and pay attention to the basics and you should have success in method transfer.

References

(1) General Chapter <621> "Chromatography" in *United States Pharmacopeia 37–National Formulary 32* (United States Pharmacopeia, Rockville, Maryland, 2014).

John W. Dolan

"LC Troubleshooting" Editor John Dolan has been writing "LC Troubleshooting" for LCGC *for more than 30 years. One of the industry's most respected professionals, John is currently the Vice President of and a principal instructor for LC Resources in Lafayette, 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*

For more information on this topic, please visit www.chromatographyonline.com/column-lc-troubleshooting

PERSPECTIVES IN MODERN HPLC

New HPLC Systems and Products Introduced at Pittcon 2015: A Brief Review

This installment highlights noteworthy high performance liquid chromatography (HPLC) and related products introduced at Pittcon 2015 in New Orleans, Louisiana, as well as in the year prior. It summarizes the technical aspects of new HPLC and supercritical fluid chromatography (SFC) systems, modules, software, and product extensions. The focus is on innovative products, major upgrades, and novel features with substantial user impact.

Michael W. Dong
Perspectives in Modern HPLC Editor

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) is the world's largest conference on laboratory science. The 66th Pittcon was held this year at the Ernest N. Morial Convention Center in New Orleans, Louisiana, from March 8 to 12. Prior years that Pittcon was held in the same city were 1988, 2001, 2002, and 2008. New Orleans, Chicago, Illinois, and Orlando, Florida, are the three top locations for Pittcon, supplemented by venues in other cities such as Philadelphia, Pennsylvania, and Atlanta, Georgia (1). New Orleans is located on the bank of the Mississippi River and is famous for its Cajun cuisine, jazz and blues heritage, and the annual festivity of Mardi Gras. The subtropical climate at Pittcon this year contrasted greatly with the austere wintry temperatures of Pittcon 2014, which was held in Chicago.

Pittcon 2015 attracted 14,200+ attendees from more than 90 countries to attend more than 2000 technical presentations, ~150 short courses, and a huge exposition of analytical instruments. Once again, Pittcon 2015 provided a premier meeting place for analytical chemists and a global showcase for instrument manufacturers.

Megatrends in HPLC Equipment

2015 has been a productive year so far for new high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) product introductions. A little more than 10 years after the commercial introduction of the first ultrahigh-pressure liquid chromatography (UHPLC) system in 2004 (2,3), second-generation UHPLC systems are steadily making their debuts. Following are a few of the megatrends in HPLC equipment:

- Improved second-generation UHPLC systems that offer higher operating pressure ratings, lower dispersion, innovative ovens or autosamplers, and faster cycle times (4–6).
- Many manufacturers offer a single instrument platform with multiple-tiered pressure ratings (Agilent 1290 versus 1260 or 1220) or configurations (modular versus integrated). Modular systems typically have higher system performance, while integrated systems generally have lower pricing for quality control applications (for example, Shimadzu Nexera X2 versus Nexera-i).
- New systems can be brand-new platforms (such as the JASCO LC-4000 and Thermo Vanquish) or upgraded versions of existing systems (for example, Agilent 1290 Infinity II). They can also be specialized application systems (for example, two-dimensional [2D]-LC, as exemplified by Shimadzu's Nexera-e system).
- While hardware specifications remain important, purchasing decisions continue to be dominated by considerations of the choice of the chromatography data system (CDS) (7).

SFC is emerging as a preferred technology for chiral separation and purification with an increasing number of manufacturers appearing in the marketplace (JASCO and Shimadzu). Table I lists prominent new product introductions (arranged alphabetically by vendor name) at Pittcon 2015 or during the prior year, followed by more-detailed descriptions and comments for each product.

New HPLC and UHPLC Systems and Line Extensions

Several new HPLC and UHPLC systems or specialized applications systems and

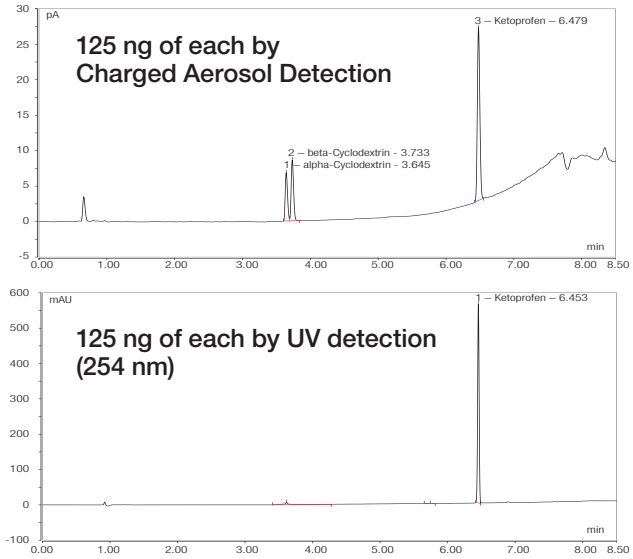
Icon image: Joe Zugcic, Joe Zugcic Photography

Universal UHPLC Detection in an Integrated Solution

Charged aerosol detection (CAD) is a well-established, near universal LC detection technology that provides a consistent response that is independent of the analyte's chemical structure. This is especially useful for detecting substances without chromophores and the quantification of unknowns without a comparable chemical standard. The technology is now available as a detection module for the Thermo Scientific™ Vanquish™ UHPLC system.

Vanquish UHPLC System with Charged Aerosol and Diode Array detection modules

Quantify Active Ingredients and Excipients in a Single Run



Ketoprofen (API) and α/β Cyclodextrin (excipient) were separated on a Thermo Scientific™ Vanquish™ Acclaim™ PA2, 2.2 µm, 2.1 x 250 mm column using the Vanquish UHPLC System. Although the diode array does not detect Cyclodextrins, it does give a stronger Ketoprofen signal, providing a more sensitive limit of quantitation (LOQ= 55 pg).

Leave No Peak Unseen

Compounds separated by chromatography have such diverse nature, that often no single detector sees them all. The Vanquish Charged Aerosol and Diode Array detectors are very powerful instruments and when combined, offer superior detection abilities addressing the broadest range of analytes, including:

- **Pharmaceutical drugs and impurities**
- **Excipients**
- **Biomolecules**
- **Natural products, supplements & botanicals**
- **Foods and beverages**
- **Surfactants and polymers**

Analytes without chromophore are hard to detect by a diode array detector but will typically be "seen" by a charged aerosol detector. With both, effectively, no sample component will be missed. Analysts can be assured the best data for verification and reporting by choosing the most appropriate results.

Orthogonal Detector Performance

The Vanquish UHPLC System with fully integrated Charged Aerosol and Diode Array Detector modules provides the most powerful UHPLC platform on the market. Scientists can now address the widest range of challenging analytical applications with speed, resolution, sensitivity and confidence.

Learn more about the Vanquish UHPLC system with charged aerosol detection at:

thermoscientific.com/Vanquish

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Table I: New HPLC product introductions at Pittcon 2015 or in the prior year

Exhibitor/ Vendor	New HPLC or Related Product	Description and Comment
Agilent	1290 Infinity II	An upgraded UHPLC platform with higher pressure and throughput and lower system dispersion. Significant performance upgrades for the autosampler and multicolumn thermostat.
Agilent	1290 Infinity II ELSD	An upgraded evaporative light scattering detector with a wider linear dynamic range and subambient drift tube operation for thermally labile compounds.
Applied Separations	eCO_2 Chrom	A flash chromatography system using liquid carbon dioxide as the primary eluent and a secondary pump for eluent modifier.
Cecil Instruments	Merit HPLC systems	Simple, low-cost HPLC systems with built-in software for teaching, column screening, and process development.
Chromperfect	Chromperfect SEVEN CDS	Chromperfect SEVEN v.6.0.10 for Windows 7 and 8 has been tested and found compatible with a prereleased version of Windows 10.
DataApex	Clarity v.6 CDS	Updated software with enhanced user interface and system control of most GC systems and many HPLC systems.
Grace	Reveleris Prep system	A medium-pressure hybrid flash chromatography–preparative LC purification system with integrated touch-screen control, on-line detection, and automated injection and fraction collection.
JASCO	LC-4000 Series	A new HPLC–UHPLC platform for analytical and preparative LC based on 30+ stackable modules.
JASCO	SF-4000 Series SFC	Two full-featured analytical and preparative SFC systems with flow rates of 0.1–150 mL/min.
PerkinElmer	PE Altus HPLC/ UPLC	A new collaboration with Waters to market HPLC/UPLC with Empower control for non-pharma markets.
Scientific Systems, Inc.	New series of Next Gen pumps	M1, MX, LS, LU, LD, PR, and CP pumps capable of a wide range of flow rates and pressure limits.
Shimadzu	Nexera-i and Prominence-i	Two integrated HPLC–UHPLC systems complementing the existing modular Nexera/ Prominence line.
Shimadzu	Nexera-e	A "comprehensive" 2D-LC system based on Nexera-X2 with specialized control software.
Shimadzu	Nexera-UC SFE–SFC–MS	An innovative supercritical fluid extraction (SFE)–SFC–MS system for automated extraction and high-resolution analysis of labile analytes and complex samples.
SofTA	2300 ELSD	An evaporative light scattering detector with 3+ orders of magnitude in dynamic range and improved sensitivity using helium gas.
Thermo Fisher Scientific	Vanquish UHPLC	A new UHPLC system with a 1500-bar pressure rating and low dispersion and dwell volume designed for ultrafast applications.

modules are summarized in Table I and described with more details below:

Agilent 1290 Infinity II UHPLC — Five years after the introduction of the 1290 Infinity UHPLC, Agilent implemented a major upgrade to their flagship UHPLC system. All modules have a similar look and footprint (slightly wider for the column compartment) with significant performance enhancements for the autosampler and the column oven. The Agilent 1290 Infinity II has an upper pressure limit of 1300 bar with reduced system dispersion. Increased system throughput is accomplished with a new multisampler with an optional dual-needle design operating on two separate flow paths to reduce the cycle time to seconds. A higher sample capacity of up to 16 microtiter plates or 6144 samples (or 432 vials) is made possible by an integrated elevator plate feeder. Lower carryover (<9 ppm) can be achieved via external multisolvent wash to the outer needle and additional needle-seat backflush. The larger multicolumn thermostated compartment now fits up to eight short columns and accommodates column lengths of 30 cm with a temperature range of 4–110 °C. Two 1300-bar pumps are available: a high-speed (binary) pump and a flexible (quaternary) pump with improved compositional accuracy. A new diode-array detector has a higher sampling rate of 240 Hz and a smaller flow cell for improved efficiency with 2.1-mm i.d. columns. The Infinity II system uses Instrument Control Frame-work and RapidControl Drivers software to facilitate connection to any Agilent CDS (OpenLab or MassHunter) or other third party CDS. A new quick-connect fitting (fingertight up to 1300 bar) that autoadjusts tubing insertion length to eliminate void space is available.

Figure 1 shows the reduced cycle time of the Agilent 1290 Infinity II system using the double sampling needle option to eliminate time lags between injections.

Cecil Instruments' Merit HPLC systems — The Merit HPLC systems from Cecil Instruments are low-cost HPLC systems with built-in chromatography software and UV–vis or photodiode-array detection designed for teaching, quality control, column screening, and process development applications.

JASCO LC-4000 Series HPLC and UHPLC systems — The JASCO LC-4000 series is the latest LC platform consisting of many 300-mm wide, stackable modules. These available modules can be configured into conventional (500 bar), rapid analysis HPLC (700 bar), UHPLC (1300 bar), or preparative LC systems (20, 50, or 120 mL/min). The LC-4000 replaces the existing JASCO series of X-LC-3000 or LC-2000

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- Excellent first-choice column for method development
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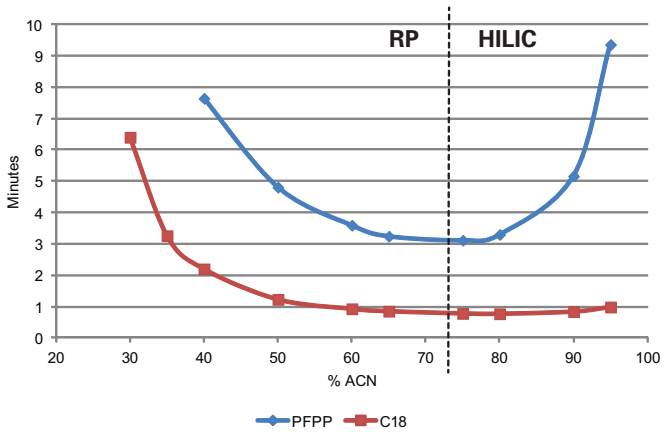

Imipramine Retention Profile based on % ACN
PFPP vs. C18

The Selectra® PFPP phase can use multiple selectivity mechanisms including hydrogen bonding, dipole-dipole, pi-pi overlap, HILIC, and hydrophobic interactions. It's ideal for pharmaceutical and natural products research and is well suited to LC/MS/MS. UCT's Selectra® PFPP HPLC column is an excellent choice for compound classes such as nucleosides, nucleotides, purines, pyrimidines, beta-blockers, taxanes, tricyclic antidepressants, and fluorinated corticosteroids.

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INNOVATION THROUGH CHEMISTRY

Figure 1: Two comparative signal traces of the reduced cycle time of the Agilent 1290 Infinity II system using the new Multisampler autosampler with a dual-needle design that eliminates time lags between injections.

systems. All are controlled by the upgraded JASCO ChromNAV 2.0 CDS.

More than 30 new modules are available for analytical or preparative-scale applications with various specifications as follows:

- Autosampler: HPLC (300 bar, 60 vial), rapid HPLC (700 bar, 180 vial), UHPLC (1300 bar, 180 vial), and preparative scale (400 bar, 10-mL injection, 40 vials).
- Column ovens: Compact, high capacity (holds six or 10 columns), preparative (temperatures ranging from ambient -15 °C to 100 °C), and a reaction oven for postcolumn derivatization.
- Pumps: Four analytical pumps (binary or quaternary, 700 or 1300 bar) with dynamic or turbulent flow mixers and built-in microdegassers. Three preparative pumps have maximum flow rates of 20, 50, or 120 mL/min rated to 500 bar and have optional solvent degassing and recycling features.
- Detectors: Available UV–vis, photodiode-array, refractive index (analytical or preparative), fluorescence, circular dichroism, and optical rotation detectors.
- ChromNAV 2.0 CDS: Operates under Windows 7 or 8.1 with a graphical user interface and controls up to four JASCO systems. This CDS is 21 *CFR* Part 11 compliant. An optional ChromNAV-FC CDS is available for preparative LC and an optional ChromNAV-GPC add-on is used for molecular weight distribution calculations.

PerkinElmer Altus HPLC/UPLC — At Pittcon 2015, PerkinElmer launched its Altus HPLC and Altus UPLC systems, controlled through the Waters Empower CDS, for the environmental, industrial, and applied markets.

Shimadzu Nexera-i and Prominence-i systems — Shimadzu introduced two identical looking integrated HPLC (Prominence-i) and UHPLC systems (Nexera-i) to complement their existing Prominence and Nexera XR/X2 modular systems. According to Shimadzu, the "i" in the i-series names stands for "integrated" (6) and also for "innovative," "intuitive," and "intelligent." These integrated units are designed for quality control and academic laboratories and are controlled by Shimadzu's LabSolutions and major third-party CDS. Each system is equipped with a built-in color touch screen to allow control of all LC parameters, including automatic on/off, manual purging, batch table sequencing functions, and chromatogram display. System status (ready, pretreatment, run, and error) is readily visible from the front panel. One can start an analysis from the instrument's front panel immediately after loading samples. Remote monitoring via smart devices is possible.

The built-in autosampler can make reproducible injections down to less than 1 µL with a 14-s cycle time and can accommodate up to 216 1.5-mL vials. The large-capacity forced-air column oven fits six 10-cm or three 30-cm columns with a temperature range of ambient -10 °C to 85 °C. The built-in quaternary pump is rated at 440 bar (Prominence-i) or 660 bar (Nexera-i) and uses a parallel double plunger design. An optional low-pressure valve allows selection of up to seven solvent reservoirs. The built-in UV–vis or photodiode-array detector is equipped with TC-Optics and flow cell to reduce signal drift because of ambient temperature changes. Shimadzu's LabSolutions CDS allows many convenient autofunctions such as start-up, purging, column equilibration, baseline check, shutdown, system validation, and email notifications.

Shimadzu Nexera-e 2D-LC system — Shimadzu Nexera-e (the "e" stands for exponential) is a comprehensive 2D LC system based on the company's Nexera X2 UHPLC system. The system is designed for the analysis of complex samples by coupling two "orthogonal" separating modes with a built-in valve–loop switching system, and detection using photodiode-array or mass spectrometry systems (8). System configuration and setup is accomplished using LC×LC Assist software. Comprehensive 2D qualitative and quantitative analysis using contour graphics is accomplished via ChromSquare software, which can also display mass spectral data. Figure 2 shows a chromatogram of a first-dimensional separation of a complex traditional Chinese medicine sample and the resultant 2D contour display of more than 200 peaks displayed with ChromSquare software.

