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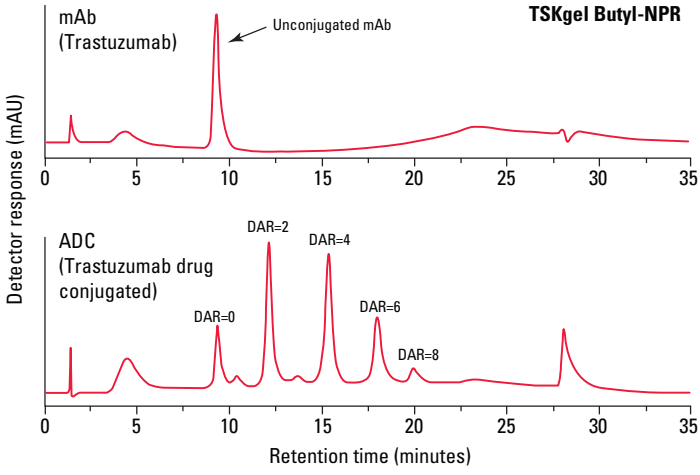

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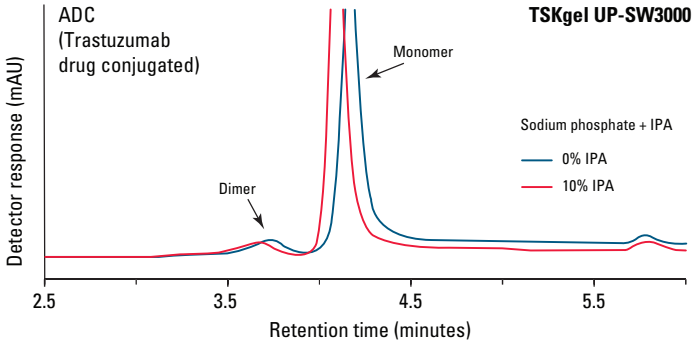

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SPECIAL FEATURES

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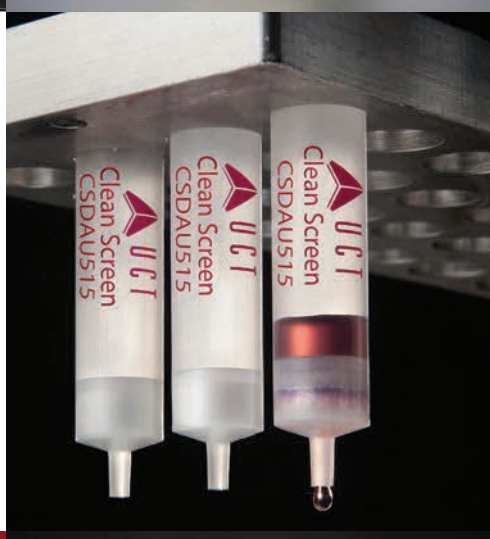

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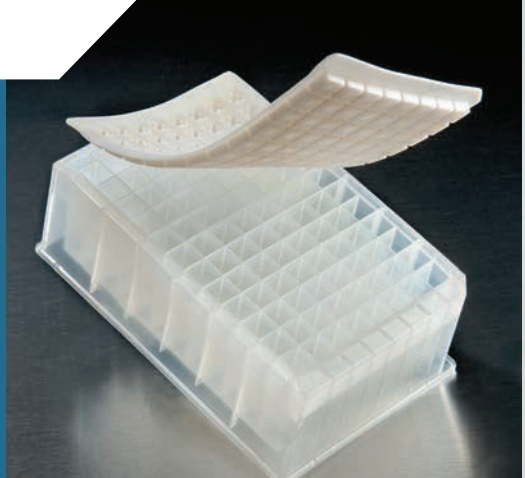

PEAKS of Interest

Debby Mangelings Joins LCGC's *Editorial Advisory Boards*

LCGC magazine is pleased to announce the addition of Debby Mangelings to the editorial advisory boards of *LCGC North America* and *LCGC Europe*.

Mangelings received her PhD in pharmaceutical sciences in 2006 from the Vrije Universiteit Brussel, in Brussels, Belgium, where she currently works as an associate professor in the Department of Analytical Chemistry and Pharmaceutical Technology. Her work focuses on chiral separations, miniaturized separation techniques, capillary electrochromatography (CEC), liquid chromatography, and supercritical fluid chromatography (SFC). She is also interested in the synthesis of in-capillary stationary phases, such as monoliths for both chiral and achiral separations in CEC.

A key focus of Mangelings's work has been defining and updating chiral separation strategies for various modes of high-performance liquid chromatography (HPLC) as well as for SFC and CEC.

More recently, she has worked on the chemometric data analysis of chiral separation data to study systems with similar or dissimilar enantioselectivity. In CEC, she is working on the evaluation of new stationary phases, such as those involved in the successful chiral separation of uncommon compounds as the boron cluster species.

Mangelings received *LCGC's* 2016 Emerging Leader Award, which was presented to her in March, at Pittcon 2016 in Atlanta, Georgia.

Trajan Scientific Acquires LEAP Technologies

Trajan Scientific (Melbourne, Australia), which develops medical devices as well as analytical systems such as gas and liquid chromatography columns and sample preparation systems, has acquired the business of LEAP Technologies (Carrboro, North Carolina). LEAP Technologies provides automated sample preparation systems for chromatography and mass spectrometry.

Trajan indicated that bringing LEAP into the Trajan Group would complement the capabilities of Trajan. Stephen Tomisich, the company's chief executive officer, said he was pleased to welcome the LEAP team into the Trajan family.

LEAP President Werner Martin predicted that the opportunity for LEAP to join Trajan would be a step forward for the application of new automation configurations for analytical chemistry laboratories worldwide, while LEAP Chief Executive Officer Sal Iacono commented that LEAP is looking forward to continuing and expanding its relationships with technology partners in integrating their offerings with Trajan's disruptive technologies. ■

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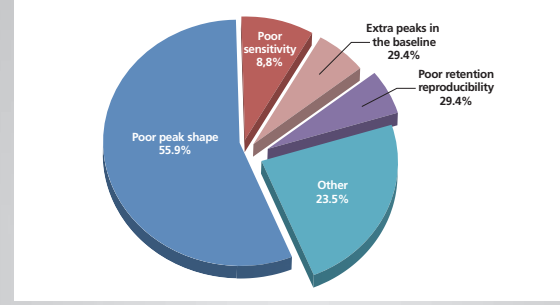
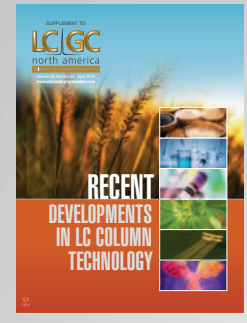


What is your biggest problem with isocratic LC methods?

LCGC ran a poll to ask liquid chromatography users about their biggest challenges with isocratic methods. As the figure shows, a majority of respondents cited poor peak shape, while a substantial portion of users indicated they had other challenges.

"Other" responses included limited selectivity, baseline noise after a long series and late eluting peaks, and non-optimized selectivity for older methods.

SPECIAL ISSUE HIGHLIGHTS



RECENT DEVELOPMENTS IN LC COLUMN TECHNOLOGY

In applications ranging from food to pharma, and biotherapeutics to biomes, advances in liquid chromatography are playing a critical role. Modern particle designs and surface chemistry treatments are continually being adopted in a variety of disciplines. Read our April 2016 supplement, *Recent Developments in Column Technology,* to learn more.

This issue's articles include

- **The Impact of Superficially Porous Particles and New Stationary-Phase Chemistries on the LC–MS Determination of Mycotoxins in Food and Feed**
- **The Synthetic Cannabinoid Chemical Arms Race and Its Effect on Pain Medication Monitoring**
- **HPLC Column Technology in a Bioanalytical Contract Research Organization**
- **Latest Advances in Environmental Chiral Applications**
- **Characterizing SEC Columns for the Investigation of Higher-Order Monoclonal Antibody Aggregates**
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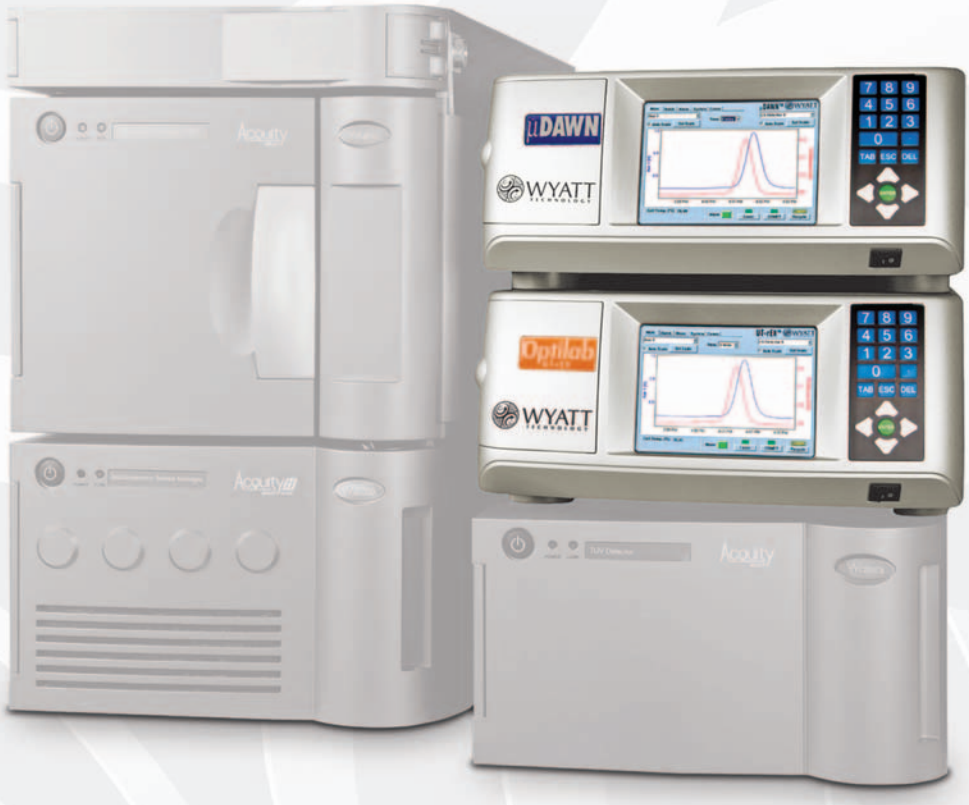

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On Maintaining Technical Proficiency

I.S. Krull and P.T. Kissinger

For scientists in any field, staying current with new developments is essential. The irony is that today, when we are flooded with information, keeping up with what matters is actually a bigger challenge than it was in the past.