Thermo Scientific Vanquish UHPLC system — The Thermo Scientific Vanquish UHPLC system is a new integrated, low-dispersion, biocompatible UHPLC system with an upper system pressure limit of 1500 bar or 22,500 psi (5). The Split Sampler HT is capable of precise injections of 0.01–100 µL. Its injection cycle time can be as short as 15 s, with carryover as low as 4 ppm. The sampler supports injections from HPLC vials as well as from 96- and 384-well plates. The optional charger unit extends sample capacity to 23 plates (8832 samples). Automated barcode reading for rack identification is available as an option. The unique column compartment H (5–120 °C) can

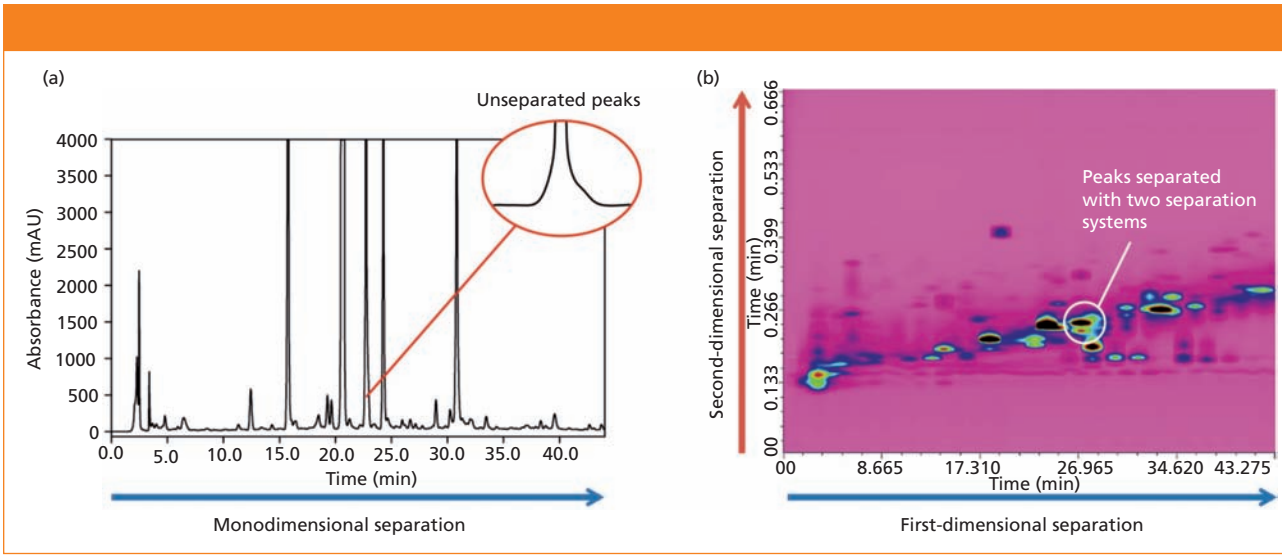

Figure 2: (a) Chromatogram in the first dimension of a separation of a complex traditional Chinese medicine sample with UV detection; (b) Resultant 2D contour display of more than 200 peaks with the ChromSquare software shown as spots in the 2D plot from "orthogonal" separations in the comprehensive 2D mode. Coeluted peaks from the first dimension (manifested as a peak shoulder in the inset of 2a) are separated as distinct spots shown in 2b.

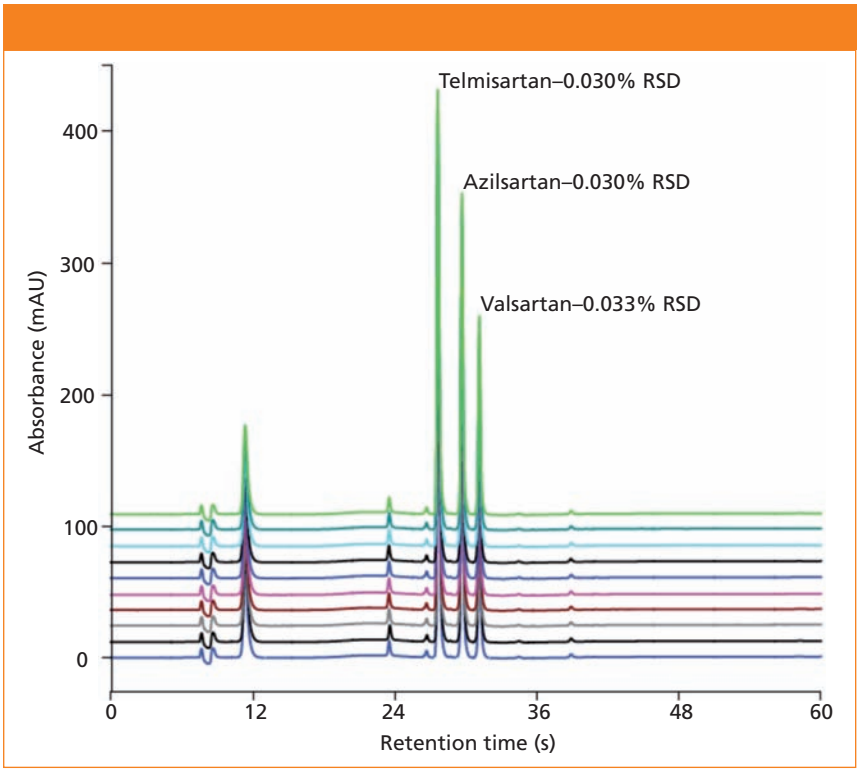

Figure 3: The excellent precision of the Vanquish UHPLC (peak area RSD of ~0.03%) in an overlaid chromatogram of 10 repetitive injections of a pharmaceutical sample containing three major drug components.

operate in two thermostating modes: a still-air mode to maximize column efficiency by reducing radial thermal gradients inside UHPLC columns or a forced-air mode to mimic column ovens of other manufacturers (9,10). The binary pump H has a unique dual-piston-in-parallel design with variable stroke volume capability. It has a flow accuracy of ±0.1% and pulsation of <0.4%. It supports binary high-pressure gradient mixing of six solvents (in a configuration of 2×3 and a total of nine combinations) with a default mixing volume of 25 µL. The built-in diode-array detector HL uses silica "LightPipe" technology, has a flow cell volume of 2 µL or 13 µL (pathlengths of 10 mm or 60 mm, respectively), and supports programmable slit widths of 1–8 nm spectral bandwidths. The system is controlled by the Chromeleon CDS with integrated mass spectrometry (MS) support.

Figure 3 shows the excellent precision performance of the Vanquish UHPLC system with an overlaid chromatogram of 10 repetitive injections of a pharmaceutical sample. Figure 4 shows the simulated thermal heat maps inside a UHPLC column operating under a still-air mode (to maximize column efficiency) or a forced-air mode (to mimic operation in other manufacturers' ovens) (9,10).

HPLC Modules

Several new HPLC modules were introduced this year:

Agilent Infinity II ELSD — An upgraded evaporative light-scattering detection (ELSD) system with an improved linear dynamic range is capable of subambient drift tube operation for detection of thermally labile compounds.

SofTA 2300 ELSD — The upgraded 2300 ELSD system from SofTA has more than three orders of magnitude in dynamic range and an option of using helium nebulizer gas for improved sensitivity.

Scientific Systems Next Gen pumps — Scientific Systems, Inc. (SSI) introduced a new family of Next Gen pumps: M1, MX, LS, LU, LD, PR, and CP pumps capable of a wide range of flow rates (0.001–300 mL/min) and pressure limits (50–25,000

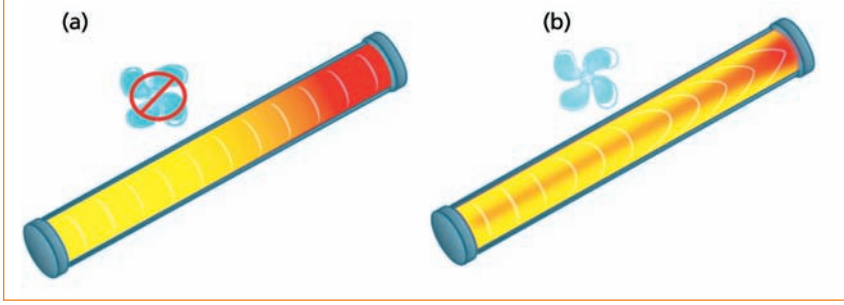

Figure 4: Simulated thermal heat maps inside a UHPLC column operating at very high pressure in a Thermo Scientific Vanquish UHPLC system: (a) Using the column oven operating under a still-air mode (with the fan off to maximize column efficiency without any radial thermal gradients); (b) using a forced-air mode with a circulating fan. Note that radial thermal gradients in the column under the forced-air mode can be highly detrimental to column efficiency performance.

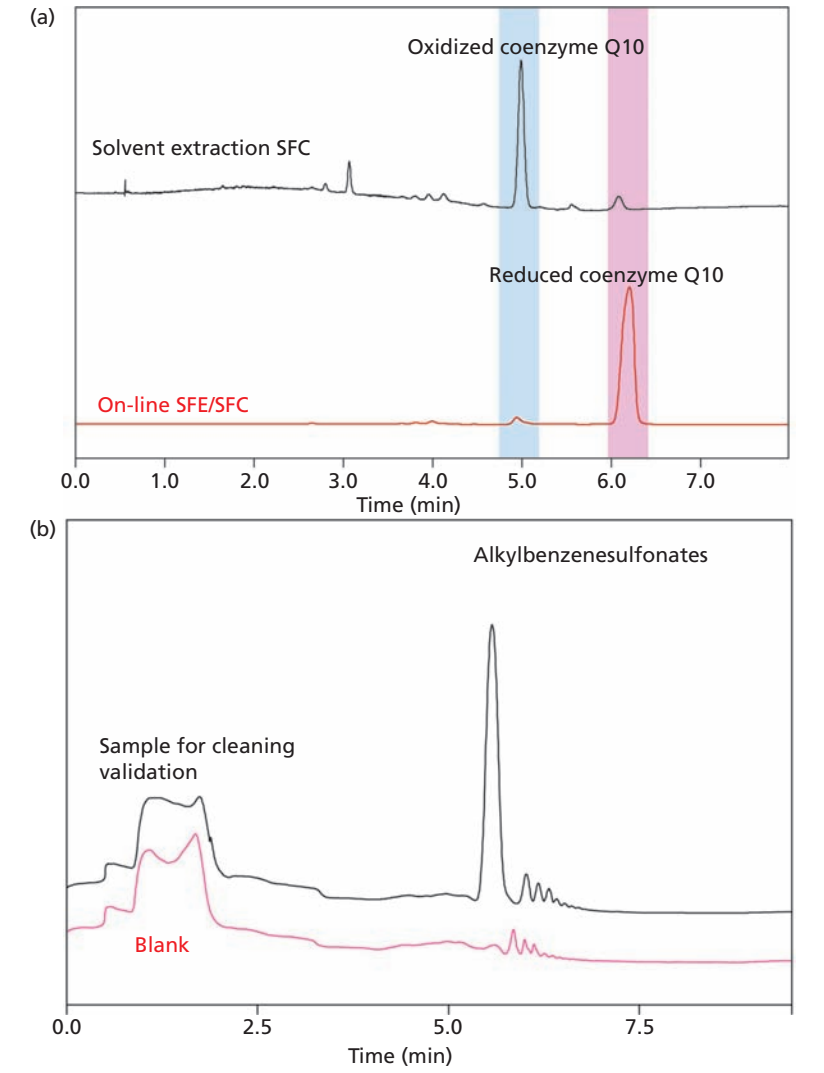

Figure 5: The results of two applications of the Nexera UC SFE–SFC–MS system: (a) An on-line SFE and SFC analysis of reduced coenzyme Q10 extracted from a dietary supplement shown in the bottom chromatogram is preferred over analysis using off-line extraction. (b) SFC chromatogram of residual detergents (alkyl benzenesulfonates) from a cleaning verification swab sample extracted directly from the swab with SFE versus a blank swab.

psi). The company's product line consists of pumps for analytical and preparative scales, flash, SFC, mini-metering, process, UHPLC, and biocompatible applications.

Chromatography Data Systems

A number of new updates in chromatography data systems were introduced this year:

Agilent OpenLab CDS — The improved OpenLab CDS software has a more intuitive user interface and faster time for data processing, reviewing, and reporting. It is 21 *CFR* 11 and European Union Annex 11 compliant and has a new graphical tool for sample entry for the new Infinity II Multisampler autosampler.

Chromperfect SEVEN CDS — Chromperfect SEVEN CDS v.6.0.10 for Windows 7 and 8 was found to be compatible to a prereleased version of Windows 10. Both Chromperfect SEVEN client-server and small laboratory (SL) CDS are 21 *CFR* part 11 compliant and can control most gas chromatography (GC) and many HPLC systems.

DataApex Clarity v.6 CDS — DataApex introduced an updated version of Clarity v.6 CDS with an enhanced user interface, sequence functions, GC×GC extension, and higher linearity ranges for MS and flame ionization detectors as well as system controls for Hitachi HPLC systems and Advion MS systems.

Preparative LC, SFC, and Other Related Products

Several new preparative LC, SFC, and other related products were also introduced this year, such as:

Grace Reveleris Prep system — The Grace Reveleris Prep is a medium-pressure (1700 psi), hybrid flash chromatography–preparative LC purification system, with integrated control software displayed on a 12-in. touch screen. It has a maximum flow rate of 200 mL/min with a built-in automated injector and fraction collector that uses three UV–vis plus evaporative light-scattering detector signals. This simple, yet flexible system is ideally suited for use in an organic synthesis or sample purification laboratory.

JASCO 4000 Series SFC — JASCO introduced five SF-4000 Series systems for semimicro, analytical, analytical or semipreparative, and semipreparative and preparative SFC. The systems have pumps capable of flow rates ranging from 0.2 mL/min to 150 mL/min at pressures up to 500 bar, variable loop injections of up to 180 samples,

multiple column or solvent selection, detection by UV, circular dichroism, photodiode array, flame ionization, ELSD, and MS, and enclosed automated fraction collection.

Shimadzu Nexera-UC SFE–SFC system — The Shimadzu Nexera-UC (unified chromatography) SFE–SFC–MS system is an innovative on-line system designed for automated extraction and ultrafast analysis of a variety of analytes including labile compounds in complex samples (11,12). The automated Nexera SFE system can extract 48 samples (in 0.2- or 5-mL extraction vessels, at temperatures up to 80 °C and pressures as high as 40 MPa) with subsequent on-line SFC analysis with MS and UV detection. The system is uniquely suited for analysis of difficult samples such as multiresidual pesticides and polycyclic aromatic hydrocarbons in foodstuffs or soil, labile lipids or disease biomarkers in biofluids or dried bloods, swabs for cleaning verification (12), and additives in organic polymers. The Nexera SFC system includes the LC-30AD pump (5 mL/min to 66 MPa) with a back-pressure control unit and can be configured for chiral screening (12 columns with four organic modifiers) or as an SFC-UV analytical system. The system's automated extraction, coupled with the direct splitless SFC–MS interface, leads to highly reproducible and sensitive assays of labile or water-sensitive analytes in complex sample matrices.

Figure 5 shows results from two applications of the SFE–SFC–MS system: automated analysis of a labile analyte (reduced coenzyme Q10 from a dietary supplement) and residual detergent analysis from a cleaning verification swab sample.

Applied Separations eCO_2 Chrom — Applied Separations' eCO_2 Chrom is a flash chromatograph using liquid (not supercritical) carbon dioxide as the primary eluent. A standard carbon dioxide cylinder provides the pressure to drive the system. A supplementary pump is used for delivering an eluent modifier (such as methanol). This purification system is equipped to use disposable and reusable columns, and comes with integrated software, a UV detector, and an automated fraction collector.

Concluding Remarks

Many analytical chemists, including myself, come to Pittcon for professional and personal reasons. My schedule this year (my 16th consecutive year) included several short courses on HPLC and UHPLC and the drug development process, attending the plenary lecture by Dr. Halas, and two of my own oral presentations and a poster on low-cost MS. My networking events included an *LCGC* editorial advisory board lunch meeting and the Chinese American Chromatography Association (CACA) dinner with ~100 attendees (featuring speeches by professor Yafeng Guan of Dalian Institute and Dr. Richard Henry from Supelco). I spent two days on the exposition floor gathering confirmatory information for this installment. The excellent organization of Pittcon allows me to learn about emerging analytical trends, meet with old colleagues, and make new acquaintances. I look forward to attending future Pittcon conferences to be held in Atlanta, Chicago, Orlando, Philadelphia, Chicago, and back to the "Big Easy" (New Orleans) in 2021.

Summary

This installment summarizes new HPLC and SFC product introductions (systems, modules, CDS, software, and related products) launched at Pittcon 2015 and in the prior year. It focuses on innovative features from a user's perspective. Since the coverage is brief and nonexhaustive, the reader is referred to manufacturers for additional technical details. Note that new HPLC columns introduced at Pittcon are presented and discussed separately in *LCGC*'s annual reviews (13). The opinions expressed in this installment are the author's own and bear no reflections of those from *LCGC*, the Pittsburgh Conference, or any other organizations.

Acknowledgments

I would like to thank the Pittcon staff for organizing this global conference so predictably well, where everything runs like clockwork. Also, a special thanks to the marketing personnel of manufacturers for their timely responses to our requests for information. The author is particularly grateful to Drs. Davy Guillarme and Szabolcs Fekete of the University of Geneva, Mengling Wong from Genentech, Dr. Raphael Ornaf of Vertex Pharmaceuticals, and John Batts of IDEX Health & Science for their invaluable editorial and technical input.

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MISER LC–MS Analysis of Teas, Soft Drinks, and Energy Drinks

In this study, we describe a simple and rapid liquid chromatography–mass spectrometry (LC–MS) method for the evaluation of caffeine, taurine, and aspartame in teas, soft drinks, and energy drinks using high performance liquid chromatography (HPLC) coupled with electrospray ionization (ESI)-MS detection and multiple injections in a single experimental run (MISER) analysis. The misergrams obtained from the injection of one sample per minute allows the convenient visualization of the outcomes of multiple analyses, allowing rapid comparison of analyte levels among a variety of products and brands.

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The technique of liquid chromatography–mass spectrometry (LC–MS) is evolving into a workhorse technology used across many industries and disciplines for routine chemical analysis and problem solving (1–7). Recent trends in miniaturization and cost reduction suggest an opportunity for even more widespread adoption of LC–MS beyond the traditional laboratory setting (8–13). Consequently, studies that familiarize students and other newcomers with the practical aspects of LC–MS are becoming increasingly important. While mastery of LC–MS may require many years of training and experience, a working knowledge can be picked up in a short time, especially when simplified versions of the technique are used.

Multiple injections in a single experimental run (MISER) (14) is a simplified form of LC–MS that is well suited to fast analysis of the presence of a given component within multiple samples (14–19). Minimal chromatographic separation is used, allowing fast analysis while separating the component of interest from substances that could potentially interfere with MS detection. The injection of multiple samples in the same experimental run allows a straightforward interpretation of the MS detector response (or "misergram") providing ready interpretation without the need for difficult and time-consuming peak integration, data processing, and graphing.

We previously investigated the application of MISER LC–MS to a study of the level of capsaicin in chili peppers and hot sauces, finding the experimental approach to be easily grasped by students (19). We now report an investigation into the use of MISER LC–MS for the analysis of caffeine, aspartame, taurine, and other components within teas, soft drinks, and energy drinks, with the hope that this approach may be utilized by students and others interested in rapidly developing a familiarity with LC–MS and high-throughput analysis.

Experimental

Instrumentation

Reversed-phase high performance liquid chromatography (HPLC) experiments were performed on an Agilent 1100 system. The Agilent stack comprised a G1312A binary pump, a G1367A WPALS autosampler, a G1315B diode-array detector, and a 6120 quadrupole LC–MS detector with electrospray ionization in the positive mode. The system was controlled by Chemstation software, with the flow injection analysis (FIA) mode enabled.

Chemicals, Reagents, and Stationary Phases

Acetonitrile (HPLC grade) was purchased from Fisher Scientific. Formic

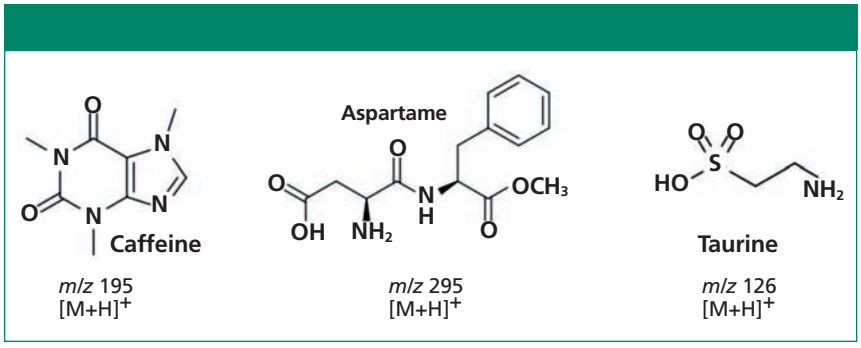

Figure 1: Structures of caffeine, aspartame, and taurine.

acid (HCOOH), ammonium formate (NH_4HCO_2), caffeine, aspartame, taurine, theanine, vitamin C, vitamin B5, and sucralose were purchased from Sigma–Aldrich. Ultrapure water was obtained from a Milli-Q Gradient A10 water purification system from Millipore. The 50 mm × 4.6 mm, 2.5-µm XBridge Phenyl column was purchased from Waters Corporation.

Preparation of Buffer Solutions

Solutions containing 2 mM ammonium formate in water (pH 3.5) and 2 mM ammonium formate in acetonitrile (pH 3.5): 12.6 g ammonium formate and 7.9 mL formic acid were dissolved in 1 L of Millipore water. A 100-fold dilution of this stock solution was performed in either pure water or a 90:10 acetonitrile–water mixture to prepare the 2 mM solutions.

Sample Preparation

Tea — Time Course

A single Lipton All Natural tea bag was placed in 8 oz (approximately 240 mL) of boiling water, and the bag was submersed using a kitchen thermocouple. A 2-mL sample was taken at $t = 0$ s (before tea bag addition), 30 s, 60 s, and every minute thereafter up to 8 min of total steeping time. (Note: The tea bag was not intentionally moved during the course of the experiment.)

Table I: Experimental tea samples

Tea Description	Amount
Lipton All Natural	Single bag
Pure White Tea	Single bag
Green with Jasmine	Single bag
White Tea, Frutto Blanco Pearls	5.159 g
White Tea, Lavender Dreams	2.301 g
The Bright Tea, English Breakfast	3.030 g
Lipton Decaffeinated	Single bag
Matcha Green	0.676 g

Tea — General

Brewed tea samples were achieved by placing the noted amount of tea in a Williams–Sonoma Open Kitchen Tea Ball, or a single tea bag where noted, and placing the ball or bag in 8 oz of boiling water for 5 min. The temperature was recorded at the start and end of the steeping, and a 2-mL

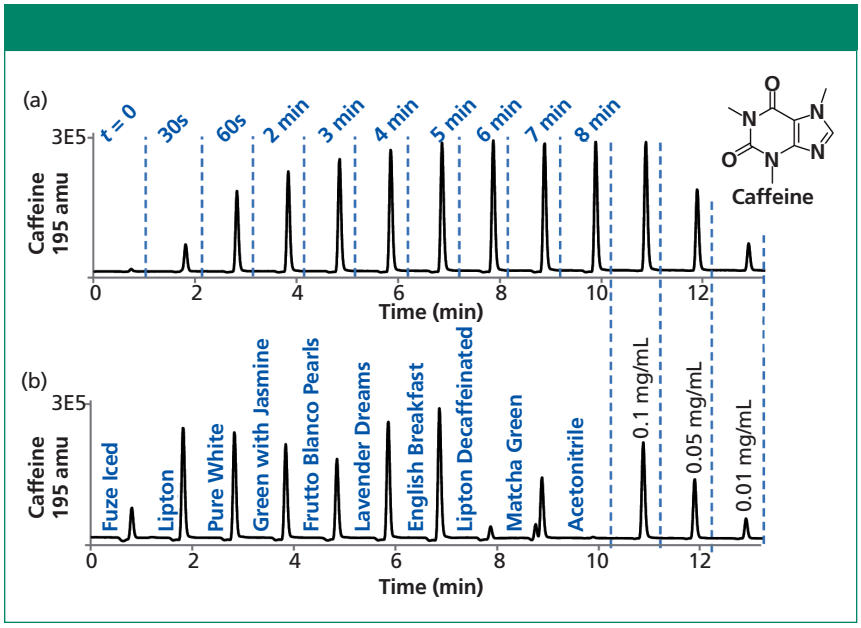

Figure 2: MISER HPLC–MS analysis of caffeine in various teas. Analysis of samples and authentic standards by MISER HPLC–MS with selected ion monitoring of the caffeine molecular ion at 195 amu reveals relative caffeine levels of time-course extraction study from (a) Lipton all natural tea and (b) nine different teas. Chromatographic and MS conditions were as described in the experimental section. Detection: ESI-MS(+) at *m/z* = 195 amu; injections: 0.6 µL every 1 min.

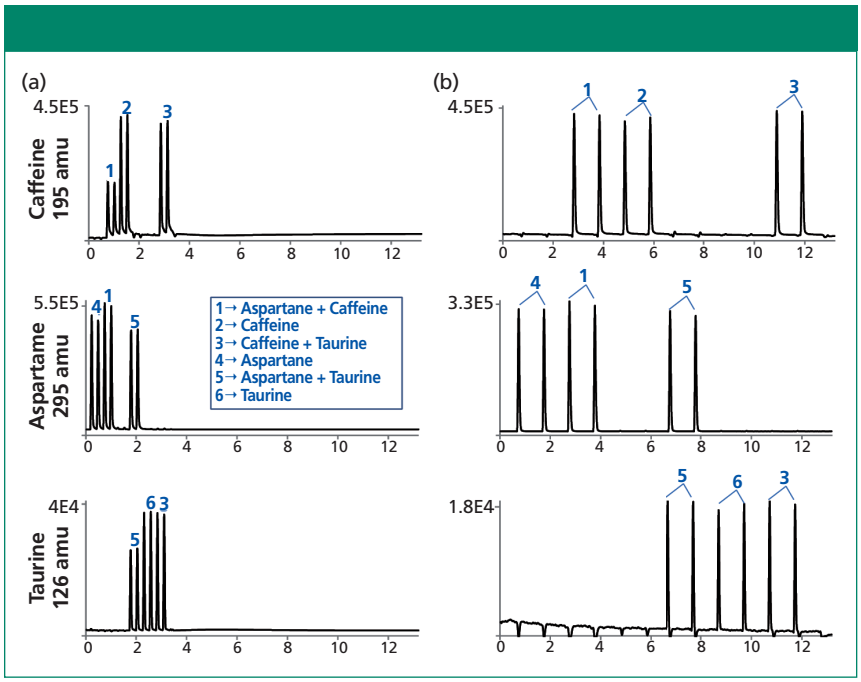

Figure 3: Signal response comparisons (*m/z* 195, 295, and 126) for caffeine, aspartame, and taurine standards and the respective two component mixtures: (a) MISER HPLC–MS profiles obtained from a fast method: column: SB-C18 (20 mm × 3.0 mm, 1.8 µm); temperature: 40 °C; detection: SIM ESI-MS(+) at *m/z* = 195, 295, and 265 amu; sample: 0.6-µL injection of each component solution at 0.25 mg/mL in acetonitrile–water; flow rate: 1 mL/min; isocratic mobile phase: 10% eluent A: 2 mM ammonium formate in water (pH 3.5) and 90% eluent B: 2 mM ammonium formate in 10:90 water–acetonitrile (pH 3.5). Time between injections: 22 s. (b) MISER HPLC–MS profiles obtained from the standard method as describe in the experimental section. Time between injections: 1 min.

sample was taken for analysis at the end of brewing. (Note: The tea ball [or bag] was not intentionally moved during the course of the experiment.) The observed average brewing temperatures were T_{init} = 97 °C, to T_{final} = 80 °C.

Store Beverages

Samples of various beverages, including Fuze iced tea, were obtained from a local supermarket by high school interns and directly analyzed without treatment. Authentic standards of caffeine, aspartame, and taurine were prepared by serial dilution and analyzed along with the beverage samples.

HPLC–MS Conditions

HPLC separations were carried out on a 50 mm × 4.6 mm, 2.5-µm XBridge Phenyl column by isocratic elution at a flow rate of 1 mL/min. The LC eluents were 60% solvent A (2 mM ammonium formate in water, pH 3.5) and 40% solvent B (2 mM ammonium formate in 90:10 acetonitrile–water, pH 3.5). The column and samples were maintained at a temperature of 25 °C. The misergrams were obtained from continuous sample injections (1 µL) every 1 min. The positive ion electrospray ionization (ESI) parameters were as follows: skimmer, 45 V; desolvation gas, nitrogen; temperature, 350 °C; and flow rate, 12 L/min. The nebulizer was adjusted to 35 psig, and the fragmentor and the capillary voltages were adjusted to 150 and 2500 V, respectively. G1969-85000 ESI-L Low Concentration Tuning Mix (Agilent Technologies) was used for tuning and calibration of the mass spectrometer.

Ion Suppression and Enhancement Between Target Analytes

Six sets of samples were prepared at 0.25 mg/mL. Set 1 contains aspartame and caffeine; set 2, caffeine; set 3, caffeine and taurine; set 4, aspartame; set 5, aspartame and taurine; and set 6, taurine. All six samples were analyzed per duplicated using the standardized MISER LC–ESI(+)-MS method (as described above). In addition, a faster MISER LC–ESI(+)-MS method was evaluated for comparison: Column: Zorbax Stablebond SB-C18 (20 mm × 3.0 mm, 1.8 µm, Agilent); temperature: 40 °C; detection: selected ion monitoring (SIM) ESI-MS(+) at *m/z* = 195, 295, and 265 amu; sample: 0.6-µL injection of each component solution in acetonitrile–water; flow rate: 1 mL/min; isocratic mobile phase: 10% eluent A: 2 mM ammonim formate in water (pH 3.5) and 90%

eluent B: 2 mM ammonium formate in 10:90 water–acetonitrile (pH 3.5). Time between injections was 22 s. The extent of ion suppression and ion enhancement effects are clearly visualized in the corresponding misergrams.

Results and Discussion

Students learning elementary principles in chemistry can find ready subject matter for study in the modern world. In addition to the practical advantages of sample availability, convenient disposal, and reduced requirements for personal protective equipment, the study of materials that can be found in a local supermarket provides a quick connection with the everyday world, reinforcing the important message that chemistry is omnipresent in the modern world. The analysis of caffeine in beverages is a popular laboratory experiment with a proven ability to engage students (20,21). Bier and Grabowski (22) have even implemented virtual MS approaches. In this study, we apply the MISER LC–MS technique to the measurement of caffeine and other substances in teas, soft drinks, and energy drinks collected at local supermarkets.

Samples of commercial teas were brewed before analysis (see the experimental section). A time-course study of the appearance of caffeine in brewed tea upon the addition of water is shown in Figure 2a. Sample aliquots were removed at designated timepoints and analyzed using MISER LC–MS with a sample injection period of 1 min and detection at *m/z* of 195, corresponding to the caffeine molecular ion. The resulting misergram shows the kinetic profile of the appearance of caffeine in the brewed tea. Inclusion of several caffeine standards allows approximate quantitation. A maximum caffeine concentration of about 0.1 mg/mL is reached by about 5 min. It should be noted that the steeping tea samples were not stirred, and that more rapid extraction of caffeine may be possible when teabags are stirred or "dunked."

A comparison of several different commercial teas, each prepared with 5 min steeping is shown in Figure 2b. An iced tea beverage is also included for comparison. While most of the teas show a caffeine level of about 0.1 mg/mL, the level in the iced tea is significantly less, and perhaps not surprisingly, only a trace amount of caffeine is observed in the sample of decaffeinated tea included in the study. In addition, it should be noted that the loose teas studied differed in particle size from large (Frutto Blanco Pearl), to small (English Breakfast tea ground for the Flavia brewing system), to almost powder-like (Matcha green tea). These results are in agreement with previous studies where caffeine concentrations in white, green, and black teas ranged from 14 to 61 mg per serving (6 or 8 oz) (23). Taken together, the MISER LC–MS experiment provides an easily interpreted graphical depiction of the experimental results.

An advantage of the MISER LC–MS analysis is the ability to simultaneously monitor the presence of several different analytes. We next set out to exploit this capability, focusing on the simultaneous

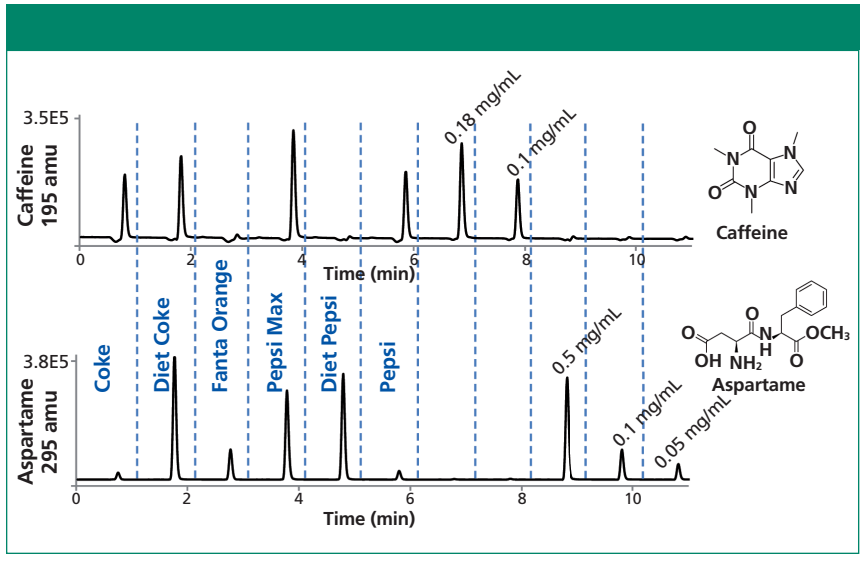

Figure 4: MISER HPLC–MS analysis of caffeine and aspartame in various sodas. Analysis of samples and authentic standards by MISER HPLC–MS with selected ion monitoring of molecular ions at 195 and 295 amu. Experimental conditions as described above. Detection: ESI-MS(+) at *m/z* = 195 and 295 amu; injections: 0.6 µL every 1 min.