If you are young, you may think that you have nothing to learn from us, as we have both passed the usual retirement age. It's true that things have changed drastically since we started our careers. At that time, there were fewer journals, very few trade publications, and no electronic communications devoted to science. "Keeping up with the literature" meant weekly browsing sessions in the quiet of a local chemistry library, admiring the latest journals as they arrived week by week. The information reviewed was at least a year old.

But like you, we live in today's information tsunami. And like you—we hope—we maintain a deep desire to continue learning. In our case, our focus is on analytical chemistry applicable to health care advances, but the same challenge exists regardless of your specialty.

Drinking from a Fire Hose

In this Wiki-search-engine-internet age with daily tables of contents, free educational videos, trade publications, webinars, e-books, and websites of innovative vendors, "keeping up with the literature" has an entirely new meaning. Although almost all of these resources are free in a financial sense, they overwhelm us by taking our most valuable asset—time. Identify the sources most useful to you and focus on those.

Your university or company pays substantial sums to give you unencumbered access to peer-reviewed science. Today, there seems to be a "bubble" of journals, and many are pretenders with similar titles. One must be discerning.

The Special Case of Analytical Chemistry

The field of analytical chemistry is perhaps a special case. The vast majority of published papers have essentially the same title: "Determination of X in Y Using Z." While we've both published plenty of "Determination of X in Y Using Z" papers, such papers are rarely of interest unless X or Y is of interest. What really excites analytical innovators is when Z is both new and productive. The most important literature to keep up with is in the 1%, where those new measurement technologies are explored. For 95% of the papers we see, we read only the abstract, figure captions, and the concluding paragraph.

Our field has advanced so far in the last five decades that we are not exaggerating much if we say that with modern instrumentation we can determine any relevant substance at a relevant concentration in any relevant sample. Of course, what is relevant has changed a lot in those five decades, typically by a factor of 1000 or more in concentration, volume, and spatial resolution. The greater the magnification, the more we can see and the more challenging the validation. These advances are one source of the widely described "replication crisis in science and medicine."

Browse, Too

On the other hand, while we are talking about the importance of triage and carefully choosing the information we consume, we also want to put in a plug for randomness. Few of us read intact journals today; most search for relevant articles with search engines. This is a logical and necessary approach, but it also means that we miss the unexpected connections that come from browsing. We recommend subscribing to at least one multidisciplinary general science journal such as *Science*, *Nature*, *The Scientist*, or *Scientific American*. Advances often come from unanticipated connections of the previously unconnected. The more you see, the more you'll find. Serendipity often beats planning.

Joining Still Matters

Both of us woke up one day to discover we were 50-year members of several science organizations. Why did we join groups such as the American Chemical Society (ACS), Sigma Xi, and the American Association for the Advancement of Science (AAAS)? We joined for the networking and the information resources. Back then, these organizations were more exclusive, and joining was necessary to get personal access to the journals. That was also how job opportunities came to the forefront. We went to conferences to hear about and see the latest innovations.

But even if the reasons for joining those organizations have changed, it still makes sense to meet peers face to face locally, nationally, and globally. Don't just focus on the national and international conferences. Look for local scientific organizations and discussion groups. Some of these are chapters of national associations, such as ACS, American Association of Pharmaceutical Scientists (AAPS) or the Association of Analytical Communities (AOAC), and others—such as the Minnesota Chromatography Forum—are independent. These groups often meet in the evening, bringing together scientists from companies, government, and academia to review new developments. There often are a keynote speaker, a few posters, and a sponsoring vendor. In addition, keep your eye out for talks at local colleges and universities.

Another growing concept is that of a journal club, analogous to a book club. Members read a selected paper and comment on it, ask each other questions about it, and come to conclusions together. In some variations, members concentrate on particular components of the paper and report to the group.

Every productive scientist is a node in a dynamic human network that grows and evolves over time. This network must be nurtured by maintaining existing technical relationships (such as pals from graduate school) and developing new ones.

For young scientists, the biggest problems are failing to be aware of work that came before and thinking that answers will just fall into your lap in the first screen of your search.

Conclusion

Maintaining and growing technical competencies requires effort. We both remember scientists several decades ahead of us who failed to keep up with important developments. For example, there were some who never fully grasped the development of liquid chromatography (LC) circa 1970 or LC coupled with tandem mass spectrometry (MS-MS) circa 1995.

For young scientists, the bigger problem is not failing to change with critical new developments, but failing to be aware of work that came before (reinventing the wheel) and thinking that answers will just fall into your lap in the first screen of your search.

In today's world, the volume of available information resources can be overwhelming. It is clear that the signal-to-noise ratio has gotten worse rather than better. Each of us must triage carefully. There is no alternative to carefully selecting the information resources most relevant to your work, while acknowledging the fact that opportunities for surprise insights may be lost. Supplementing your carefully selected sources with one broad science read can help compensate for that loss. Good networking with your peers can also help broaden your horizons.

I.S. Krull *is a professor emeritus in the Department of Chemistry and Chemical Biology at Northeastern University in Boston, Massachusetts.*
P.T. Kissinger *is a professor in the Department of Chemistry at Purdue University in West Lafayette, Indiana. Direct correspondence to: petekissinger@gmail.com and irask@aol.com* ■

A Q&A

Hormone Analysis by LC–MS and Water Impact

Joseph Plurad,
North America Field
Marketing Manager,
MilliporeSigma

The increased use of hormone-based therapies in health care throughout the world has resulted in hormones finding their way into municipal water supplies. The potential health risks of unintended consumption of hormones through drinking water have significantly increased the interest in identifying these compounds in our water supplies. The Milli-Q system incorporates a combination of purification processes that provides ultrapure, hormone-free lab water for the LC–MS techniques used for interference-free analysis of these contaminants.

LCGC: I know we've heard it before, but why is water quality important in liquid chromatography and mass spectrometry?

PLURAD: Water is probably the most used chemical in a laboratory. In liquid chromatography and mass spectrometry, water is used throughout the entire workflow. Any contaminants that remain in the purified water used in an analysis that have a direct impact on the separation or detection are of concern.

Also, if there are any traces of the molecules you're trying to analyze in the water you're using, you may have inaccurate results. Water quality is important to avoid interference with the analytes you're measuring or identifying as well as for optimizing instrument operation.

LCGC: Which specific contaminants can affect the LC–MS process?

PLURAD: The most obvious are organics, and in LC–MS, that's typically what you're looking for. Reduction to trace levels is key, particularly if the organics are similar to what you're analyzing.

Water that's heavy with organics can also cause issues with column efficiency by coating the separation media, resulting in poor peak resolution and shifting peaks. Ions can be a problem. Certain metals can create adducts resulting in noisy mass spectra.

Particle-free water is important to ensure proper flow through the system. With shrinking columns and tubing, as well as improvements and changes to separation media and higher pressures, the impact of particles clogging an LC–MS becomes even more magnified.

Bacterial contamination is a two-headed monster. Bacteria behave as particles, so you run the risk of blocking and clogging tubing or columns. But as bacteria die off, they leech out and reintroduce various organic and ionic contaminants into the previously clean water.

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LCGC: Regarding the work MilliporeSigma recently completed, why is there so much interest in analyzing for hormones in water today? And how did you pick the water samples you chose to analyze?

PLURAD: In the last 20 years, a lot of attention has been given to "persistent organic pollutants" in drinking water sources. Much of the original focus in this area was on organic molecules and species that came from so-called chemical sources such as pesticides and solvents.

With advances in health care, however, pharmaceutical sources of these persistent organic pollutants have become more significant. As many of our listeners may remember, we were once told to dispose of our expired or unused pharmaceuticals by flushing them down the toilet, which in retrospect was not the best idea because this water, now carrying these drugs, eventually finds its way back into the municipal drinking water supply.

With the escalation of use of hormone-based therapies such as topical steroids, birth control, and hormone-replacement therapies, there may be long-term effects if these therapeutics exist in our drinking water, such as effects on human fertility and actual embryo development, as well as endocrine and other general health issues.

Consequently, there's extremely high interest in identifying what's in the water and at what levels to determine imminent or long-term health risks. Because our lab water systems rely on potable tap water as a feed source and are used throughout the world, we felt it was important to understand what influence hormones could have on our ability to provide ultrapure water. We also wanted to demonstrate that our purification techniques can provide high-purity water for the detection of hormones in drinking water via LC–MS.