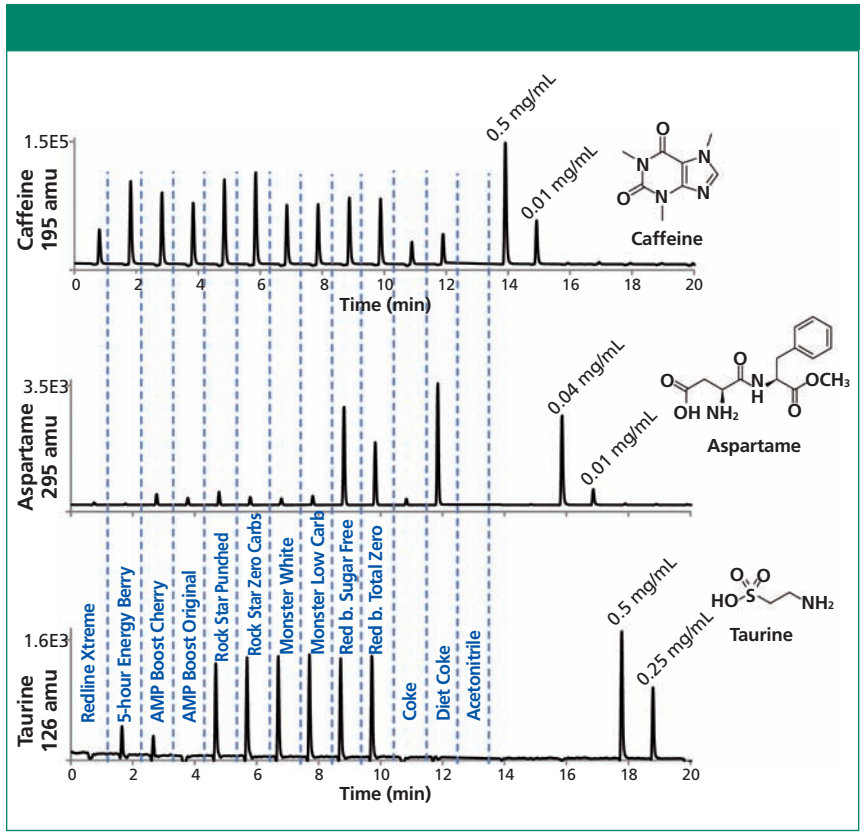

Figure 5: MISER HPLC–MS analysis of caffeine, aspartame, and taurine in various energy drinks and sodas. Analysis of samples and authentic standards with selected ion monitoring of the caffeine, aspartame, and taurine molecular ions at 195, 295, and 126 amu, respectively. Experimental conditions were as described in the text. Detection: ESI-MS(+) at *m/z* = 195, 295, and 126 amu; injections: 0.6 µL every 1 min. The first two energy drink samples (Extreme Triple Berry and 5 Hour Energy Berry) were 100× diluted, while all of the other samples were 10× diluted.

evaluation of caffeine, aspartame, and taurine in soft drinks and energy drinks. Accurate quantitation with the MISER LC–MS technique requires analytes of interest to be chromatographically resolved from potentially interfering substances in the sample mixture. When several analytes are simultaneously analyzed, it is important to demonstrate that the short analysis times for MISER LC–MS do not lead to sample interference. Model experiments can help to rapidly assess potential interference issues, identifying any possible "crosstalk" between the principal analytes under investigation. Figure 3a shows the results of such a method development study, clearly illustrating that the shorter method leads to a significant ion suppression of caffeine and taurine MS signal in the presence of aspartame, while the longer 1-min injection period is free from such problems.

The simultaneous evaluation of caffeine and aspartame in various soft drinks is shown in Figure 4. No caffeine signal was observed for the orange soda or Pepsi caffeine-free products, which was consistent with the labels on these products. The levels of caffeine observed in Coke, Diet Coke, and Pepsi were found to be slightly higher than 0.1 mg/mL. Pepsi Max was shown to have about 0.2 mg/mL, in keeping with reported values (24).

The levels of aspartame in Diet Coke and Diet Pepsi were found to be about 0.5 mg/mL, in keeping with reported values (25). Very small peaks for aspartame (<< 0.05 mg/mL) were observed in both Coke and Pepsi, although neither of these products contain aspartame as a listed ingredient. This finding highlights one of the potential pitfalls of using MISER LC–MS for detecting trace amounts of analytes in very complex mixtures: It may well be the case that the complex caramel mixtures or other components in these colas may contain a component that gives rise to a small amount of a coeluted component with mass 295 amu. Such interference problems could potentially be reduced or eliminated by the use of high-resolution MS detection using exact mass or multiple reaction monitoring (MRM), or the application of selected reaction monitoring (26,27) to multiple product ions from one or more precursor ions (28,29).

We next investigated the analysis of caffeine, aspartame, and taurine in a variety of different "sports energy" drinks, which have risen tremendously in popularity in recent years. In addition to caffeine, energy drinks often contain additives ranging from high-fructose corn syrup (for non-diet versions) to taurine, B vitamins,

sucralose, aspartame, maltodextrin, glucuronolactone, carnitine, creatine, inositol, and various extracts of açaí, ginseng, and *Ginkgo biloba* (30).

The misergrams shown in Figure 5 show the relative levels of caffeine, taurine, and aspartame in a variety of different energy drinks, with Diet Coke and Coke included for comparison. Initial evaluations by direct sample injection showed much higher levels for the three components, with resulting poor baselines. Consequently, all samples were diluted 10 times with water, with the exception of Red Line Xtreme and 5 Hour Energy, which were diluted 100×. The resulting misergrams show much higher caffeine content in the energy drinks than in Coke and Diet Coke, with 5 Hour Energy showing a considerably higher amount of caffeine (similar signal response as Rock Star drinks, but the sample is 10 times more diluted). Red Bull sugar free and Total Zero show aspartame concentrations lower, but comparable to Diet Coke. Rock Star shows almost no aspartame, but checking the ingredients we found sucralose, another artificial sweetener commonly used in energy drink preparations. On the other hand, the taurine signal response from Rock Star, Monster, and Red Bull drinks were very similar at about 0.4 mg/mL, but higher than AMP Boost Cherry. All of these results are consistent with the caffeine, aspartame, and taurine levels published in the different literature sources (24,25,31–35).

During the course of our investigations it became clear that in addition to caffeine, aspartame, and taurine, many other components are present in some of these beverages. Many products contain vitamins (such as vitamins C, B5, B6, and B12), artificial sweeteners such as sucralose or even added compounds such as açaí, choline, various forms of ginseng, inositol, carnitine, creatine, and *Ginkgo biloba*. Figure 6 illustrates how the MISER LC–MS approach can potentially be used for rapid investigation of the presence of some of these additional components, although additional analysis and investigation of potential interference issues would be needed for definitive studies.

Theanine, a nonprotein derived amino acid, is a component of tea that has received considerable attention recently (36–38). The time-course analysis for extraction of this component in English Breakfast tea is shown in Figure 6a, with the level reaching a maximum value at about 5 min, similar to what was observed with caffeine (Figure 2a). The level of theanine in nine different teas is shown in Figure 6b. The level in the bottled iced tea drink is seen to be quite low, a result that is consistent with the report that theanine tends to be highest in freshly brewed teas, with degradation over time being observed in bottled teas.

The levels of vitamins C and B5 in three different vitamin waters are shown in Figure 6c. Similar amounts of vitamin B5 (about 0.2 mg/mL) are seen in all three products, whereas dramatically different amounts of vitamin C are observed. The latter result may be an artifact caused by interference, as reported values suggest that the vitamin C content of Berry Punch should be only about 30% lower than that of Orange Mango. Finally, the evaluation of sucralose in a variety of energy drinks (Figure 6d) shows the highest levels for AMP Sugar Free, Rock Star Sugar Free, and Rock Star Zero Carbs; all of them

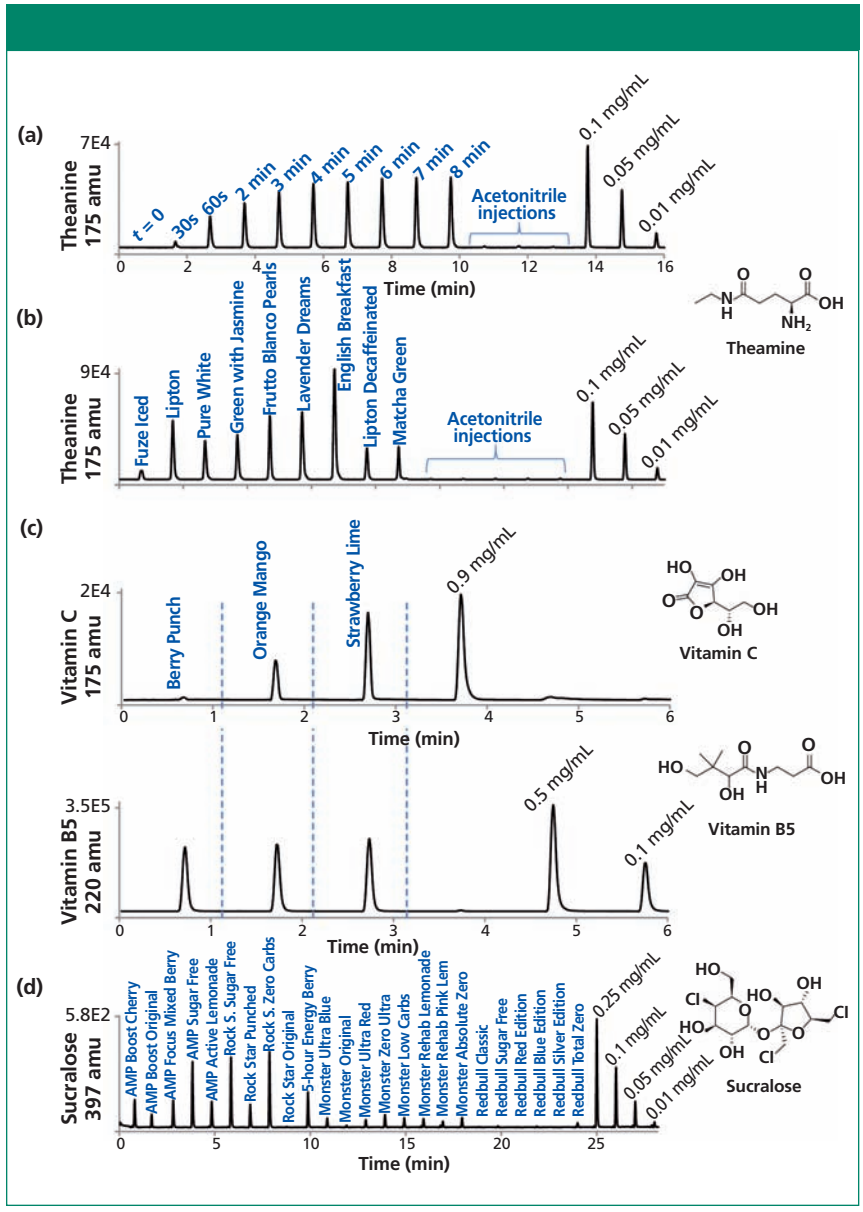

Figure 6: MISER HPLC–MS analysis of multiple analytes in different samples: Theanine levels of time-course extraction study from (a) Lipton all natural tea and (b) nine different teas. (c) Vitamin C and B5 levels of three different vitamin waters. (d) Sucralose levels in 24 different energy drinks (all samples were 10× diluted, except AMP and Rock Star drinks). Chromatographic and MS conditions as described in the experimental section. Detection: ESI-MS(+) at *m/z* = 175 (theanine) and 220 amu (vitamin B5); ESI-MS(-) at *m/z* = 175 (vitamin C) and 397 (sucralose) amu, 0.6-µL injection every 1 min. Fragmentor voltage was kept at 150 eV for all compounds, except for vitamin C (100 eV).

were reported to contain this artificial sweetener. This is also consistent with the observation that no aspartame was detected in Rock Star drinks (Figure 5).

The advantage of the MISER LC–MS (high throughput, simple readout of results) for simultaneous semiquantitative evaluation of caffeine, taurine, and aspartame levels in teas, soft drinks, and energy drinks can provide an entertaining introduction to the important analytical technique of LC–MS. While this approach is fast and simple, and easily grasped by students, it is important to note that MISER LC–MS is typically used for the analysis of relatively clean samples containing only a few potentially interfering substances, and may be less well suited for reliable quantitation when dealing with samples with highly variable and complex matrices. When more exact quantitation is required, model experiments can help to rapidly assess potential interference issues (39–41).

Conclusions

The approximate levels of caffeine, aspartame, and taurine in soft drinks and energy drinks can be easily measured by MISER LC–MS, providing a useful introduction to the important analytical techniques of liquid chromatography and mass spectrometry. Considerable variation in these analyte levels is clearly visualized among a variety of these beverages, with the energy drinks showing a higher concentration of caffeine and taurine, and the diet soft drinks containing a higher amount of artificial sweetener (aspartame).

Acknowledgments

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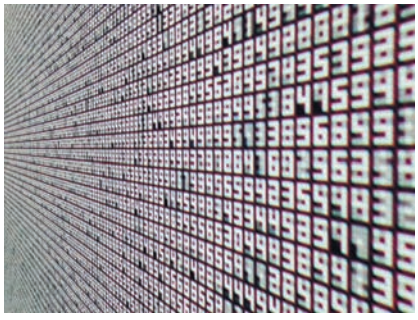
Unlocking the Power of Data



The modern analytical laboratory generates enormous amounts of data. These data are typically stored in vendor-specific, proprietary file formats. Historically, the data management problem has been addressed through custom software and patches designed only to mitigate issues without addressing the underlying problem. Here we describe the underlying data-related problems facing the analytical chemistry community and present our collaborative approach to address these problems.

James M. Vergis, Dana E. Vanderwall, James M. Roberts, and Paul-James Jones

Regardless of the type of measurement, a company's size, or industry sector, analytical data and analysis thereof underpins the fundamental processes and decision making for companies and businesses operating within the analytical community. Arguably, this makes data an asset of indispensable value for any of these companies, on comparable standing with its people and products. Because of the sheer volume and complexity of the data, the management and maintenance of this valuable asset is associated with numerous challenges, which in turn create additional challenges in fully leveraging its value. In practice, significant value remains untapped because the attention and investment today in data management tools and practices are not commensurate with the volume, complexity, and value of our analytical data. Furthermore, when compared to other industry sectors, these challenges are exacerbated for any company that works in domains with additional requirements associated with regulatory compliance, like the pharmaceutical and biotechnology industry. When considering the importance of data in daily business operations, why then is it so common that data management continues to be so difficult, time consuming, and costly — all of which affect the efficiency and subsequent profitability of companies? Can a tacit acceptance of added cost and complexity be best practice or good business? A series of articles in 2010 and 2011 described the problems associated with analytical chemistry data and proposed an approach to address them (1,2). As a direct result of these papers, a group of pharmaceutical and biotechnology companies formed Allotrope Foundation in 2012 to address the paradox between the ease of generating enormous amounts of data and the difficulty in extracting value from these data. The members of the foundation have recognized that through collaboration, sharing of resources, and expertise, it is possible to change the status quo. What follows is a description of the approach taken by Allotrope Foundation and progress toward solving these problems by addressing their root cause. Though initially conceived and initiated in the pharmaceutical industry, there is ample evidence that neither the problems nor the approach to a solution are uniquely defined by the nature of the samples being analyzed. Rather, any company in any industry using electronic data management in analytical chemistry would be subject to the same issues, and realize similar benefits from the work of Allotrope Foundation.

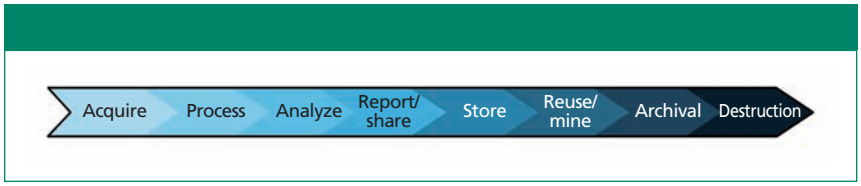


Figure 1: The analytical data life cycle. The data life cycle begins with "data acquisition" and ends with "data archival," followed by its "destruction" in some cases.

The Problem Statement

The pharmaceutical and biotechnology industries generate enormous amounts of data in all aspects of their daily operations such as research and development, and manufacturing. Even considering only the analytical experiments conducted for just one drug product, the volume of data that needs to be managed is enormous. Since the cost of data storage continues to decrease and the speed at which data can be transmitted continues to improve, it is not the quantity of data that is the problem, rather it is the means by which the data are recorded, described, indexed, and stored. The accumulation of small issues or gaps throughout the entire data life cycle (Figure 1) affects the ultimate value or usability of the measured result in a profound way. Like any imperfection in a multistep process, it can be difficult or impossible to find, let alone correct the original mistake, particularly if some information is missing or contradictory. Three fundamental points of failure or complexity are at the root of the problem and are described below: proprietary file formats, inconsistent contextual metadata, and incompatible software. Addressing these fundamental issues will positively affect the ability to use, exchange, transmit, recall, and extract value from data at every stage of their life cycle and address the underlying data management problems facing today's modern analytical laboratory (Figure 2).

Proprietary File Formats

As the science and associated technologies of analytical chemistry have evolved, the broad landscape of instruments and software applications has generated an equally diverse array of proprietary data formats and systems to consume them. Unfortunately, this diversity in data formats hinders the ability of these systems to consume and share data. The data and associated metadata stored in these proprietary file formats are effectively inaccessible outside of their native vendor application. Proprietary formats pose a serious problem for companies that wish to share data or electronic instrument methods between business units or with external collaborators and partners such as contract research organizations (CROs), particularly if each site or partner utilizes different software or hardware in their analytical workflows. To circumvent this problem, proprietary data and method files are often converted into "compatible" formats for exchange and then are subsequently converted back into the proprietary formats required by the software used by the recipient. For electronic methods, this conversion is routinely accomplished "by hand," in which the contents of a static PDF document are read and manually transcribed by a human into the instrument control software. Furthermore, static PDF documents often do not adequately reference the data or provide the complete metadata package, such as audit trail information, which would exist in the original electronic records. Each of these conversion and manual transcription steps impedes data exchange, analysis, and archival and increases the possibility for the introduction of errors and misinterpretation into the process, especially where manual steps are required.

Besides impeding facile data use and exchange, proprietary file formats also introduce significant long-term retention problems. Like the pharmaceutical industry, many industries need to archive data for regulatory, legal, business, or intellectual property reasons, often for extended periods (such as decades). Continuous and rapid technological evolution in computers, software, and hardware results in inevitable obsolescence, causing incompatibilities to accumulate over time, and in turn results in a significantly diminished ability to use archived data. To allow future access to data stored in and dependent on obsolete and unsupported file formats, companies often maintain museum-like storehouses of decommissioned hardware and software. In addition, companies regularly and on a recurring basis invest enormous resources in data migration projects to periodically move data to current systems, only to have to repeat this exercise a few years later when the "new" software system becomes "old." Nonetheless, all the steps that are undertaken today to provide future readability of archived data only increase the likelihood that these data can be accessed in the future, they do not guarantee it.

Inconsistent Contextual Metadata

Metadata capture the "who," "what," "when," "where," "why," and "how" the data were generated — that is, all the information needed to describe the data and the context in which they exist. While some software systems record certain pieces of metadata automatically, the information captured is typically not sufficient to provide the level of experimental detail required for full interpretation and contextual understanding after-the-fact, nor are these metadata readily available to downstream software applications. As a result, the burden to fill in the metadata gaps falls to the scientist. These metadata gaps are filled through a combination of free-text entry or selections from a limited, predefined set of local vocabularies that are often inconsistent between software applications. By relying on free-text entry, the possibilities of deviating from an agreed vocabulary or format, introducing spelling errors, or just conveying inaccurate information significantly increases the chance of inadvertently recording bad metadata. This combination of free-text errors and limitations of local, predefined vocabularies, coupled to the likelihood of blank metadata fields greatly hinders the usefulness of the metadata that are captured and subsequently the usefulness of the data themselves. Whereas humans can read a variety of spellings and abbreviations of a word or phrase and automatically interpret them to mean the same thing, software-based searching or aggregating data based on this kind of unstructured data is much more difficult, and in most situations requires manual intervention.

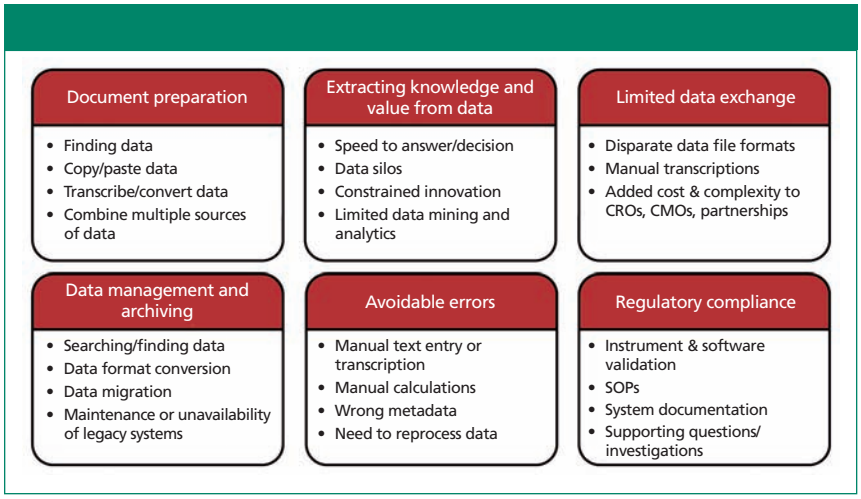


Figure 2: A representative set of downstream effects caused by the current data management problems.

Because important metadata, such as instrument methods, settings, and so on, are stored within proprietary formats, the aforementioned problems associated with proprietary formats directly and materially contribute to the reality that contextual metadata are often incomplete, inaccurate, or incorrectly captured throughout the analytical workflow. Given the number and diversity of software applications used in the typical analytical laboratory, pieces of this critical context are spread across multiple software applications and sources, electronic documents, and on paper. The probability of losing the knowledge of where these different pieces of context are stored, and how the local vocabulary and syntax are to be interpreted increases as time passes especially as data are shared and used further from their origin. The loss of this context subsequently erodes the value of the data, until finally it is deemed less trouble to repeat the measurement than find or understand the original data.

Incompatible Software

The analytical laboratory depends on a significant number of software applications including electronic laboratory notebooks (ELN), laboratory information management systems (LIMS), chromatography data systems (CDS), and data analysis packages, just to name a few. In addition, analytical instruments and even the detectors themselves may have stand-alone software required for instrument control, data capture, and data analysis. But, unlike the "app stores" for mobile devices with literally a million applications that can be downloaded and run instantly, the modern analytical laboratory is not a "plug-and-play" environment. It can cost millions of dollars and require a small team of experts to install and validate software (above and beyond the requisite license fees). The patchwork of software that needs to be interconnected to enable the acquisition, transfer, analysis, and storage of data can be numerous and complex. There are several routes available to a company to bridge software applications such as: internal developers using vendor provided software development kits (SDKs) to create adaptors to allow the output from one program to feed into another program; software integration vendors contracted to develop custom software to connect software; or the standardization within a company or department on a single vendor for their analytical laboratory needs. Typically, in practice it is a combination of several solutions used to interconnect software systems, which results in a highly customized and likely expensive undertaking that rarely, if ever, creates a fully integrated data-sharing environment. Furthermore, because of the level of customization, laboratories frequently have to deal with issues arising from software updates and version changes along with the introduction of new technology into the laboratory. The result is a perpetual cycle of creating expensive stop-gap solutions to keep the data flowing.

The Proposed Solution — "The Allotrope Framework"

Companies have historically dealt with the aforementioned data management problem by focusing on and addressing the downstream effects illustrated in Figure 2. The explosion of data coming from analytical laboratories is rendering this position increasingly unsustainable and untenable. These effects may originate in the analytical chemistry laboratory, but are felt throughout the organization, even in those areas that do not have direct or knowing contact with "data." Accordingly, Allotrope Foundation member companies devised a holistic strategy to deal with this problem and to create a solution that addresses the root cause of the problem — the incomplete, inconsistent, and potentially incorrect metadata (captured through manual entry) and the lack of a standard file format to contain the data and associated metadata. This solution forms the basis of the "Allotrope Framework," which is composed of three parts: a nonproprietary data format, metadata definitions and repository, and reusable software components to ease adoption and ensure consistent implementation of the other two components

Standard Data Format for Analytical Chemistry

Given the longevity of record retention and access requirements by regulatory agencies (such as the Food and Drug Administration [FDA], European Medicines Agency [EMA], and Pharmaceuticals and Medical Devices Agency [PMDA]), the Allotrope Framework will include the development of a publicly documented file format for the acquisition and exchange of data that will allow for easier long term storage and access to both the analytical data and associated metadata. As such, the format must not be limited to storing data; it must also be the format to store the context in which the experiment is performed as well as the method used to acquire the data. The ability to create and seamlessly share a vendor-neutral instrument method file eliminates the "PDF file-sharing method" along with the requisite manual transcription steps.

As described above, the readability of analytical data today and far in the

future is critically important, with some companies' data retention policies spanning 25, 50, or even 100 years. Allotrope Foundation recognizes that data files created today need to be directly and easily accessible to other software packages, processes, corporate entities, collaborators, and regulators today as well as in the future. This accessibility far into the future will be made possible through the evaluation, selection, federation, and subsequent implementation of today's best-in-breed data standards to create a standardized, obsolescence-resistant, nonproprietary data format. However, it is certain that despite the best intentions of Allotrope Foundation, the selected data standard will need to be updated to newer technology at some point in the future. Making the technical details of the standard format publicly known ensures that third parties could provide solutions to access or migrate the existing body of standard format data, as needed.

To facilitate adoption by the vendor community, the standard data format is being designed from first principles to meet the performance requirements of modern instrumentation and to be readily extensible. Extensibility provides resistance to technological obsolescence by allowing new techniques, combination of techniques, and technologies to be incorporated while maintaining backwards compatibility with previously released versions. This backwards compatibility is especially important when considering the archivability of the file format.

Metadata Definitions and Repository

The ability to easily read data in the future is practically useless without knowing the context around which the data were gathered or even at a more basic level, being able to find the data in the first place. It is the retention of complete, consistent, and correct metadata that allows data to be efficiently searched and retrieved. As such, the capture and storage of all relevant contextual metadata is a necessary precondition to mitigating the data management problem and is critical to an archive-ready file format and data integrity.

The metadata definitions provide the ability to consistently and accurately describe the methods, the experiment, and the context in which they occur. To address the shortcomings in the current metadata capture process, which relies heavily on free-text manual entry, we envision that many manual entry steps can be automated so that standardized, predefined metadata terms are obtained from authenticated sources, initiating their look-up via the barcode of a sample, or near-field communication (NFC) of an employee's badge with password authorization, or triggering a pick-list of values from the software user interface to select appropriate parameters (retrieved from a controlled vocabulary source). This strategy eliminates errors associated with unverified free-text entry since it no longer occurs. It also eliminates inconsistent metadata because scientists are restricted to predefined and approved terms that are relevant to the experiment at hand. In turn, this reduces the burden to record experimental details, including those details unknown or not easily accessible to scientists. The automation is carried one step further in the metadata approach created by the Allotrope Framework, in which metadata stored alongside the data will grow and will be augmented as the data move through the product life cycle in an automated fashion. This allows a "data map" of the laboratory to be created and searched in ways not possible today.

The approved or predefined vocabularies are provided to users through an extensible "metadata repository." The Allotrope Framework will contain integrated metadata repositories containing common or core terms, company-specific terms, and vendor-specific terms. This modular approach to the metadata repository allows for future extension of terms as the underlying science advances, new techniques and technologies become available, or business processes change through evolution, acquisition, or divestiture. In addition, industry-specific metadata repositories can also be created and incorporated into the framework allowing companies outside the pharmaceutical industry to implement the Allotrope Framework.

Reusable Software Components

Although the standard data format and metadata components of the Allotrope Framework are relatively simple and straightforward at a conceptual level, at the level of a practical implementation these components are all highly detailed and complex. As any solution becomes more complex, the barrier to implement and adopt that solution increases dramatically, thus reducing the likelihood of its use. Moreover, consistency is critical — an inconsistently implemented or unused standard of any kind is not a standard. To significantly lessen the complexity associated with implementing the Allotrope solution, while at the same time ensuring consistency of the implementation, a third component is needed: readily available software tools that will allow developers to easily and consistently embed the Allotrope Framework into their software without requiring an underlying, detailed knowledge of the technical aspects of the standards or analytical techniques. By lowering the barrier to implement the standards, the framework provides the means to drive their widespread adoption.

The software tool set provided will consist of application programming interfaces (APIs), class libraries, and associated documentation. This approach is analogous to APIs provided by iOS, Android, and Windows: toolkits that enable applications that allow us to exchange texts, hyperlinks, and images using different mobile devices, all of which use underlying standards that are largely invisible to software developers. Similarly, vendor-provided SDKs can be employed today by end-users to create applications for, or extensions to the vendor software, both of which are external to the original software application. The goal of Allotrope Foundation in providing the Allotrope Framework "toolkit" is to allow vendors to integrate the Allotrope Framework, and the standards it encompasses, directly into their software products. During the early stages of deployment and integration of the Allotrope Framework in everyday use it might be necessary in some cases to use the vendor SDKs to create adaptors to allow the current generation of software (as well as legacy systems) to interface with the Allotrope Framework. We envision that in the future new versions of software will be provided by vendors in an "Allotrope Ready" format, with Allotrope Framework components or APIs embedded directly into their

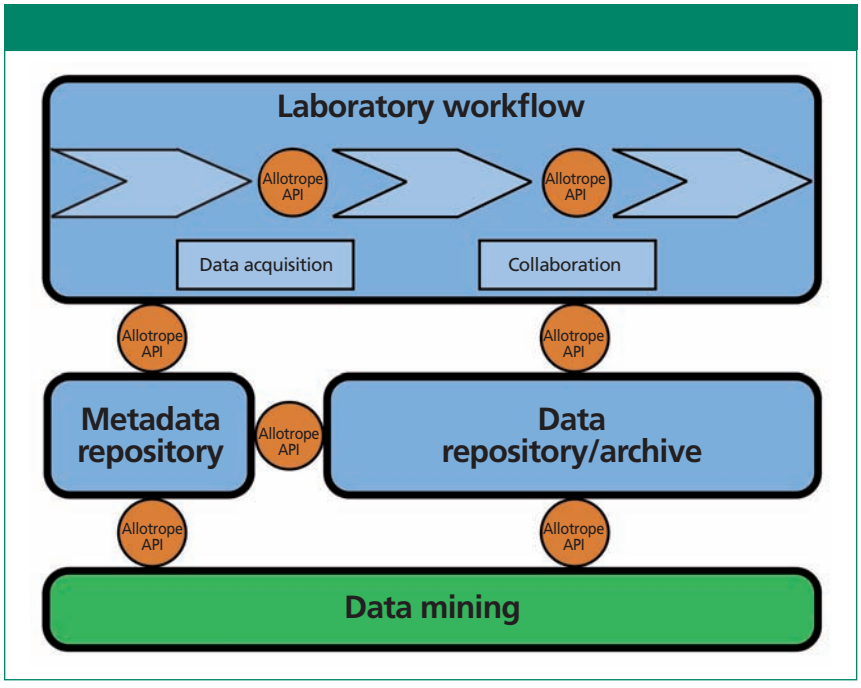


Figure 3: The future analytical laboratory. A schematic illustrating a proposed reference architecture incorporating the Allotrope Framework is shown and consists of: the laboratory workflow, metadata repository, and data repository or archive (blue boxes); the interconnectivity of all the laboratory components and steps within the laboratory workflow (light-blue chevrons) is provided by the Allotrope Framework APIs and class libraries (orange circles). Additional components that are typically required and provided through vendor-supplied applications are not illustrated for clarity. The new level of data mining capability made possible by implementation of the Allotrope Framework and concepts is represented as a green box.

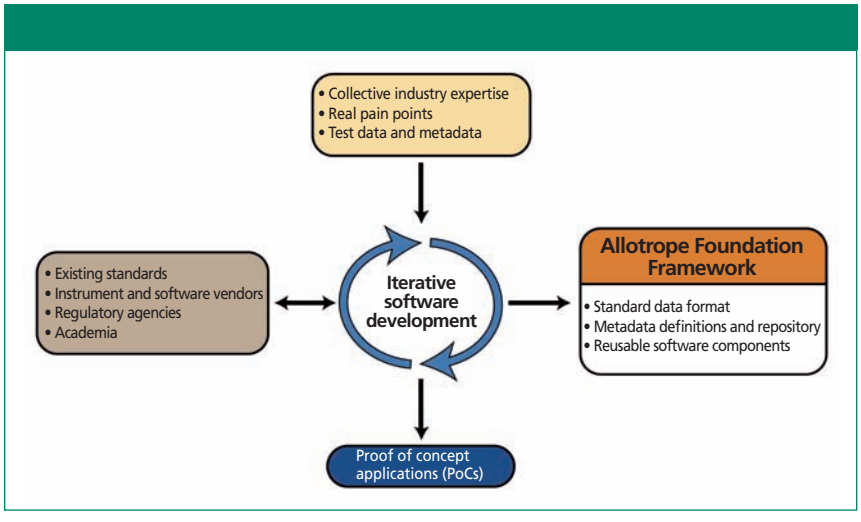


Figure 4: The sources of information utilized in an iterative development approach to PoCs and ultimately the Allotrope Framework components.

applications and solutions. In this future state, Allotrope Foundation foresees that the Allotrope Framework toolkit will evolve to be a means for vendors, third parties, and end-users to innovate and exploit analytical data and metadata in ways not possible today.

A schematic illustrating a proposed reference architecture integrated with the Allotrope Framework is shown in Figure 3 and at a high level consists of: the laboratory workflow, metadata repository, and data repository or archive. Allotrope Framework APIs and class libraries provide connectivity between and within these high level objects. The laboratory workflow contains all the components including the associated instruments, software, and outsourcing required to execute experiments, acquire data, analyze data, and create reports (additional components that are typically required and are provided through vendor-supplied applications are not illustrated for clarity). In addition, the analytical laboratory requires some form of data storage element. The Allotrope Framework provides the metadata vocabularies and dictionaries to the reference architecture and supplies the means to connect all of the components to one another through the use of the Allotrope Framework APIs and class libraries. The result is a laboratory comprising interconnected components, which can be software or collaborators, all producing and using data in a common format that is able to be readily shared and stored in an archive-ready format. In addition, these data contain complete and accurate contextual metadata provided through the metadata repository, which ensures data integrity and enables data mining capabilities not possible in today's laboratory.