Because this is a global issue, we selected drinking water samples from various geographies including China, France, and Spain. We're not stating that these samples reflect the overall water qualities in these countries or the safety of the drinking water sources. These are single points of analysis chosen to show that the problem exists to some degree everywhere.

LCGC: What are the challenges in analyzing hormones at trace levels?

PLURAD: We found that these hormones are everywhere and that simple or single-stage purification techniques may not be effective in removing them. Consider deionization, for example. As a purification technique, it only works on contaminants that have an electrical charge. Most organics are neutral or very weakly charged. So deionization is not very effective at removing these contaminants.

Or, consider reverse osmosis. Although this is a workhorse in water purification, a reverse-osmosis system operating well removes only 95% to 99% of the contaminants in the water feeding that membrane. This means that in water systems that have relatively higher levels of these persistent contaminants you can expect to see some residual contaminants post purification.

Clearly, a combination of techniques is required to ensure full removal of these molecules and to have water free of hormone residues for your analytical work. We can consider purification that includes activating carbon, reverse osmosis, UV photo oxidation of organics, and ion exchange. And if that's still not enough, we can consider other purification media at the point of use such as additional activated carbon that targets specific contaminants.

LCGC: What were the results of your analyses?

PLURAD: We found hormones in all of the water sources we tested. It stands to reason that in highly industrialized and developed countries you would expect to see various hormones at various levels. Our R&D team found androsterone and estradiol in city water sources in France and Spain, and corticosterone was detected in China.

I'd like to reiterate it's understood that these municipal drinking water samples are safe and suitable and approved for human consumption. Agencies worldwide recognize the existence of this issue and are taking a hard look at the long-term effects of having these molecules in the drinking water.

The combination of purification processes embedded in a Milli-Q water purification system allows us to provide hormone-free lab water for the LC–MS techniques used to analyze for these contaminants.

LCGC: The sensitivity of analyses is constantly improving. How is MilliporeSigma responding to this laboratory market demand?

PLURAD: We now have scientists who are able to analyze and quantify trace levels of contaminants that are far below the detection limits provided by traditional quality measures for ultrapure water; in fact, by orders of magnitude at this point.

MilliporeSigma continues to develop more efficient means to remove general classes of contaminants and continues to develop purification packs that are adapted to remove very specific classes of molecules at the actual point of use. Being able to control the purification process 100% from tap water feeding the water purification unit gives users the best chance at managing the impact of persistent contaminants.

SAMPLE PREP PERSPECTIVES

New Sample Preparation Products and Accessories for 2016

This yearly report on new products introduced at Pittcon or in the preceding year covers instruments, accessories, and sorbents for sample preparation.

Douglas E. Raynie
Sample Prep Perspectives Editor

Our annual review of sample preparation products covers new product introductions made since May 2015. The primary focus is new product introductions at Pittcon, though this is not the exclusive focus. In late 2015, the *LCGC* editorial staff submitted a survey to vendors of sample preparation products. Responses to this survey are compiled in this review. Additionally, a keyword search using the terms "sample preparation" and "extraction" was conducted for exhibitors at Pittcon 2016; then each of these vendors was visited. Although attempts were made to be as inclusive as possible, we apologize for any oversight.

While attendance at Pittcon has fallen over the past decade and some vendors have diminished their presence, the current product review demonstrates the importance of regularly attending such conferences to stay up to date with the latest instrumentation. Although there were no monumental splashes of new technology, when taken as a whole, the incremental advances developed over the past year add up to significant progress in the field of chromatographic sample preparation.

In last year's product review (1), we forecast advances in sorbent technology, including QuEChERS (quick, easy, cheap, effective, rugged, and safe); activity around the end of the original solid-phase microextraction (SPME) patents, including biocompatible and liquid chromatography (LC)-compatible products; and serial and parallel sample processing, all driven by bioanalytical and food safety applications. Meanwhile, our recent survey of sample preparation trends (2) added interest in derivatization, pressurized solvent extraction (PSE), solvent evaporation, homogenization, internal standard addition, trace enrichment, matrix-solid phase dispersion (MSPD), microwave-assisted extraction (MAE), solvent exchange, supercritical fluid extraction (SFE), ultrasound extraction, stir-bar sorptive extraction (SBSE), restricted-access media (RAM), and direct analysis in real time (DART) mass spectrometry (MS).

Our batting average with these prognostications isn't too bad. Excluding applications (of which there are several significant recent publications in many of the areas described above), product introductions in the past year touched on sorbent technologies (including solid-phase extraction [SPE], MSPD, and QuEChERS); solvent evaporators and the associated trace enrichment and solvent exchange; serial and parallel processing, especially in SPE; and advanced techniques for extracting solids (including SFE, ultrasound, automated Soxhlet, and PSE). One major driver of advances in sample preparation that we did not anticipate is the buzz (pun intended) around medicinal marijuana.

This review is presented in three sections. First, we discuss advances in instrumentation for sample preparation. This instrumentation is primarily concerned with extracting solid samples, although automated SPE systems are discussed (though sorbent technology is presented elsewhere). Next, we turn to accessories and supporting technologies in the sample preparation process. Advances in sorbents is the final section. To assist readers with some of the details behind these new products, each section includes a table that summarizes the associated products.

Sample Preparation Instrumentation

New instrumentation for sample preparation can be classified into four major areas: automated SPE, enhanced extraction of solids, approaches to volatile samples, and specialty products. These are summarized in Table I.

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Automated Solid-Phase Extraction

As we discussed in our trends survey (2), automation, including serial and parallel sample processing, is of strong interest to those working in the laboratory. Four instruments centered on automated SPE appeared this year. PromoChrom introduced the RT-01 system, LabTech had the Sepaths UP system, Reeko Instruments announced the Auto SPE-60 Plus system, and Fluid Management Systems presented the EconoPrep system. These systems are primarily in response to demand from the environmental and bioanalytical communities. A detailed look at the products shows parallel processing appears to be the norm, a wide range of solvent volumes and solvent switching is available, and both the disk and cartridge approaches to SPE are accommodated. For specific applications, the systems may be associated with specific SPE products. One criticism with automation and instrumentation in general is the associated acquisition cost. Fluid Management Systems announced a rental program aimed at getting clinical laboratories into the automated SPE field. Although equipment rental programs are not new, they are also not commonplace. It will be interesting to see if this program targeting clinical laboratories will gain traction.

While not automated SPE, Gerstel introduced two products coupling robotic automation (via their gas chromatography [GC] autosampler platform) with GC injection. Regarding analyte isolation, these systems have liquid sampling, headspace sampling, SPME, and SBSE capabilities. For sample treatment, capabilities are as varied as derivatization, internal standard addition, weighing, and solvent evaporation. Additionally, a new module accommodates ease of replacing injection syringes.

Enhanced Extraction of Solids

The application of heat or energy to enhance extraction efficiency beyond that observed with Soxhlet extraction has been of interest for the past generation. Perhaps the most straightforward approach is what has been termed *automated Soxhlet* or the *Randall method*. This approach combines leaching approached by immersing the sample thimble into the boiling solvent, then the more conventional Soxhlet approach is used to wash the sample. Velp Scientifica introduced such a system this year that also features recovery of the extraction solvent. This approach uses much less solvent and takes significantly less time than conventional Soxhlet.

The next conceptual advance in extraction of solids is the application of pressure so that temperatures greater than the atmospheric boiling point can be used. This pressure may be applied, as in PSE, or via heating a solvent in a closed system, as in MAE or hot-block approaches. The Gemini High Pressure Solvent Extractor system uses both high pressure and high temperature and may be integrated with extract concentration or SPE. The application of ultrasound energy to influence extraction efficiency is well-established. Bath-type sonicators tend to be lower energy, so ultrasonic probes (horns) are often used in extraction. Elmasonic has developed a bath-type ultrasound system with higher frequencies, up to 80 kHz.

A final approach to extracting solids is SFE. The environmental, and other, benefits of supercritical carbon dioxide abound. Extractions at greater scale than analytical are driven by the isolation of cannabidiol from medicinal marijuana, even in states where medicinal marijuana itself may be illegal. Two analytical instruments, from Waters and Applied Separations, are based on the same technology used at larger scale. The Applied Separations Helix system is also engineered for use with subcritical water, to extend the available solvent polarity range of the extracting fluid. Of course, SFE has myriad applications beyond cannabidiol isolation.

Approaches to Volatile Samples

By the nature of the analyte, these systems are typically directly coupled with GC. CDS Analytical added preheating capabilities to thermal desorption to better accommodate wet samples. Teledyne Tekmar and OI Analytical are two companies already established in purge-and-trap technology, and both extended their product range with an eye toward increased throughput. Finally, as noted, the Gerstel autosampler systems are used for GC systems.

Specialty Products

"Specialty" is not meant to imply small markets, but rather unique applications. For example, water analysis via Environmental Protection Agency (EPA) methods is a huge industry segment. The LC Tech Freestyle Xana system performs parallel extractions of up to 10 L of water. Lipid interferences are important concerns in some analyses. DeltaChrom uses a gel-permeation chromatography (GPC) approach to remove lipids during the analysis of environmental solids. Meanwhile, Polymer Char has an external filtration system for sample clean-up associated with polymer synthesis. Also on the synthesis front, the SiliCycle MiniBlock system is applied to peptide synthesis and screening by including an SPE step.

Sample Preparation Accessories

Summarized in Table II, these accessories include products centered on sample handling, solvent evaporation, and sample treatment.

Sample Handling

Related to sample handling, the ChemCob-One robotic pipetting system is coupled with a gravity-chromatography platform for trace enrichment of analytes. For liquid samples subject to SPE, Orochem presented positive pressure processing for sample handling with SPE. Technology for handling solid samples differs from that for liquids, especially when considering frozen biological samples. CyroXtract developed technology for the sample handling of frozen tissue aliquots while minimizing deleterious freeze–thaw cycles by using temperatures as low as -80 °C.