Delivering the Solution

Allotrope Foundation is guided by the principle that the best learning comes from doing. This thinking pervades all aspects of the organization. As part of the Allotrope Framework development, components are first tested through proof-of-concept applications (PoCs) specifically designed to test concepts, approaches, implementation details, and existing standards for data, metadata, and software. This allows for concepts and hypotheses pertaining to the Allotrope Framework to be rapidly tested and refined based on the collective industry expertise from Allotrope Foundation subject matter experts (SMEs), industry best practices, and the vendor community. This approach is consistent with the agile, iterative software development approach being used for the Allotrope Framework and PoC development (Figure 4). The PoCs allow for feedback and input from these various information sources to be readily accommodated into the development process. The extensive use of PoCs and "real-world" testing and evaluation as an integral part of the development process reduces the risk of creating a solution that will have limited practical applicability. In addition, there

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ON-DEMAND WEBCAST Originally aired March 24, 2015

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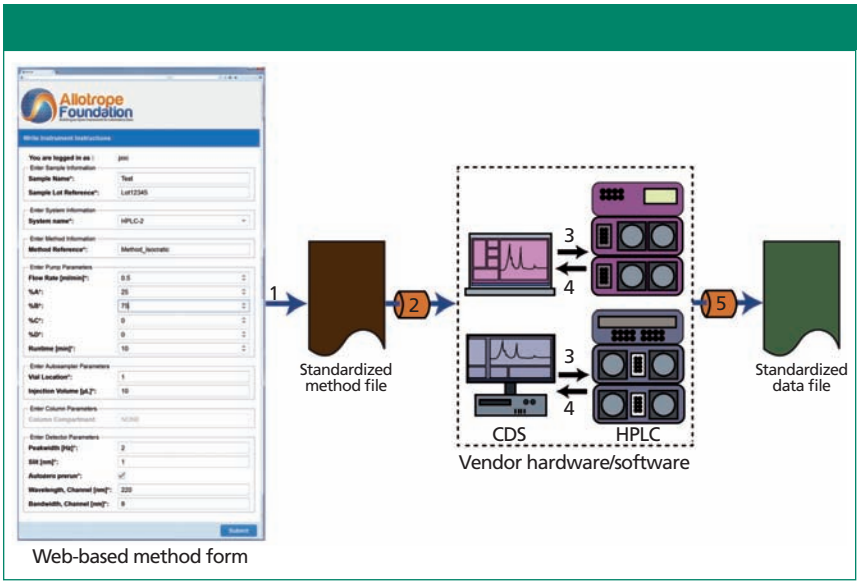


Figure 5: The flow diagram of the instrument software integration PoC. Step 1: HPLC-UV method parameters are input into a web form to create a standardized method file. Step 2: this method file is passed through a vendor-specific adaptor used to convert the standardized instructions into vendor-specific instructions, which are then passed to the CDS for execution. Steps 3 and 4: the CDS executes the run and captures the data. Step 5: the raw data are passed through a second adaptor to convert it into the defined standard format.

is a dedicated team tasked to ensure that all Allotrope Framework development throughout the entire software development life cycle (SDLC) is carried out in accordance with our understanding of regulatory and best practice expectations, including appropriate documentation and controls (beginning even with the PoCs described below). Furthermore, the Allotrope Foundation's iterative approach allows for instrument and software vendors, regulatory agencies, and academia to contribute to the development cycle. During the first year of development, the main objective of Allotrope Foundation was to verify the main Framework concepts through the creation and evaluation of three PoCs: instrument software integration, capture of contextual workflow metadata, and archiving support. The Analytical Information Markup Language (AnIML) was used as the initial standard based on its maturity and the fact that it includes concepts from previous standards such as the Joint Committee on Atomic and Molecular Physical data – Data Exchange (JCAMP-DX), Generalized Analytical Markup Language (GAML), mass spectrometric data extensible markup language (mzML), and others (3). These PoCs demonstrate not only the functionality of the framework, but also represent the beginning and end in the data life cycle — data creation and data archiving (Figures 5–7).

Instrument Software Integration PoC

The purpose of the instrument software integration PoC application was to test the ability of a standardized, nonproprietary file format to provide method instructions to native instrument control software from multiple vendors; to use the prototype framework to direct that control software to utilize the supplied method to acquire analytical data on a real analytical instrument; and to store the resulting data back in the standardized format (Figure 5). This PoC demonstrated the ability to create and share methods and provide the resulting data in a standardized format to be read and used regardless of the specific software version or specific vendor used. This concept is rooted in a common real-world scenario — the need to share methods and data between entities, for example, between a sponsor and a CRO.

For this PoC, high performance liquid chromatography with UV detection (HPLC–UV) was chosen as the analytical technique to be tested because of its ubiquitous use in the laboratory. As mentioned above, AnIML formed the basis of the standardized data format. To demonstrate the vendor-neutrality of the standardized method and data format, the CDS software from both Waters (Empower 3) and Agilent (OpenLAB ChemStation) were used in this PoC. To interact with each vendor's CDS, Allotrope Foundation created adaptors using the vendor SDKs to allow standardized instructions to be sent to each of these CDS platforms and back-convert the CDS-captured data into the standardized data format. The instrument software integration PoC was successfully deployed and implemented at two Allotrope Foundation member companies in early 2014 and subsequently distributed to all Allotrope Foundation member companies for deployment and testing in their individual environments. Future extensions of this PoC will enable the much more complex HPLC–UV experiments that are typically run in modern laboratories, eliminate manual free text entry, and provide a mechanism to support vendor-specific hardware in addition to processed results (for example, peak tables).

Capture of Contextual Workflow Metadata PoC

The ultimate value of data is only realized when the experimental data are associated with accurate and complete contextual metadata. This not only provides the means by which data can be searched and retrieved, it also forms the basis for fueling laboratory automation, data mining, and downstream business intelligence applications. In the metadata capture PoC, a very simple repository containing a standard set of instrument names was created and linked to the instrument software integration PoC. This linkage of a defined dictionary to the web form that was used to define the experiment method allowed the scientist to see the list of available instruments for that experiment, only allowed those instruments to be selected, and ultimately associated the acquired data to that instrument. The metadata capture PoC thus demonstrated the concept to automatically capture metadata provided from a metadata repository and associate these metadata with the original experimental data.

Providing a specific instrument name as standardized, contextual metadata is an illustrative example of the approach the Allotrope Framework is meant to enable, but it clearly doesn't constitute

sufficient content to be of any practical utility. As a mechanism to explore which contextual metadata may be most relevant and prioritize its inclusion in the first implementations of the Allotrope Framework, a large body of simulated data was created that included an idealized representation of complete, consistent, and correct contextual metadata for 14,000 analytical experiments representing the typical variety generated in a pharmaceutical company over a decade. This body of simulated data is used to illustrate a variety of ways in which the Allotrope Framework might facilitate the kind of analytics that could deliver insight into a company's data in a way that is a significant challenge and very costly in the current situation.

An illustration of the diversity of such a dataset is presented in Figure 6a. Through the use of suitable analysis tools, the scientist can leverage the complete, consistent, and correct metadata to filter the view, search for specific data, or generate any one of a large number of different aggregate views in seconds as illustrated in Figures 6b–6d. Furthermore, the full integration of the metadata with the experimental data and results will enable facile access to underlying data from the macro-level views. For example, Figure 6e illustrates all the HPLC–UV drug substance stability tests for a given compound, including the ability to visualize individual chromatograms. An impurity threshold can be set interactively to automatically flag individual experiments and by selecting a particular experiment, provide the ability to display the actual HPLC–UV trace. Today, such an analysis using an equivalent amount of real data of a similar broad scope would be exceedingly difficult, laborious, costly, and time-consuming. All of this interactive drill down and data mining in the simulated dataset are achieved with only a few mouse clicks, and hints at just a little of what will be possible once the Allotrope Framework is in widespread use. This detailed level of data mining and analysis is only possible when complete and accurate metadata are preserved with the data and the metadata terms themselves are standardized and predefined across an enterprise.

Archive PoC

As previously mentioned, data are among the most valuable assets of any company. Because the aforementioned problems with contextual metadata are widespread in the current state for many companies, including Allotrope Foundation member companies, the difficulty to find specific data has a profound negative impact on the ability of those companies to preserve and retrieve retained analytical data effectively. This is an acute pain point for Allotrope Foundation member companies, and as a result, the benefits provided by the framework as they relate to long-term data integrity and preservation were deemed an important priority to demonstrate among the first PoC applications.

As such, the purpose of the archive PoC was to demonstrate the ability to store data automatically enriched with the contextual metadata in an archive and to automatically set a retention period based

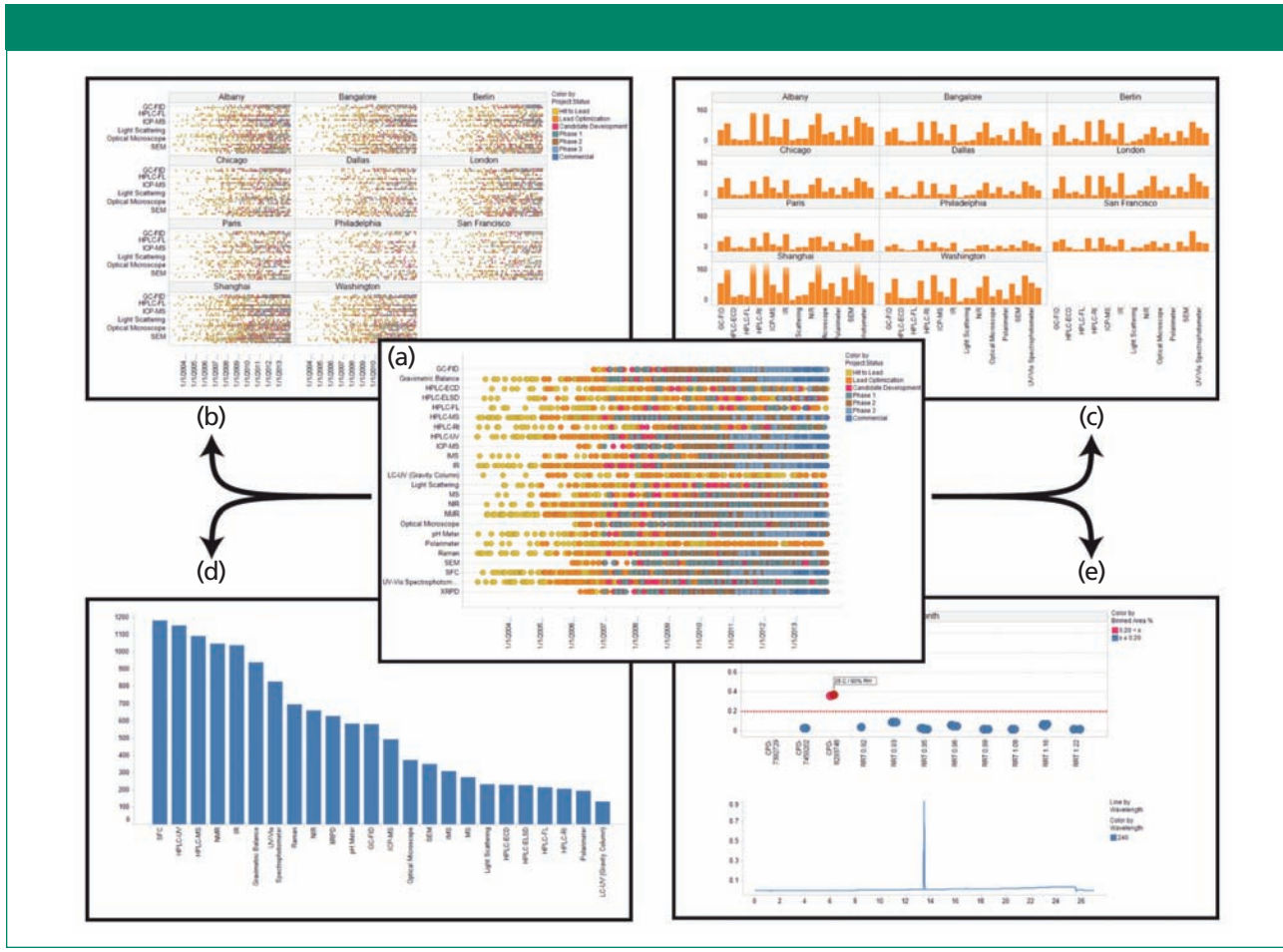


Figure 6: Illustrative data-mining example when complete and accurate metadata are captured. (a) Representation of a simulated dataset that illustrates the diversity of analytical data generated in a typical pharmaceutical company over a 10 year period; Approximately 14,000 simulated analytical experiments are represented, one per dot, organized by technique and color coded according to phase of drug development. (b) All data by site; (c) instrument utilization at each site; (d) instrument utilization globally; and (e) the demonstration of an interactive "drill-down" into the dataset where the selection of any one point in one of the other views displays the details of the corresponding experiment including the data traces.

on application of business rules to the contextual metadata associated with the data trace. This PoC also demonstrates the integration with the instrument software integration PoC and metadata repository PoC, as shown in Figure 7, and represents the functional demonstration of three fundamental pieces of the reference architecture described above. As the standardized document containing the acquired data is read into the PoC archive, the contextual metadata contained within it is used to determine the retention period (which itself is stored as an additional piece of contextual metadata in the document), and to index that file so the rich, standardized metadata can now be leveraged for powerful and intuitive searching downstream.

Though the PoCs described here are considered "throwaway" applications, all the capabilities described were executed using the underlying framework, which is being developed to a level of quality suitable for commercial use. Furthermore, while the integration of these PoCs represents a vastly simplified version of the analytical laboratory dataflow, it demonstrates the potential utility of the Allotrope Framework throughout the data life cycle (Figure 1) — data generation through archiving and storage. Based on this result, we are confident that all of the intermediate steps between these two endpoints can be enabled via the standard data format, metadata repository, and standard APIs.

Analytical Community Engagement

Allotrope Foundation was created specifically to build the Allotrope Framework and as such, is organized to support the collaborative project team (Figure 8). The current Foundation membership comprises a significant cross section of leading pharmaceutical and biopharmaceutical companies: AbbVie, Amgen, Baxter, Bayer, Biogen Idec, Boehringer Ingelheim, Bristol-Myers Squibb, Eisai, Eli Lilly, Genentech/Roche, GlaxoSmithKline, Merck, and Pfizer. The member companies provide the project funding and SMEs, along with governance and oversight through a Board of Directors while the Consortia Management Team of Drinker Biddle & Reath LLP coordinate and provide legal, scientific, and logistical support. Allotrope Foundation working groups comprised of SMEs focus on defining business requirements and tackling specific tasks or issues, both technical and non-technical. In addition, Allotrope Foundation has partnered with OSTHUS, a leader in data and systems integration

in research and development (R&D) to be the framework architect, working under the direction of Allotrope Foundation with member company SMEs that provide the necessary analytical chemistry knowledge and user requirements. Yet, it is clear that the success of the framework depends on broad adoption and therefore requires the engagement of and input from the greater community of stakeholders. The only way the Allotrope Framework will solve the data management problem of the community is if all the stakeholders in that community contribute to its development.

Vendors and Academicians Collaborate Via the Allotrope Partner Network

Although the various industries generating data comprise one dimension of the analytical community, the vendors of the instrumentation and software play a critical role since the easiest path to widespread adoption of the Allotrope Framework (and associated standards) is through directly embedding the Allotrope Framework components into the products they offer. This requires cooperation and collaboration between Allotrope Foundation and the vendor community to ensure that the framework being developed is the right framework and robust enough to accommodate specific vendor needs such as compatibility and performance. Academia represents another community of consumers of instruments and software, but they are also an important source of new methods and technologies driving innovation. To provide a venue for these groups to collaborate in the Allotrope Framework development, the Allotrope Partner Network (APN) (4) was created. The APN program was modeled after similar collaborative programs used by companies to garner input into upcoming products and technologies during the development stage. Members of the partner network benefit from access to common industry requirements, prerelease materials and software, technical support, and can engage directly in collaborative technical work with the Allotrope Foundation project team.

Alignment with Regulatory Agencies

Numerous industries are required to adhere to rules and regulations set forth by governments and enforced by various government regulatory agencies such as the Environmental Protection Agency (EPA) and the FDA in the United States. The ability to find specific analytical data, create reports using those data, and even provide those data upon request to regulatory agencies is a daunting task in the modern analytical laboratory. This is difficult enough with recent data, but is made even more challenging when data from 10 to 20 years ago, or even older, are requested and needed in a short time frame. This challenge is further complicated by the problems associated with reading old data that were acquired on now obsolete technology. Implementing the Allotrope Framework will address these challenges for all future data since the data format will be backwards compatible and always readable. However, to ensure that regulatory agencies agree with the approach taken in creating this framework, Allotrope Foundation welcomes the opportunity to directly engage these agencies and solicit their feedback and support.

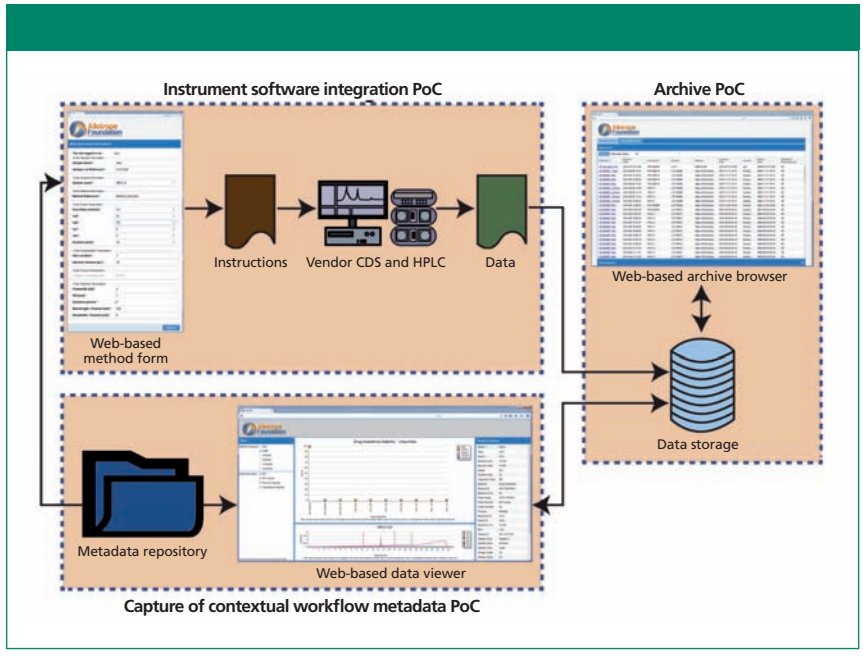


Figure 7: The functional integration of three PoCs demonstrating the potential to impact the data life cycle from data generation through archiving or storage.

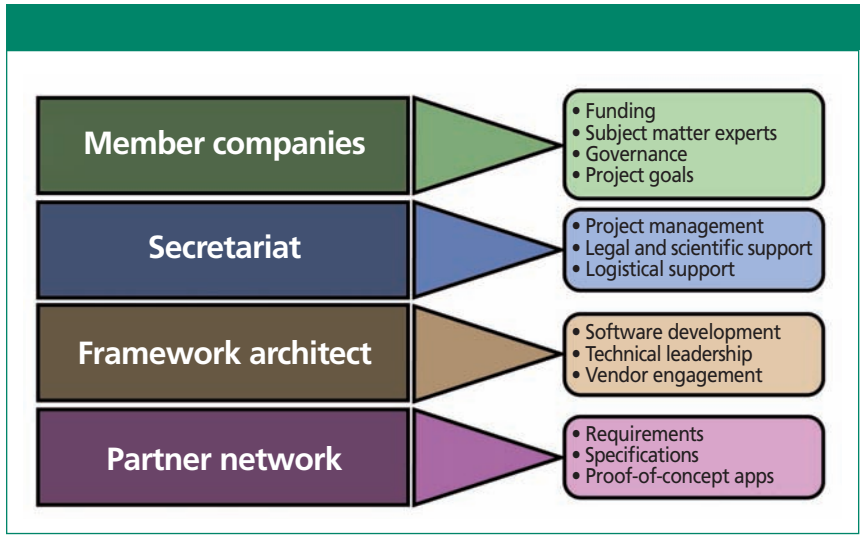


Figure 8: Allotrope Foundation organization. For each of the functional components illustrated, representative roles and responsibilities are shown.

Since Allotrope Foundation is primarily made up of pharmaceutical and biotechnology companies, initial outreach efforts have focused on the FDA. In 2010, the FDA Center for Drug Evaluation and Research (CDER) established the CDER Data Standards program and published the CDER Data Standards Strategy Action Plan (5), in which a project entitled "CMC Data Standardization" is described. In addition, the draft of the FDA Strategic Priorities 2014–2018 (6) mentions the FDA's commitment to foster improvements in data management and the use of standards. The goals of Allotrope Foundation are well aligned and consistent with those articulated by the FDA with regard to the data standards strategy and the associated benefits from their adoption and use.

Other Industries Invited to Join Allotrope

Although it was companies from the pharmaceutical and biotechnology industries that formed Allotrope Foundation, it's clear that CROs and contract manufacturing organizations (CMOs) along with other industries such as consumer goods, agrochemical, petrochemical, and environmental health use the same analytical chemistry instrumentation and software within their companies and likely experience challenges similar to those already highlighted above. This was reinforced through recent cross-industry workshops in which representatives from these other industries met with Allotrope Foundation to discuss the Allotrope Framework concept and their analytical data management problems. As a result, membership in Allotrope Foundation is now available to any company that engages in analytical chemistry testing for research, development, or manufacturing of commercial products.

Closing Remarks

As described above, Allotrope Foundation has made tangible progress in creating a sustainable solution to address the analytical data management problems that persist in companies spanning numerous industries. Software development started in September 2013, and in less than one year the project moved from the vision articulated in 2011 to demonstrating that the core concepts of the Allotrope Framework are technically feasible, built functional versions of novel components that did not exist previously, and developed an approach to break a complex problem into tractable pieces. In the process, a deeper understanding has been developed of what it will take to put a version of the Allotrope Framework into production in our companies along with the capabilities exemplified by the PoCs; this will be the subject of the next phase of the project.

Ultimately, what we have learned from the PoCs and our understanding of the requirements to solve this problem is that no one single standard format will meet all the requirements; these technique-specific standards work well for their intended application (for example, mzML for mass spectrometry), but are generally limited in their extensibility for other uses. Furthermore, none of the existing analytical data standards provide an adequate mechanism to preserve the contextual metadata that is as fundamental as the file format itself to solving the problems described above.

As a result, the solution provided by the Allotrope Framework will use a federation of existing standards, choosing those best suited for the intended purpose. In addition, the extensible design of the framework allows it to readily accommodate future needs and analytical techniques.

Year Two of Development

The next phase of the project will focus on maturing the established framework and standards, as well as developing the path forward to deliver the framework into production in member companies. Foundation member companies have compiled a list of analytical techniques, software, and initial capabilities required to implement the Allotrope Framework in their laboratories. In addition to furthering the work on HPLC–UV, development for the near term will focus on expanding the list of supported techniques, including mass spectrometry, weighing, and pH measurement. This set of techniques will be supported in the first release of the Allotrope Framework. Numerous standards such as Hierarchical Data Format 5 (HDF5) (7), AniML (8), Batch Markup Language (BatchML) (9), Sensor Model Language (SensorML) (10), and mzML (11) have been systematically evaluated to determine their suitability and ability to accommodate the data and metadata associated with these analytical techniques. As described above, the format will be extensible so as to accommodate additional analytical techniques and standards in future versions.

New PoCs are being defined by Allotrope Foundation and APN members alike that will be developed with collaborations between APN members and the Allotrope and OSTHUS team. A governance model is in place to ensure the PoC process encompasses a wide range of requirements and perspectives, and guide the integration of any subsequent new software components in the Allotrope Framework. Anticipating the availability of the Allotrope and compatible software, Allotrope Foundation members have already begun devising the Allotrope Framework integration and deployment strategy for their individual companies, with projects targeted to begin in 2015.

Invitation to Join Allotrope

Through the participation of a wide and diverse array of analytical community representatives, the Allotrope Framework being developed will be able to meet the individual needs of members of this analytical community. By participating in this endeavor, through membership in Allotrope Foundation or as a member of the APN, you can contribute your expertise toward the development of the Allotrope Framework and help ensure its existence, utility, and quality. The only way to solve an industry-wide problem is to do it industry-wide. Allotrope Foundation invites you to join us and help create this sustainable solution to persistent data management problems.

Acknowledgments

The authors would like to acknowledge the valuable contributions of Allotrope Foundation member company representatives and SMEs, the team at OSTHUS, and members of the Allotrope Foundation Secretariat, all of whom work extremely hard to help ensure the success of this ambitious project while making this a fun and exciting endeavor. We also appreciate advice from the reviewer who provided helpful suggestions to improve the manuscript.

This article was prepared by Allotrope Foundation, which is an association of companies developing a common, open framework for analytical laboratory data and information management. For details, please contact the Secretariat: more.info@allotrope.org.

References

(1) J.M. Roberts, M.F. Bean, W.K. Young, S.R. Cole, and H.E. Weston, *Am. Pharm. Rev.* **13,** 60–67 (2010).
(2) J.M. Roberts, M.F. Bean, C. Bizon, J.C. Hollerton, and W.K. Young, *Am. Pharm. Rev.* **14,** 12–21 (2011).
(3) B.A. Schaefer, D. Poetz, and G.W. Kramer, *JALA* **9,** 375–381 (2004).
(4) http://partners.allotrope.org.
(5) http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/ElectronicSubmissions/UCM400873.pdf.
(6) http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Reports/UCM403191.pdf.
(7) http://www.hdfgroup.org/HDF5/.
(8) http://animl.sourceforge.net/.
(9) http://www.mesa.org/en/BatchML.asp.
(10) http://www.opengeospatial.org/standards/sensorml.
(11) http://www.psidev.info/mzml_1_0_0.

James M. Vergis *is with Drinker Biddle & Reath LLP and is the Allotrope Foundation Secretariat.* ***Dana E. Vanderwall*** *is with Bristol-Myers Squibb, an Allotrope Foundation Member Company.* ***James M. Roberts*** *is with GlaxoSmithKline, an Allotrope Foundation Member Company.* ***Paul-James Jones*** *is with Boehringer Ingelheim, an Allotrope Foundation Member Company. Direct correspondence to: james.vergis@dbr.com* ■

For more information on this topic, please visit www.chromatographyonline.com

PRODUCTS & RESOURCES

Laboratory management software

The NuGenesis laboratory management system from Waters is designed as an alternative to a traditional laboratory information management system. According to the company, the system combines data, workflow, and sample management capabilities to support the product lifecycle from discovery through manufacturing.

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

GC valve oven

Agilent's Large Valve oven for its 7890 GC system is designed with the capability to be configured to support complex, multivalve ASTM International and EN (European Standard) GC applications. According to the company, the external valve oven provides a homogeneous isothermal environment with up to six positions for columns and valves.

Agilent Technologies, Inc., Santa Clara, CA. www.agilent.com

HPLC columns

Silica-based and polymeric HPLC columns from Hamilton include 17 polymeric HPLC columns for reversed-phase, anion-exchange, cation-exchange, and ion-exclusion separations, and two silica-based columns for reversed-phase separations. According to the company, the polymeric columns provide inertness and pH stability (pH 1–13) with the pressure stability of silica-based columns.

Hamilton Company, Reno, NV. www.hamiltoncompany.com

SEC system

The OMNISEC gel permeation–size exclusion chromatography system from Malvern is designed with a combination of detectors to measure the molecular properties of polymers and proteins, including absolute molecular weight, intrinsic viscosity, molecular structure, and size. According to the company, the system and software have been designed to minimize user intervention while improving productivity.

Malvern Instruments Ltd., Malvern, UK. www.malvern.com/omnisecgpc

UHPLC system

The Altus UPLC system from PerkinElmer is designed for use in food, environmental, and industrial laboratories for detecting adulterants, contaminants, and pollutants. According to the company, the system combines advanced fluidics with hybrid particle columns and is controlled through Waters' Empower 3 software.

PerkinElmer, Inc., Waltham, MA. www.perkinelmer.com

Supported liquid extraction sorbent

Phenomenex's Novum Simplified Liquid Extraction sorbent is available in 1-, 3-, 6-, and 12-cc tubes , in addition to the original 96-well plates. According to the company, the synthetic supported liquid extraction sorbent in the tubes can be used for larger format applications such as food safety and quality, personal care products, and environmental analysis.

Phenomenex, Torrance, CA. www.phenomenex.com

Sample cleanup system

Pickering's DEXTech automated Sample-Clean system for Dioxin and PCB samples reportedly uses a multistage process that reduces the throughput to 96 min and the solvent consumption to less than 640 mL. According to the company, the system was developed in cooperation with the German governmental laboratory, Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA MEL), and LCTech GmbH Germany.

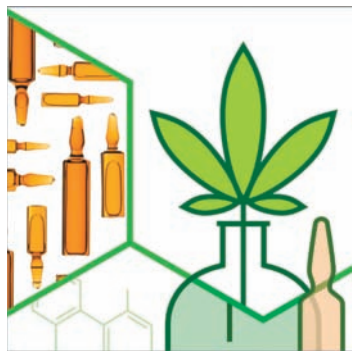
Pickering Laboratories, Mountain View, CA. www.pickeringlabs.com

Capillary tubing

Tight Tolerance Vision System flexible fused-silica capillary tubing from Polymicro Technologies, offered by Molex, is designed to provide precise internal diameter tolerances. According to the company, the tubing is drawn from high-purity synthetic fused-silica preforms and has industry-standard outer diameter dimensions.

Polymicro Technologies, Phoenix, AZ. www.molex.com/polymicro/capillarytubing.html

Terpenes standards



Multicomponent terpenes standards for medical cannabis analysis are available from Restek. According to the company, the certified reference materials meet strict ISO requirements and were developed specifically for cannabis analysis.
Restek Corporation,
Bellefonte, PA.
www.restek.com/medical-cannabis

SPE column

The FASt solid-phase extraction column from UCT is designed to provide improved sample preparation for LC–MS analysis. According to the company, the column employs a one-step process to prepare urine samples for the analysis of multiple drugs and metabolites and can extend the time between maintenance calls on LC–MS-MS equipment by minimizing contaminants that foul HPLC columns and detector sources. **UTC, LLC,** Bristol, PA.
www.unitedchem.com

Micro LC columns

Micro LC columns from YMC are designed to reduce solvent consumption, compared to conventional HPLC. According to the company, the columns are offered with various stationary phases in four inner diameters (75, 100, 300, and 500 µm), and four column lengths (50, 75, 100, and 150 mm). Guard columns are also available.
YMC America, Inc.,
Allentown, PA.
www.ymcamerica.com

UHPLC system

The 1290 Infinity II LC system is designed to enable operators, scientists, and laboratory managers to perform enhanced UHPLC analyses. According to the company, the system's analytical, instrumental, and laboratory efficiency are maximized, and the instrument has eight new modules and a variety of A-line accessories.
Agilent Technologies,
Santa Clara, CA.
www.agilent.com

GC autosampler

The Flex autosampler from EST Analytical is designed for GC and GC–MS applications. According to the company, the autosampler has liquid injection capability with an upgrade path to headspace and SPME analysis, and wireless technology for expansion.
EST Analytical,
Fairfield, OH.
www.estanalytical.com

Automated sample preparation system

FMS's EconoPrep automated sample preparation system for trace analysis is designed for persistent organic pollutants analysis. According to the company, the system offers consumable kits for environmental and food matrices, and automatically performs extraction, sample cleanup, and concentration, allowing for same-day results.
FMS, Inc.,
Watertown, MA.
www.fms-inc.com

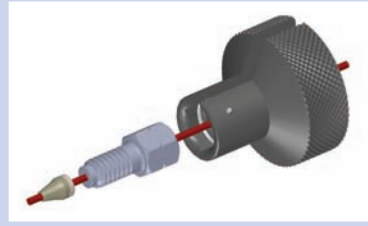
HPLC systems

JASCO's LC-4000 HPLC systems are designed for semi-micro or preparative, low pressure or high pressure, and basic or complex chromatography. According to the company, the system platforms provide 500-, 700-, and 1300-bar pressure ratings for conventional HPLC, rapid HPLC, and UHPLC, respectively.
JASCO,
Easton, MD.
www.jascoinc.com

Combustion ion chromatograph

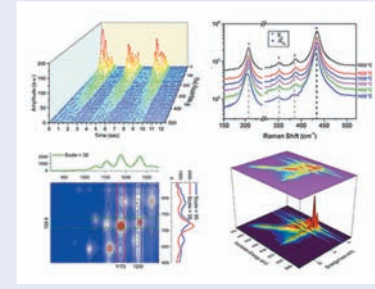
Metrohm's combustion ion chromatograph (CIC) is designed to automate the determination of halogens and sulfur. According to the company, the system's autosampler can run both solid and liquid samples, and flame sensor technology is used to measure the light intensity from the pyrolysis oven during combustion.
Metrohm USA,
Riverview, FL.
www.metrohmusa.com/CIC

UHPLC nano fittings



EXP2 UHPLC fittings from Optimize Technologies are designed with titanium hybrid Ti-LOK ferrules for PEEKsil tubing. Male fittings reportedly feature a hex head with a removable slotted knurled wrench for hand tightening to more than 15,000 psi, depending on the tubing internal diameter. According to the company, uses for the fittings include nano and UHPLC connections as well as sample loops.
Optimize Technologies, Inc., Oregon City, OR.
www.optimizetech.com

Data analysis and graphing software



Origin and OriginPro 2015 data analysis and graphing software from OriginLab add more than 100 new features and improvements. According to the company, enhancements include collapsible menus, project file search for string, thumbnail previews of graphs, and tooltips that display folder or window comments in Project Explorer. **OriginLab,** Northampton, MA.
www.originlab.com

2D-LC system

Shimadzu's Nexera-e comprehensive two-dimensional liquid chromatograph is designed for use with complex sample matrices. The system reportedly incorporates the company's Nexera X2 photodiode-array detector for sampling rates up to 200 Hz and can be used with its LCMS-80 triple quadrupole LC–MS-MS, for ultrahigh-speed data scanning.
Shimadzu Scientific Instruments, Columbia, MD.
www.ssi.shimadzu.com

HPLC columns

Shodex's HILICpak VG-50 HPLC columns are designed to support the analysis of saccharides and reducing sugars such as fructose, mannose, glucose, and sucrose. According to the company, applications are available from the company's database.
Showa Denko America, Inc., New York, NY.
www.shodex.net