Solvent Evaporation

Although solvent evaporation is conceptually well known, performance of evaporation while avoiding loss of semivolatiles and accommodating high-throughput analysis is difficult. New systems by Glas-Col and Reeko Instruments address these concerns for environmental analysis (Reeko) or with 96-well plates (Glas-Col). Two products from Horizon Technology consider other important issues with solvent evaporation. One product, DriPure, is used to avoid solvent bumping during the evaporation process. It is also compatible with well-plates. Another Horizon product, LyoSpeed, is used with gummy or oil samples to facilitate dry powder formation during solvent evaporation.

Sample Treatment

Sample treatment approaches are currently addressing biological samples. Notably, deglycosylation reactions are of prime interest. Kits from Agilent

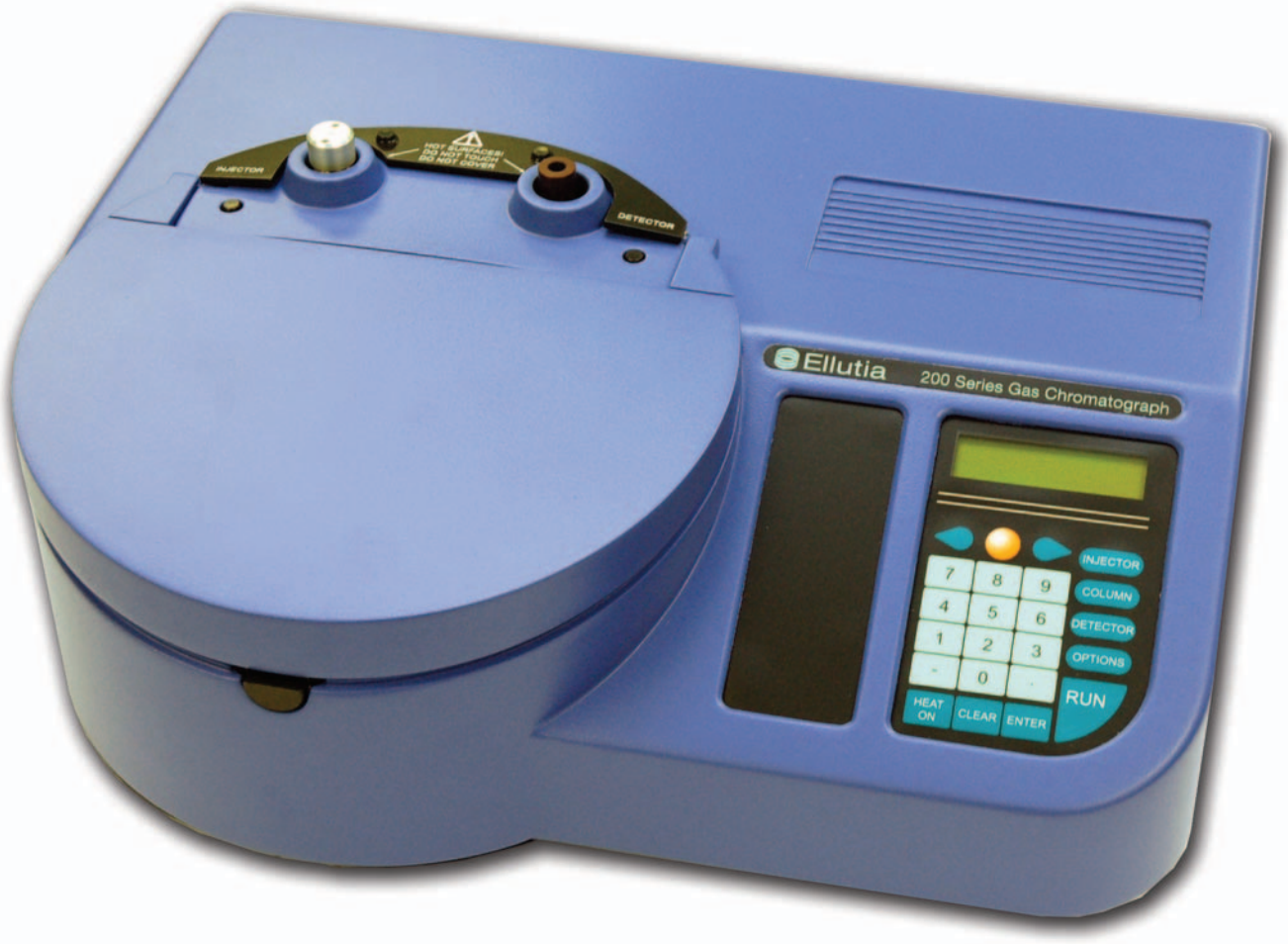

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Table I: Sample preparation instrumentation

Supplier	Product Name	Application	Main Use	
Applied Separations	Helix	SFE	Extraction of organic samples, such as astaxanthin, cryptoxanthin, lutein, and zeaxanthin	
CDS Analytical	CDS-7500S	Thermal desorption autosampler	Sample treatment by thermal desorption before GC	
DeltaChrom	SCS-200	Sample cleanup	GPC	
Elma Ultrasonics	Elmasonic P	Ultrasonic extraction	Liquid–solid extraction and other laboratory processes requiring use of ultrasound	
Fluid Management Systems	EconoPrep	SPE-based sample cleanup	Dioxin and PCB analysis	
Gerstel	MPS Robotic Probe Autosampler	Rail-type autosampler for GC	Automated GC injection	
	Dual-Head Single-Rail Workstation	GC autosampler workstation	GC injection	
LabTech, Inc.	Gemini High Pressure Solvent Extractor	Multichannel solvent extractor	Extraction of PCBs, pesticides, dioxins and furans, heavy metals, petroleum hydrocarbons, PAHs, antibiotics, drug residues, arsenic, bromide compounds, natural products, pharmaceutical, and packaging materials	
	Sepaths UP AutoSPE	Automated SPE	Environmental, clinical, biological, and food samples	
LCTech	Freestyle Xana	Preparation of 1–10 L water samples	Automated sample preparation for water analysis	
OI Analytical	Eclipse 4760	Purge-and-trap concentrator	VOC analysis	
Polymer Char	External Filtration System (EFS)	Filtration	Sample filtration before chromatographic injection	
PromoChrom	RT-01	Automated SPE	Purification of natural products and other targeted components	
Reeko Instrument USA	AutoSPE-06 Plus	Automated SPE	Extraction and concentration of trace organic compounds in aqueous samples	
SiliCycle	SiliCycle MiniBlock	Peptide synthesis	Allows solid-phase or solution-phase synthesis and purification to be carried out on the same equipment	
Teledyne Tekmar	Lumin	Purge-and-trap concentrator	Stripping VOCs with delivery to a sorbent trap for EPA, ASTM, and related methods	
Velp Scientifica	SER Automatic Solvent Extractor	Automated liquid–solid extraction	Randall extraction and related AOAC, EPA, and other regulatory methods	
Waters	SFE Bio-Botanical Extraction System	SFE	Large-scale fractionation of natural products	

	Important Features	Comments
	Extension of SFE to pressures of 1000 bar	Capable of extraction with water or carbon dioxide. Increasing supercritical carbon dioxide extraction pressures up to 1000 bar may upgrade existing industrial processes operating at suboptimal conditions as well as lead to the discovery of effective supercritical conditions for new products.
	Preheat function offers better handling of wet samples and chromatographic injection	Stand-alone version of a thermal desorption autosampler. Does not require a separate sample concentrator.
	Removal of lipids from samples during environmental analysis	Styrene-DVB copolymer optimized for lipid removal and switching valve for sample cleanup before chromatography.
	Two available frequencies (37 or 80 kHz) in a single unit	Bath-type ultrasound system with higher frequencies and temperature-controlled auto-start.
	Supports several EPA methods	Processes eight samples simultaneously using ABN silica, alumina, carbon, or high-capacity acid silica columns. Equipment rental program for clinical laboratories.
	Liquid, headspace, and SPME injection standard. Other optional sampling modes.	May be combined with thermal desorption, automatic injection liner exchange, and desorption from stir-bar sorptive extraction. Available Universal Syringe Module offers rapid replacement of syringes with 1–1000 µL capacity.
	One rail is an automated liquid sample handler, while the other rail accommodates multiple GC injection techniques	May be configured to perform a multitude of tasks including dilution, derivatization, standard addition, solid-phase extraction, and disposable pipette tip cleanup and extraction, weighing, filtration, centrifugation, or solvent evaporation.
	Dual channels and supports eight different solvents. Combines high performance solvent extraction, on-line concentration, and solid-phase extraction.	Allows the quantitative separation of a substance or a group of substances from a mixture of solids or semisolids at high temperature and high pressure. The extraction is performed in two phases with a final recovery of the used solvent, allowing a reduction of atmospheric pollution, of the extraction time, and of the costs of analysis. The top model integrates on-line sample concentration and solid-phase extraction.
	Supports both disk-based and cartridge-based SPE	Accommodates 1-, 3-, 6-, and 30-mL SPE cartridges or 47- and 90-mm SPE disks in up to six channels. Access to as many as eight solvents with 1–80 mL/min flow.
	Parallel processing procedures of three simultaneous samples	Well suited for use in both smaller and higher-throughput laboratories, with treatment of up to 65 samples in 24 h unsupervised. Positive pressure of up to 4 bar.
	Direct resistance heating of trap at over 1000 °C/min	Fourth-generation system with faster cycle times, higher throughput, and improved reliability. Optional foam sensor and pH monitor.
	Fully automated, no solvent handling or manual vial transfer	Automated, fast, and innovative apparatus for eliminating carbon black, catalysts, or other small particles present in, for example, polyolefin samples. No cross contamination. Avoids the damage of light scattering detectors and extends the life of GPC columns. The process takes around 2 min per vial. Once the sample is filtered, it is ready to be injected into GPC.
	Processes eight samples in parallel mode	Sample components can be separated into a well plate. Sample volumes of 0.5–4000 mL. On-line blending of two solutions for gradient elution. Uses SPE cartridges and flash columns.
	Combines nitrogen purge and vacuum pumping for improved drying and time savings	Fully automatic, improved drying, and anhydrous sodium sulfate column to remove moisture on-line.
	Complete platform for 6–48 parallel reactions (40–4 mL) with its own orbital shaker and heating (up to 120 °C) or cooling (down to -20 °C)	Allows parallel syntheses, screening reaction conditions, reaction optimization, scavenging studies, and removal of excess reagents, side-products, and catalysts by SPE. Usual reactions include acylation, alkylation, biaryl coupling, Diels-Alder, enolate formation, Grignard reaction, Heck reaction, heterocycle formation, metallation, nucleophilic aromatic substitution, reduction, reductive amination, saponification, Sonogashira reaction, Stille reaction, sulfonylation, and Suzuki coupling.
	Electronic mass flow controller reproducibly delivers extraction gas to sample at rates of 5–500 mL/min	Moisture control system reduces moisture by 60% compared with previous systems. Interfaces to nearly all commercial GC systems with 1 ppm maximum sample concentration.
	Processes three or six samples simultaneously	Automated Soxhlet where sample is immersed in boiling solvent followed by conventional Soxhlet, resulting in significant savings of time and solvent. Recovery of over 90% of solvent used.
	Up to 5-L extraction vessels and 200-g/min flow	Featured three cascaded cyclone separators for isolation of extracted matter with computer control of automated back-pressure regulators.