HPLC columns

BIOshell™ U/HPLC Columns

BIOshell Glycan HPLC columns from Sigma-Aldrich are designed for separations of released and labeled glycans. According to the company, the Fused-Core columns provide improved resolution when compared to conventional LC methods, allowing reliable quantitation and identification of glycans.
Supelco/Sigma-Aldrich, Bellefonte, PA.
www.sigma-aldrich.com/BIOshell

Mass spectrometer

Thermo Fisher Scientific's Q Exactive Focus LC–MS-MS system is designed for laboratories performing food and environmental testing, clinical research, forensic toxicology, pharmaceutical and biopharmaceutical measurements, and other applied analyses. According to the company, the instrument delivers up to 70,000 resolution at *m/z* 200, and a scan speed of up to 12 Hz.
Thermo Fisher Scientific, San Jose, CA. www.thermofisher.com/qefocus

N-Glycan kit

The GlycoWorks RapiFluor-MS N-Glycan kit from Waters is designed for deglycosylation and labeling. According to the company, the kit enables mass detection for biotherapeutic characterization and development, and sample preparation for released N-glycans can be completed in 30 min.
Waters Corporation, Milford, MA.
www.waters.com/glycans

SEC–MALS detector for UHPLC

The µDAWN multiangle light-scattering detector from Wyatt Technology is designed to be coupled to any UHPLC system to determine absolute molecular weights and sizes of polymers, peptides, and proteins or other biopolymers directly, without column calibration or reference standards. According to the company, the detector connects to its Optilab UT-rEX.
Wyatt Technology Corp., Santa Barbara, CA.
www.wyatt.com

CALENDAR

25–28 April 2015
25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)
Copenhagen, Denmark
www.eccmid.org

26–30 April 2015
60th Annual Analysis Division Symposium
Galveston, TX
adsymposium.org/Symposium/AD_2015.htm

3–7 May 2015
3rd International Symposium on Green Chemistry
La Rochelle, France
www.isgc2015.com

4–5 May, 2015
Inverse Gas Chromatography Symposium 2015
Newark, NJ
surfacemeasurementsystems.com/igc-symposium-2015/

4–6 May 2015
Minnesota Chromatography Forum (MCF) Spring Symposium: The Upper Midwest's Largest Chromatographic Instrumentation and Supplies Exhibition
Minneapolis, MN
www.minnchrom.com/?page_id=4

17–21 May 2015
39th International Symposium on Capillary Chromatography and 12th GCxGC Symposium I
Fort Worth, TX
www.isccgcxgc2015.com/home.html

20–21 May 2015
3rd Nordic Symposium on Convergence Chromatography
Gothenburg, Sweden
www.waters.com/waters/eventInstance.htm?eiid=134826144&locale=en_SE

31 May–4 June 2015
63rd ASMS Conference on Mass Spectrometry & Allied Topics
St. Louis, MO
www.asms.org/conferences/annual-conference/annual-conference-homepage

2–4 June 2015
Microbiology Week
Philadelphia, PA
www.cbinet.com/conference/PI15056#.VPSASUtNv1o

21–25 June 2015
42nd International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2015)
Geneva, Switzerland
www.hplc2015-geneva.org

22–24 June 2015
IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine
Paris, France
www.paris2015.org

28 June–1 July 2015
RDPA 2015: Recent Developments in Pharmaceutical Analysis
Perugia, Italy
rdpa2015.chimfarm.unipg.it

30 June–3 July 2015
21st International Symposium on Separation Sciences
Ljubljana, Slovenia
www.isss2015.si

22–24 July 2015
SFC 2015 — 9th International Conference on Packed Column SFC
Philadelphia, PA
www.greenchemistrygroup.org/Registration.html

26–29 July 2015
PREP 2015: 28th International Symposium on Preparative and Process Chromatography
Philadelphia, PA
www.prepsymposium.org

28–30 September 2015
4th International Conference on Forensic Research & Technology
Atlanta, GA
forensicresearch.conferenceseries.com

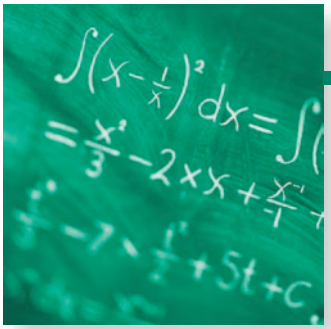
5–7 October 2015
International Symposium on Applied Chemistry (ISAC)
Bandung, Indonesia
situs.opi.lipi.go.id/isac2015/

16–20 October 2015
Native Mass Spectrometry-Based Structural Biology
Pacific Grove, CA
www.asms.org/conferences/asilomar-conference/asilomar-conference-homepage

3–6 November 2015
7th International Symposium on Recent Advances in Food Analysis
Prague, Czech Republic
www.rafa2015.eu/RAFA_2015_1st_flyer.pdf

12–15 November 2015
GPSS: Gazi Pharma Symposium Series
Antalya, Turkey
www.gpss2015.org

15–17 November 2015
12th International Symposium on Persistent and Toxic Substances (ISPTS)
Riverside, CA
pts2015.ucr.edu



SHORT COURSES

GC

5–8 May 2015
Practical Gas Chromatography
Chicago, IL
www.chem.agilent.com/en-US/training-events/en-us/R1915A/Pages/default.aspx

19–21 May 2015
Modern Practice of Gas Chromatography
West Chester, PA
www.cfdv.org/course/modern-practice-gas-chromatography-0

10–13 June 2015
Forensic GC
Chicago, IL
www.forensicchromatography.com/forensic-chromatography/

HPLC

10–15 May 2015
Protein Chromatography
Charlottesville, VA
faculty.virginia.edu/shortcourse/CourseDescription.html

1–4 September 2015
High Performance Liquid Chromatography: Fundamentals, Troubleshooting, and Method Development
Chicago, IL
proed.acs.org/course-catalog/courses/high-performance-liquid-chromatography-fundamentals-troubleshooting-and-method-development/

14–15 October 2015
How to Develop Validated HPLC Methods: Rational Design with Practical Statistics and Troubleshooting
Edison, NJ
proed.acs.org/course-catalog/courses/how-to-develop-validated-hplc-methods-rational-design-with-practical-statistics-and-troubleshooting/

Simplifying Carbohydrate Testing in Food and Beverages to Meet Food Quality and Labeling Requirements Using Ion Chromatography and Pulsed Amperometric Detection

ON-DEMAND WEBCAST Originally aired April 2, 2015

Register for free: www.chromatographyonline.com/lcgc/simplifying

EVENT OVERVIEW:

Carbohydrates are widely used in food and beverage products and have numerous functions: energy sources, sweeteners, as a creator of physical properties (like texture and viscosity) and as dietary fiber. This use has led to a need for accurate, high-quality analyses of the carbohydrate composition and content in food and beverages, including raw materials. Due to the diverse use of carbohydrates in food and beverage products, and the legal requirements for product labelling and quality control, the food and beverage industry has a high demand for testing methods which are quick, easy to use, and highly reproducible. Traditional testing methods for carbohydrates are often not suitable anymore. They can lead to faulty results and therefore inaccurate declarations on product labels and can also lead to food quality issues and product recalls. In this presentation we will discuss the use of ion chromatography with pulsed amperometric detection as a technique to provide fast, reproducible, and simple methods to determine the carbohydrate content in food and beverages and address product labeling requirements and quality control testing.

Key Learning Objectives:

- Learn about the importance of food quality and labeling testing in the food and beverage industry
- Learn the theory of ion chromatography with pulsed amperometric detection for the determination of carbohydrates
- Learn how you can simplify carbohydrate analysis to provide fast, reproducible and simple-to-use methods in food and beverage products

Who Should Attend:

- Food and beverage laboratory operators, food quality managers, and lab managers responsible for implementing labeling requirements
- Lab operators wanting to learn how to simplify carbohydrate analysis
- Food scientists working in the area of carbohydrate research
- Lab managers who are considering performing or implementing carbohydrate analysis

Presenter:

DR KHALIL DIVAN
Director of Food and Beverage Market, Chromatography and Mass Spectrometry, Thermo Fisher Scientific

Moderator:

LAURA BUSH
Editorial Director, LCGC & Spectroscopy

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LCGC

For questions, contact Kristen Moore at kmoore@advanstar.com

AD INDEX

LCGC PESTICIDE RESIDUE ANALYSIS WEBINAR SERIES
Tips and Tricks for the Whole Workflow

Workflow Guide for the Use of LC–MS

LIVE WEBCAST

Wednesday, April 29, 2015 at 8 am PDT/ 11 am EDT/ 4 pm BST/ 5 pm CEST

Register free at www.chromatographyonline.com/lcgc/pesticide_residue_analysis_series

Series Part 2 Event Overview:

Safeguarding the environment and the global food supply requires continuous monitoring of more and more compounds, and at lower levels than ever before. Pesticide residues analysts are challenged to detect, identify, and quantify hundreds of different pesticides from diverse sample types with a fast turnaround time.

As regulations change, quantitation methods must have the ability to adapt to meet these needs. Truly comprehensive monitoring requires analysis using both targeted and non-targeted approaches. The latter is required to detect illegal usage, since targeted approaches will not detect pesticides not programmed into the acquisition method. For targeted analysis, SRM (selective reaction monitoring) is still the gold standard technique. Screening and quantitation using high resolution accurate mass (HRAM) technology is also a highly accurate and sensitive method and has the advantages of reduced method development time and reduced false positives and negatives.

This webinar will provide pesticides residue analysts with valuable information on the development and optimization of chromatographic separations and mass spectrometry methods for the analysis of pesticide residues in food. The expert speakers will share their knowledge in understanding the critical aspects of the method, assisting analysts in optimizing their methods for the most challenging analyses.

Who Should Attend:

- Researchers and analysts in pesticide analysis
- Food scientists interested in learning the latest technologies for sample preparation of food matrices
- Anyone struggling with sample preparation challenges for pesticide residue analysis in food

Series Moderator

Richard Fussell, Ph.D.
Global Vertical Marketing Manager, Food and Beverage, Chromatography & Mass Spectrometry Division
Thermo Fisher Scientific

Presenter:

Claudia, P.B. Martins, Ph.D.
Applications Program Manager, Environmental and Food
Thermo Fisher Scientific

LCGC Moderator:

Laura Bush
Editorial Director
LCGC

Key Learning Objectives:

- Understand the important role of liquid chromatography in multi-analyte pesticides methods
- Learn about the applicability of triple-quadrupole and Orbitrap™ orbital trap high resolution accurate mass technologies for targeted and non-targeted analysis
- Understand the benefits and pitfalls of targeted vs non-targeted workflows

Register free at www.chromatographyonline.com/lcgc/pesticide_residue_analysis_series

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Presented by

LCGC

Part 1: Sample Prep Tips and Tricks Using QuEChERS and Accelerated Solvent Extraction
ON-DEMAND WEBCAST, originally aired March 24, 2015

Part 2: Workflow Guide for the use of LC-MS
Wed., April 29, 2015 at 8 am PDT/ 11 am EDT/ 4 pm BST/ 5 pm CEST

Part 3: Maximizing Analysis Efficiency through New GC-MS Approaches
Wed., June 17, 2015 at 8 am PDT/ 11 am EDT/ 4 pm BST/ 5 pm CEST

Part 4: Latest Developments & Future Directions in Data Processing & Analysis Software for LC-MS/MS & GC-MS/MS
Wed., July 15, 2015 at 8 am PDT/ 11 am EDT/ 4 pm BST/ 5 pm CEST

Register for the Pesticide Residue Analysis Webinar Series

For questions, contact Kristen Moore at kmoore@advanstar.com

THE ESSENTIALS

Excerpts from LCGC's professional development platform, CHROMacademy.com

Understanding Electron Ionization Processes for GC–MS

Many of us use electron ionization (EI) in gas chromatography–mass spectrometry (GC–MS) without a good understanding of the technique and how we might manipulate the process to give more appropriate results or a better understanding of the analytes under investigation. Here, we explain the fundamental principles of EI.

In the ion source, high-energy electrons (typically 70 eV) are created via thermionic emission from a resistively heated metal filament and accelerated across the source (typically using potential difference in the range 5–100 V) at right angles to the stream of neutral analyte molecules in the gas phase.

Ionization takes place because of the vast disturbances in the electrical field due to close passage of the energetic electron, which causes ionization of suitable bonds. No impacts actually occur, and hence the term *electron impact ionization* is now considered archaic. In fact, a single high-energy electron may cause several ionizations. The general scheme for EI is shown in equation 1:

$$M_{(g)} + e^- \rightarrow M^{+\bullet}_{(g)} + 2e^- \quad [1]$$

Typical ionization energies of common organic analytes will range from 5 eV to 15 eV and the ease of "extraction" of the electron will depend on the analyte, and more specifically the bond type. Ionization energy tends to increase in the following order: lone pair electrons (aniline 7.7 eV) < π bonding electrons (ethene 10.5 eV) < σ bonding electrons (ethane 11.5 eV).

So why use an electron energy of 70 eV? The ionization cross-sectional areas (the area through which an electron must pass to induce ionization) of the most common organic molecules is below 70 eV and curves of electron energy versus ionization cross sectional areas for methane (CH_4) all report a maximum at around 70 eV. Having higher energy than is required to affect ionization also improves the efficiency of the ionization process to around 0.1% — that is, around one in every 1000 analyte molecules in the ion source will be ionized and the deBroglie wavelength of the electron "wave" will match typical organic bond lengths at around 0.14 nm. Furthermore, the repeatability of the ionization is good at this value, and small changes in electron energy typically won't alter the absolute or relative intensities of ions within the spectrum — which is good when using libraries for analyte identification. Using electron energies of 60–80 eV will bring little difference to ionization efficiency, but as the energy increases, the molecule becomes transparent to the electron as its deBroglie wavelength decreases.

When using 70 eV electrons, the excess energy imparted to the analyte molecule can cause various unimolecular reactions through electronic and vibrational excitement.

Note that only the charged species in Figure 2 will be manipulated by the mass analyzer and detected. Neutral species are lost to the analyzer walls or the vacuum system. The relative intensity of each fragment indicates the relative stability of that fragment that can aid with structural elucidation.

Further fragmentation or rearrangement's may occur depending on the excess energy that remains within the molecule.

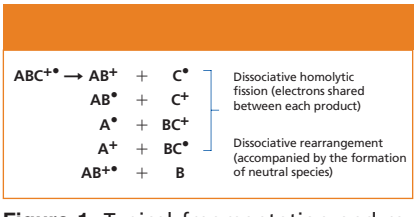

Figure 1: Typical fragmentation and rearrangements in EI GC–MS.

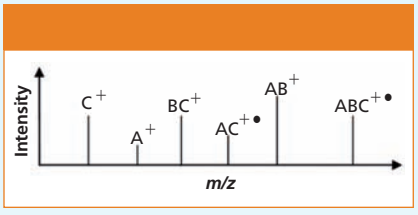

Figure 2: Typical EI GC–MS spectrum.

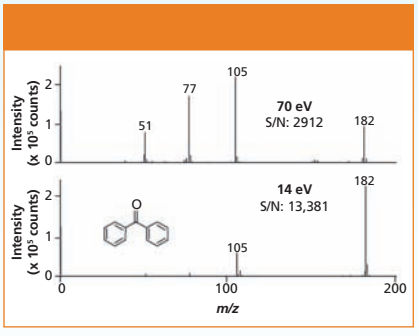

Figure 3: Promotion of molecular ion using lower ionizing electron energy without traditional loss in signal intensity (Markes International Ltd.).

However, the time domain for ionization and analysis (5 µs in the ion source and 10 µs in the analyzer, typically) means that concerted reactions are not usual and the "typical" fragmentation and rearrangement possibilities are limited to a few fairly well classified reaction types such as alpha and inductive cleavage, retro-Diels–Alder, and the so-called "McLafferty Rearrangement." Rearrangement reactions typically result in an even mass fragment, which is a significant "clue" when interpreting spectra.

At low source pressures (10^{-4} Pa) the mean free path between molecules means that reactions with background species or bimolecular reactions do not occur.

Reducing the ionizing electron energy to between 20 and 60 eV can sometimes lead to the promotion of the molecular ion (M^+), which will give an indication of the validity or identity of the molecular ion, useful when dealing with labile analytes. Technology is available that allows the use of a lower electron energy without suffering from catastrophic loss of ionization efficiency.

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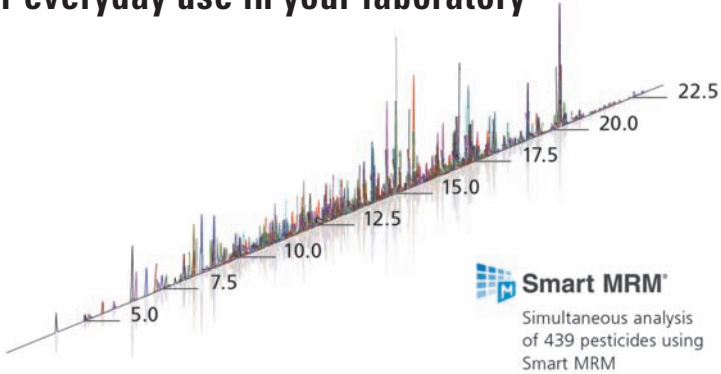

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