Table II: Sample preparation accessories

Supplier	Product Name	Application Area	Product Type
Agilent	AdvanceBio Glycan Sample Prep Kits	Deglycosylation of glycans	Kit for deglycosylation, SPE, and labeling
CleanChemLab	ChemCob-One	Trace-element separation	Automated pipetting and gravity chromatography
CryoXtract Instruments	CXT 353	Transfer of frozen sample aliquots	Sample handling
Genevac	DriPure	Solvent removal	Solvent evaporator
	LyoSpeed	Powder formation	Lyophilizer
Glas-Col	ZipVap4	High-throughput analysis using 96-well plates	Solvent evaporator
Horizon Technology	DryDisk-R	Drying organic extracts	Membrane drying accessory
MicroSolv Technology	U-2D	96-well plates for HPLC and LC–MS	96-well plates
Optimize Technologies	EXP2 Nano Trap System	General sample cleanup, sample concentration, and removal of detergents or salts at UHPLC pressures	Online sorbent traps
Orochem	Ezypress HT 96-C	Positive pressure processor	SPE
Phenomenex	Novum Simplified Liquid Extraction (SLE) Tubes	Assisted liquid–liquid extraction	Supported-liquid extraction
Reeko Instrument USA	AutoEPA	Food analysis, water treatment, environmental samples, agrochemicals, pharmaceuticals, forensic samples	Solvent evaporator
Restek	Resprep PPT^3 96-well plate	Protein precipitation	Sample cleanup
Shimadzu	Noviplex Duo Plasma Prep Card	Plasma preparation via removal of red blood cells	Blood spot analysis
Waters	GlycoWorks RapiFluor-MS N-Glycan 24-Sample Kit	Deglycosylation of glycans	Kit for deglycosylation, SPE, and labeling

and Waters are used to deglycosylate glycans, such as glycoproteins, using a 96-well plate format, sample cleanup by SPE, and derivatization for LC–MS or fluorescence. Removal of proteins via precipitation, in a 96-well plate, is facilitated by a new product from Restek. Meanwhile, the Shimadzu Noviplex product removes red blood cells for plasma analysis by LC–MS-MS. Unique sample cleanup products for ultra-high-pressure liquid chromatography (UHPLC) were also introduced such as the EXP2 Nano Trap on-line sorbent trap by Optimize Technology and processing 96-well plates by MicroSolv. Finally, Phenomenex has been a leader in the development of supported liquid extraction (SLE) and this continues with the introduction of a new sorbent in the Novum product line that targets clinical and food safety analysis.

Sorbent Products

New sorbent technologies are shown in Table III and address dispersive SPE, SPE cartridges and disks, and 96-well plates. These appear to be driven by interest in bioanalysis and food safety laboratories.

Dispersive SPE

Bulk sorbents for use in QuEChERS and MSPD have seen renewed inter-

	Suggested Application	Comments
	Bioanalytical	Deglycosylation of glycans (24 or 96 samples), followed by clean up and labeling with 2-AB solution. Deglycosylation clean up by SPE cartridges (24 or 96 samples), followed by 2-AB labeling with 2-AB solution and reductant solution (24 or 96 samples) and 2-AB labeling work up with SPE cartridges (24 or 96 samples). Before the chromatographic analysis, the N-linked glycans are cleaved and isolated from the reaction mix, then derivatized with 2-AB label before final cleanup and formatting for LC.
	Ion-exchange and low-resolution chromatography	System couples robotic pipetting with dedicated chromatographic platform to process up to 12 samples simultaneously.
	Allows transfer of frozen aliquots for a wide range of sample types including tissue, feces, plasma, whole blood, urine, and other biofluids, enabling the automated extraction of multiple frozen cores from a single frozen sample while maintaining the parent and extracted samples at temperatures below -80 °C	The ability to extract frozen aliquots while preserving the parent sample prevents degradation because of thawing by eliminating freeze–thaw cycling. An increased range of aliquot volumes (10–250 µL) and single-use coring probes from 1.5 mm enable smaller core volumes for precise, targeted tissue acquisition. Faster cycle times and new actuated ejection enhances cold chain sample processing and increases precision in core deposition. Stabilizes labile small molecule compounds, peptides and proteins for bioanalysis and preserves sample quality for additional testing or reanalysis.
	Natural products	Relieves bumping during solvent removal. Compatible with microtiter plates.
	Fast lyophilization	Used with solvent evaporator to form dry powder with samples, like essential oils, which typically produce gums and oils.
	High-throughput analysis	Expandable to 384 wells and features microprocessor control.
	Replacement for sodium sulfate in routine laboratory work	Membrane separation, rather than sodium sulfate, does not adsorb analytes or contaminate extract. Unlimited capacity for water.
	High-throughput analysis	Even thermal distribution for better analysis and reactions in the plate. Glass inserts can be easily removed from racks and placed in vials with caps for storage or transportation.
	On-line sample preconcentration, detergent removal, desalting, as well as protein, peptide, and small-molecule concentration	Provides low-volume hardware and connections to minimize extracolumn effects and sample dispersion. Multiple bed volumes and bonded phases available that allow customizable formats to achieve separation, cleanup, and concentration.
	Environmental, clinical, or bioanalytical sample preparation, or proteomics desalting or affinity purification	Dual flow regulators allows two different pressures for extraction and column drying. Processes SPE columns of 1-, 3-, and 6-mL capacity in batches of 1–96 samples.
	Clinical research, forensic toxicology, pharmaceutical testing, and food safety testing	Unique, synthetic SLE sorbent provides reliable, more consistent results compared with traditional diatomaceous earth sorbent. Available in 1-, 3-, 6-, and 12-mL tubes.
	Sample concentration of up to 60 samples	High-throughput, low-gas-consumption sample concentrator.
	Plasma, serum analysis in clinical or forensic labs with high throughput	Optimized dual layer nondrip filter membrane for faster filter speed with greater than 99% protein removal from plasma and serum samples. Versatility in filtration method including vacuum, positive pressure, and centrifugation.
	Blood plasma preparation for LC–MS-MS	Collects about 8 µL of plasma, providing the ability to perform two different types of LC–MS assays with a single application of blood.
	Bioanalytical	Available in either 24- or 96-sample formats, allows laboratories to rapidly go from native glycoprotein to ready-to-analyze sample. Kit includes enhanced fluorescent and MS performance reagent. Confirming glycan assignment via mass data provides information previously unavailable.

est since the advent of QuEChERS. Silica-based sorbents from MilliporeSigma, UCT, and SiliCycle remove chlorophyll from heavily pigmented botanicals (QuE Verde by MilliporeSigma) and extend the useful analyte range (UCT and SiliCycle). Another challenging issue is the removal of lipid matter from fatty samples for trace analysis. Agilent addresses this with a proprietary technology in its Bond Elut Enhanced Matrix Removal-Lipid sorbent.

SPE

Three SPE products are of special interest to bioanalytical laboratories. MilliporeSigma developed the Discovery Glycan SPE cartridge for glycan cleanup using a proprietary sorbent phase. The Waters Oasis-Prime sorbent utilizes the company's hydrophilic-lipophilic balance (HLB) sorbent technology and notably provides a product with superior water wettability. Hilicon AB reapplied the hydrophilic-interaction chromatography (HILIC)–LC approach for separation of hydrophilic samples to its iSPE product aimed at the purification of glycopeptides, glycans, and hydrophilic metabolites. Other new SPE sorbents demonstrate improvements to reversed-phase separations.

Table III: Sample preparation sorbent products

Supplier	Product Name	Product Type	Mode	Base Material	Functional Group	
Agilent	Bond Elut Enhanced Matrix Removal-Lipid	Dispersive SPE	Not supplied by vendor	Not supplied by vendor	Proprietary	
Hilicon AB	iSPE-HILIC	SPE	HILIC	50-µm, 60-Å high purity spherical silica	Hydroxyethyl amide, sulfate, quaternary ammonium	
MilliporeSigma	QuE Verde	Dispersive SPE	QuEChERS	Silica, carbon	Graphitized carbon black (GCB), Z-Sep+, and primary-secondary amine (PSA)	
	Discovery Glycan SPE	SPE	Reversed phase	Silica	Polyamide	
Orochem	Matrikleen	96-well plate	Mixed mode	Membrane and alumina acid, and silica-based ion exchange	Proprietary	
Phenomenex	Strata-X Microelution	96-well plate	Reversed phase or ion exchange	Polymer	N-Vinyl pyrrolidone (reversed phase); sulfonic acid or carboxylic acid (cation exchange); quaternary amine or amino (anion exchange)	
SiliCycle	Silia*Quick* QuEChERS	Dispersive SPE	QuEChERS	Silica	Primary-secondary amine, C18, and others	
	SiliaPrep C18 Plus	SPE	Reversed phase	Silica	C18	
	Silia*Prep* Metal Scavengers	Guard column	Metal scavenging	Silica	10 metal scavengers available: thiol, cysteine, DMT, TAAcOH, TAAcONa, thiourea, imidazole, triamine, AMPA, and DEAM	
UCT	Clean Screen FASt EtG	SPE	Filtration	Silica	Proprietary	
	QuEChERS Blend	Dispersive SPE	QuEChERS	Silica	Proprietary	
	Enviro-Clean HL DVB	SPE	Reversed phase with polar enhancement	Polymeric divinylbenzene	Proprietary	
	Scaled Down Enviro-Clean Method 8270/625	SPE	Dual adsorption cartridge cleanup using proprietary cartridges in-line with activated carbon cartridges	Polymeric divinylbenzene and activated carbon	Proprietary	
	Abalonase and Abalonase+	Hydrolytic enzyme	Purified β-glucuronidase formula for enzyme hydrolysis	Not applicable	Not applicable	
Waters	Oasis Prime HLB	SPE	Reversed phase	Polymeric	Hydrophilic-lipophilic balance	
Wheaton	AntiBind Microplates	96-well plates	Protein cleanup	Polypropylene	Proprietary hydrophilic phase	

Well Plates

Three new 96-well plate products, from Orochem, Phenomenex, and Wheaton, in addition to those previously mentioned, use reversed-phase or ion-exchange mechanisms individually or in a mixed-mode format for high-throughput biochemistry laboratories.

Specialty Sorbents

Metal scavenging for the removal of particulates and complexes of heavy metals is developed into a high performance liquid chromatography (HPLC) guard column, as well as SPE cartridges, well plates, and pipette tips from SiliCycle. The Abalonase products from UCT present a purified β-glucuronidase formula for enzymatic hydrolysis, especially for drug metabolites.

Conclusion and Future Directions

We have noted that new sample preparation products have been heavily driven by market needs, and that trend is likely to continue in the foreseeable future. The vast array of new products introduced in

	Dimensions	Comments
	Not supplied by vendor	Unique sorbent selectively removes lipids in complex matrices and challenging high-fat samples. Universally applied to the analysis of polar, mid-polar, and nonpolar target analytes, providing effective matrix removal.
	1, 3, 6 mL with 25–1000 mg HILIC material	Purification of glycopeptides, glycans, hydrophilic metabolites, PSP toxins, and neurotransmitters using tailor-made bonded HILIC products for sample preparation of hydrophilic compounds.
	2- and 15-mL centrifuge tubes	Analysis of planar analytes in green food matrices. Designed to provide high recovery for all pesticides, including problematic planar pesticides, in samples containing high levels of chlorophyll.
	50 mg/1 mL, 250 mg/3 mL, 250 mg/6 mL, 500 mg/6 mL, 1 g/12 mL, 2 g/20 mL, 5 g/60 mL, 50 g bulk	For cleanup of glycan samples after reductive amination labeling or enzymatic digestion. The cartridge is equilibrated with acetonitrile, then loaded with a maximum of 20 µg of a glycan sample. The glycans are bound while excess dye and salts are removed by washing with acetonitrile.
	3-mL cartridge or 2-mL/well 96-well plate	Pharmaceutical plasma–serum–blood samples, and clinical diagnostic samples. Universal extraction tool for polar and nonpolar chemicals, both acidic and basic. Eliminates matrix effect from phospholipids and proteins; high recovery for most drug compounds.
	96-well plate	The microelution format allows elution with volumes as low as 25 µL. The low elution volume results in an ultraconcentrated sample without performing a drying step, maximizing recovery of target analytes which may stick to the walls during dry down (such as peptides) and protect thermally labile analytes.
	50-mL, 15-mL, and 2-mL polypropylene centrifuge tubes	High-purity sorbent provides high recoveries of a large array of pesticides, drugs of abuse, veterinary drugs, antibiotics, hormones, and more.
	Cartridges: 1 mL/30 mg to 25 mL/5 g; Well plates: 2 mL/50 mg and 2 mL/100 mg; Tips: 10 µL/30 µg to 1000 µL/50 mg	Irregular silica, 40–63 µm, 60 Å, 500 m^2/g, 17% C, pH stability: 3.0–8.0, proprietary endcapping. The homogeneous coverage of the silane on the surface results in a strongly hydrophobic and nonpolar sorbent. Uses include isolation of acidic, neutral, basic compounds, or VOCs from aqueous solutions and drugs and metabolites from physiological fluids.
	Cartridges: 1 mL/30 mg to 25 mL/5 g; Well plates: 2 mL/50 mg and 2 mL/100 mg; Tips: 10 µL/30 µg to 1000 µL/50 mg	Irregular silica, 40–63 µm, 60 Å, endcapped (except TAAcOH and TAAcONa). Particulate matter will be filtered and heavy hydrophobic compounds will stick to the sorbent. Lowers the residual metal concentration of various metal complexes (Pd, Pt, Rh, Ru, Ni, Sn) to parts-per-million levels, with a simple SPE step before HPLC.
	200 mg/3 mL cartridge; 100 mg/ well	Solution to combat the significant ion suppression EtG/EtS urine analysis typically suffers using a standard dilute and shoot approach.
	Mylar pouch/50 mL centrifuge tube containing QuEChERS salts for THC potency and pesticide testing; 2-mL dispersive tube containing QuEChERS sorbents for pesticide testing in edibles	Optimized QuEChERS salt–sorbent mixtures developed for detection of cannabinoids and pesticides in marijuana and cannabis-infused products.
	500 mg/6 mL cartridge	Highly cross-linked divinylbenzene-based sorbent featuring enhanced hydrophobic retention and capacity. Universal sorbent for acidic, neutral, and basic compounds. Used to extract compounds with diverse physiochemical properties.
	500 mg EC8270 sorbent/6 mL cartridge; 1000 mg activated carbon/6 mL cartridge	Cartridge retains the majority of the target analytes including acids, bases, and neutrals. Meanwhile the carbon cartridge, connected downstream from the main cartridge, captures several very polar compounds.
	10, 25, 50, and 100 mL	Maximized enzyme performance so that half the activity units provide the same conversion rate as a traditional abalone-derived enzyme. Rapid hydrolysis buffer included. Deconjugation of major metabolites of interest including benzodiazepines, opioids, natural and synthetic cannabinoids, and steroids.
	Microelution plate, 96-well plate, 30–200 mg cartridges	Copolymeric sorbent is water-wettable, so it does not require conditioning, saving time and solvent. Two to six times faster than comparable materials with cleaner sample extracts.
	0.5-mL 96 deepwell plate or 120-µL 384 plate	For assays conducted with low-abundant proteins, the hydrophilic surface of the polypropylene microplates reduces surface binding, improving protein recovery. Hydrophilic treatment will not leach.

just the past year requires responsible laboratory managers and scientists to continually stay on top of developing trends.

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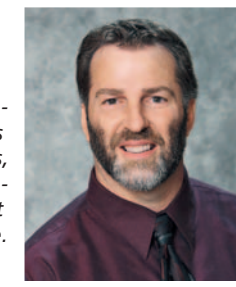
Douglas E. Raynie

"Sample Prep Perspectives" editor Douglas E. Raynie is an Associate Research Professor at South Dakota State University. His research interests include green chemistry, alternative solvents, sample preparation, high resolution chromatography, and bioprocessing in supercritical fluids. He earned his PhD in 1990 at Brigham Young University under the direction of Milton L. Lee.



LC TROUBLESHOOTING

How Does It Work? Part I: Pumps

Understanding how liquid chromatography pumps operate can help streamline solving pump problems.

John W. Dolan
LC Troubleshooting Editor

It is possible to obtain very satisfactory quantitative results from a liquid chromatography (LC) analysis by following an established method, preparing the samples, placing them on the LC system, and after they have been analyzed, processing the collected data. However, when things don't go as expected, I believe you'll be most successful at troubleshooting if you have two knowledge sets in your mental toolbox: knowledge of how the various instrument components work and knowledge of the basic principles of the chromatographic process. Unfortunately, when problems occur, time constraints often do not allow us to take the time to obtain this knowledge. Thus, it is best to make a habit of gradually gaining this knowledge and reviewing it periodically, so it will serve you well when you need it. For this reason, I'm embarking on a series of discussions of how the components of an LC system work. Most of this description will be generic in nature, but it should be obvious how it applies to particular brands and models of equipment in your laboratory, whether these have been in use for 20 years or they are the latest ultrahigh-pressure LC (UHPLC) systems.

This month's "LC Troubleshooting" discussion focuses on the pump that drives the mobile phase through the system. We also consider a little history on pump development and describe the two most common pump designs in use today.

Basic Design

All LC pumps in use today are based on the reciprocating piston design shown in Figure 1. The basic elements of the pump are a cylindrical pump chamber that holds the piston, a motor that operates a driving cam, a pump seal, and a pair of check valves. As the motor rotates, the piston is moved in and out of the pump chamber. In most pump designs, the pistons are made of sapphire, but stainless steel and graphite are sometimes used. The check valves serve to control the direction of flow of mobile phase through the pump (flow is from bottom to top in all the figures). In their simplest form, the valves comprise a ruby ball and a sapphire seat that is slightly ground ("lapped") to ensure a leak-free seal when the ball rests on the seat. The valves open and close in response to pressure and gravity. The pump seal keeps mobile phase from leaking out around the piston when the pump is under pressure and keeps air from leaking in when the pump is filling.

On the inlet stroke of the pump (Figure 1a), the piston moves out of the pump chamber. This creates a low-pressure region in the chamber, which allows the outlet check valve to settle onto its seat, and a slight siphon pressure from the mobile phase reservoir plus the low pressure inside the pump chamber cause the inlet check valve to rise off of its seat and allow mobile phase to enter the pump chamber.

The piston reverses direction during the pressure, or delivery stroke of the pump (Figure 1b). The increased pressure inside the pump chamber causes the inlet check valve to close, and when the pressure inside the pump exceeds the pressure downstream, the outlet check valve is forced open and mobile phase flows toward the column.

The pump seal comprises a polymeric ring encircling the piston. The cross-section of the seal in Figure 1c shows a groove that contains a spring on the high-pressure side of the seal. The spring helps to pull the lip of the seal against the piston to make better contact and is aided by the high liquid pressure in the pump chamber that also pushes the lip of the seal against the surface of the piston. Thus, the seal acts much like a squeegee to keep the mobile phase within the pump chamber. However, the sealing process isn't perfect, but allows a thin film of mobile phase to remain on the piston surface

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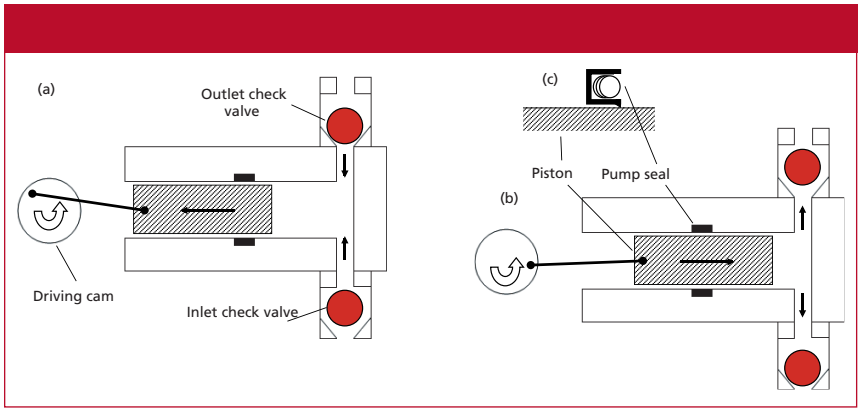

Figure 1: The single-piston pump: (a) suction or fill cycle; (b) pressure or delivery cycle; (c) detail of pump seal. See text for details.

and thus lubricate the piston-seal interface so the seal does not wear out prematurely.

A pump that was not much more sophisticated than that of Figure 1 was a common component of early LC systems. The first LC system I used in 1972 was a lab-built system that contained a Milton-Roy Mini-Pump that was distributed by Laboratory Data Control. The flow rate was controlled by adjusting the stroke of the piston using a large micrometer mounted on the pump.

One huge problem with the simple single-piston reciprocating pump is that it spends half its time filling and half the time delivering mobile phase. This means that it will have large pulses in flow and pressure—both defects that are not desirable for the constant flow and even pressure required for satisfactory detector performance. Early systems that relied on the MiniPump often incorporated pulse dampers in the form of a gas ballast, Bourdon tube, or mechanical pressure gauge. Later innovations included elaborately shaped driving cams and variable-speed motors that reduced the fill portion of the pump cycle and smoothed the delivery. Although the designs were clever and were great improvements over the original pumps, they could not fully correct the flow and pressure problems inherent in single-piston pumps.

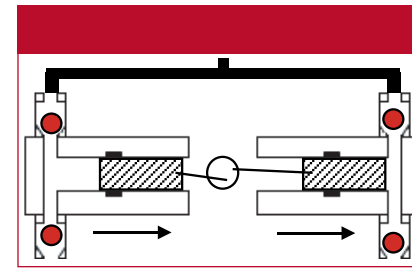

Figure 2: Dual-piston pump.

Enter the Dual-Piston Pump

There is a very simple way to overcome the flow and delivery pulses of the single-piston pump—just use two pumps operating 180° out of phase. A conceptual diagram of this setup is shown in Figure 2. In this case, two pumps are driven from a single cam. While one pump head fills (left in Figure 2), the other delivers (right). The combined output of the pumps (top of Figure 2) should be constant: when one pump head fills, the other is delivering. With a bit of fine-tuning of the design, this type of LC pump is very effective. For example, the pistons usually are mounted parallel to each other, much like an automobile engine, and are driven off of a single camshaft. The dual-piston pump is at the center of many of today's LC and UHPLC systems.

An Alternate Design

Another way to use two pistons in a single pump is to operate them in tandem instead of parallel. This usually is referred to as the accumulator-piston design and is shown in Figure 3. Here, the two pistons deliver at different rates. For example, let's say that we want a flow rate of 1 mL/min; the top piston will operate at 1 mL/min and the bottom one at 2 mL/min. There are three check valves, an outlet (top) and an inlet (bottom) plus a middle check valve that acts as in inlet check valve for the top piston, but an outlet check valve for the bottom piston. In the cycle shown in Figure 3, the top piston delivers 1 mL/min to the column, with the outlet check valve open and the middle check valve closed, just as if it were a single-piston pump.

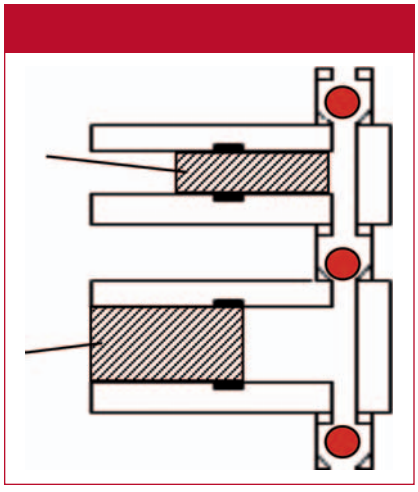

Figure 3: Accumulator-piston pump.

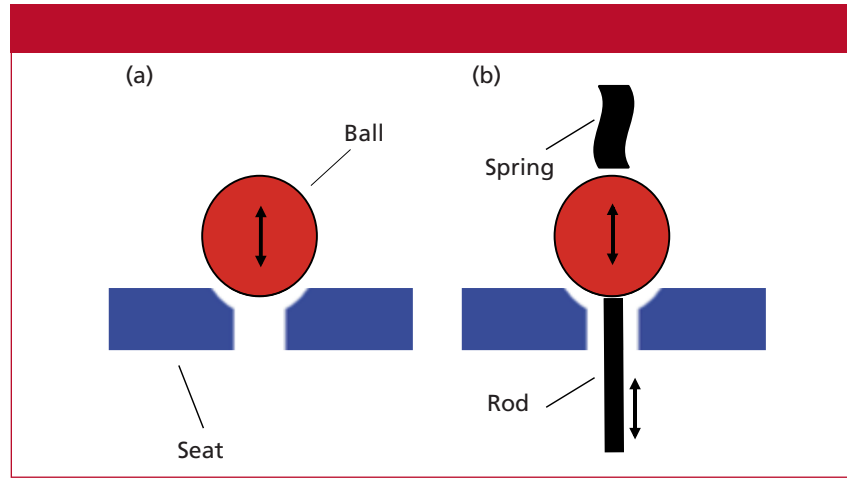

Figure 4: Check-valve designs: (a) traditional ball and seat; (b) active inlet check valve. See text for details.

Meanwhile, the bottom piston fills at 2 mL/min with an inlet check valve open and the middle check valve closed. On the alternate cycle (not shown), the bottom piston delivers at 2 mL/min; 1 mL/min serves to fill the top piston and the other 1 mL/min flows to the column. Thus, 1 mL/min of mobile phase always flows to the column. The accumulator-piston pump is also a very popular pump in modern LC and UHPLC systems.

More on Check Valves

The check valves are critical to the operation of the pump, yet they tend to be the least reliable parts. Let's take a closer look at the check valves next. The most common check valve design comprises a ruby ball and sapphire seat, as illustrated in Figure 4a. The seat is slightly ground or "lapped" to match the curvature of the ball for better sealing (this is exaggerated in the figure for illustrative purposes). When the pressure below the check valve is higher than above it, the ball is lifted from the seat and solvent flows through the hole in the seat. When the pressure is equal on both sides of the valve or higher on the top, the ball settles onto the seat and provides a seal. As long as the surfaces of both components are clean, this combination can provide effective sealing,

even at UHPLC pressures of >1000 bar (>14,500 psi).

Even with reliable sealing, it is desirable to minimize the number of check valves in a pump. It can be seen in Figure 3 that there are only three check valves in the accumulator-piston pump as compared to four in the dual-piston design (Figure 2). Fewer check valves should mean better reliability, at least in theory, and this is certainly argued by the manufacturers of accumulator-piston pumps.

Another problem that can arise with check valves is related to acetonitrile as a solvent. "Pure" HPLC-grade acetonitrile has minor contaminants that can polymerize on the sapphire surface of the valve seat, causing the ball to stick to the seat because of surface tension effects. One way to overcome this problem is to occasionally sonicate the inlet check valves in methanol. Another approach is to use another check-valve material, such as ceramic, but this approach does not seem to give as leak-free a seal as the ruby-sapphire combination. (Read more about the acetonitrile problem in reference 1.)

Sticking usually is a problem only with the inlet check valves, because the pressure within the pump is sufficient to force open the outlet check valve. Many pumps today contain "active" check valves on the pump inlet. The principle of this design is shown in Figure 4b, where mechanical assistance is added to the normal ball-type valve. A spring above the ball helps ensure that it is seated properly when the valve is closed. Below the ball, a solenoid-actuated lifting device is added. In the illustration, a rod is pushed against the bottom of the ball, forcing it off the seat. This rod moves up and down under solenoid control to open the valve or allow it to close with the assistance of the spring. The active check valve is not subject to sticking when using acetonitrile, so it is more reliable. The inlet check valves of many of today's LC and UHPLC pumps use active check valves, whether the dual-piston or accumulator-piston design is used.

One additional observation about the accumulator-piston pump (Figure 3) is that the outlet (top) check valve should always be open. This is because either the top or bottom piston is always supplying flow to the column. If this is the case, there is no need for an outlet check valve, and often it is not included on this pump design. So with no outlet check valve and an active inlet check valve, the accumulator-piston pump often has only one traditional ball-type check valve between the two piston chambers. The comparable dual-piston pump would have two active inlet check valves and two traditional outlet check valves. Although it would seem from this description that the accumulator-piston design is superior, there are other nuances of pump design that are important too. In my experience, the two designs give very similar performance. If you want more information on why one design is superior to the other, just talk to the appropriate manufacturer . . . but you may gather more information than you know how to use.

Maintenance

Before we close this discussion, let's touch on a few maintenance-related topics. As with any other part of the LC system, the pump requires periodic maintenance for reliable operation. It is a good idea to perform annual preventive maintenance on all LC pumps, even if they are not used very heavily. For laboratories that use their systems continuously, semiannual or

even more frequent maintenance may be desirable.

Cleanliness is the primary key to reliable pump operation. For this reason, you should be sure that the pump is never stored in solvents that will promote microbial growth or corrode the system. Usually this practice means removing any aqueous solutions or buffers from the system when it is not in use. Buffers can evaporate and leave deposits in the system, and buffer solutions can be ideal growth media for bacteria. Biological contaminants or insoluble materials can coat the check-valve balls or seats, preventing proper sealing. Physical blockage of the column or contamination of the column packing can occur under these conditions, as well. A simple way to avoid most of these problems is to flush salt- and buffer-containing solutions from the LC system when it is not in use. Rinse the system with high performance liquid chromatography (HPLC)-grade water, then store it in the organic solvent used in the mobile phase (usually methanol or acetonitrile).

Pump seals will often last a year or longer if the pump is rinsed regularly. I like to replace the seals annually at a minimum. Be sure to replace the seals with the correct part number item; some vendors sell seals of different composition for different applications and sometimes the wrong seal can create additional problems. If you are using UHPLC and the manufacturer recommends a seal-wash routine, use it. This procedure provides a small flow of solvent (often water) on the low-pressure side of the pump seal. This helps to dissolve contaminants from the piston surface, give additional lubrication, and, in the case of UHPLC, may cool the piston so that the seal does not melt due to the heat generated under the extreme pressure of UHPLC operation.

Check valves should not require special care if the pump is flushed regularly. If your pump has the traditional ball-type valves (Figure 4a) and you use 100% acetonitrile, you may have to institute a sonication routine if check-valve sticking becomes a problem. Check valves can be replaced, but with proper care, this is seldom required.

When setting up a preventive maintenance program, be sure to consult the operation and service manual for the pump. Some designs of pumps require periodic lubrication or other routine service.

Summary

The basic components of HPLC pumps have not changed much since the introduction of modern liquid chromatography in the 1960s. Many refinements in design and manufacture over the years have made pumps much more reliable than they were in the past. Good "chromatographic hygiene" will go a long way toward keeping the pumping portion of your LC system reliable and trouble-free.

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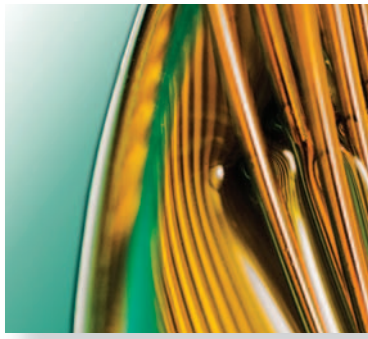
(1) J.W. Dolan, *LCGC North Am.* **26**(6), 532–538 (2008).

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*



GC CONNECTIONS

New Gas Chromatography Products for 2016

John Hinshaw presents his annual review of new developments in the field of gas chromatography (GC) seen at Pittcon and other venues in the past year.

John V. Hinshaw
GC Connections Editor

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) returned to Atlanta, Georgia, for its 67th annual meeting on March 6–10, 2016. This year's conference saw nearly 13,000 conferees and exhibitors in attendance. Interestingly, 37% of the participants categorized themselves as first-time attendees. Also of note were the 24% of attendees who hailed from foreign countries. The Pittcon exposition hosted 847 exhibitors from 37 countries in 1539 booths, including 119 first-time exhibitors. In addition, the Food Labs Conference was held in conjunction with Pittcon for the fourth year.

LCGC organized a half-day session devoted to the presentation of the 2016 *LCGC* Lifetime Achievement in Chromatography Award to Professor Milton L. Lee (Brigham Young University), and the *LCGC* Emerging Leader in Chromatography Award to Debby Mangelings (Vrije Universiteit Brussel). Detailed information on this year's awards appears in the February 2016 issue of *LCGC North America* (1).

Following its well-established rotation of host cities, next year's Pittcon will head north to Chicago's McCormick Place, March 5–9, 2017, where participants hopefully will enjoy a spate of warm spring weather. Certainly, we will get that experience in 2018 when the conference goes down south to Orlando, Florida for an early appearance from February 25 to March 1.

This annual "GC Connections" installment reviews gas chromatography (GC) instrumentation, columns, and accessories shown at this year's Pittcon or introduced during the previous year. For a review of new products in other areas of chromatography, columns, and related accessories, please see the April 2016 issue of *LCGC North America* (2,3), which is also available on-line at www.chromatographyonline.com

The information presented here is based on manufacturers' replies to questionnaires, as well as on additional information from manufacturers' press releases, websites, and product literature about the past year's products, and not upon actual use or experience of the author. Every effort has been made to collect accurate information, but because of the preliminary nature of some of the material *LCGC North America* cannot be responsible for errors or omissions. This column installment cannot be considered to be a complete record of all new GC products introduced this year at Pittcon or elsewhere because not all manufacturers chose to respond to the questionnaire, nor

Table I: Companies introducing new GC Products

Company Name
Agilent Technologies
Activated Research Company
ARM, Inc.
Hamilton Company
JEOL
Markes International
OI Analytical
Optimize Technologies, Inc.
Phemonenex
Photonis
Quadrex Corporation
Restek
Scientific Glass Technology Singapore Pte. Ltd.
Shimadzu
Supelco, part of MilliporeSigma
Teledyne Tekmar
Thermo Scientific
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Shimadzu's GCMS-QP2020 features:

- Smart SIM creation function – automatically creates a program that enables a staggered SIM of multiple components, resulting in higher SIM sensitivity
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- Advanced Scanning Speed Protocol – allows for the ability to scan up to 20,000 u/sec
- New turbomolecular pump achieves optimal performance with all carrier gases
- Front access to the ion source for easier, faster routine maintenance
- Simultaneous Scan/SIM for qualitative and quantitative data in a single run
- Specialized databases with additional retention indices support more accurate qualitative analysis, convenient quantitative method development, and screening analysis.

Table II: New GC instruments

Product	Company	Description
5977B GC–MSD with High Efficiency Source	Agilent Technologies	Agilent's High Efficiency Ion Source (HES) maximizes the number of ions that are created and transferred out of the source body and into the quadrupole analyzer. The ion source produces increased sensitivity of up to 10× compared to conventional quadrupole systems, with instrument detection limits (IDL) as low as 1.5 fg. According to Agilent, this reduces time spent on sample preparation and maintenance by requiring 1/10 the sample amount while storing, prepping, and disposing of up to 10× less material.
BenchTOF-Evolve	Markes International	Markes' BenchTOF-Evolve time-of-flight MS system for GC and GC×GC reportedly delivers "SIM-like" sensitivity with full spectral information and exceptional, "classical" quality. The system operates within existing software packages and is complemented by deconvolution software that gives analysts qualification of trace-level targets and confident identification of unknowns in a single run. The instrument is well-suited for challenging applications such as air monitoring and forensic analysis.
Eclipse 4760	OI Analytical	The Eclipse 4670 is the fourth generation of OI Analytical's Eclipse line of purge-and-trap systems. The sampler uses a patented water management system that minimizes water transfer to the GC column, with sparger heating during the bake period that reduces sample carryover. System status is viewable with OI's TruColour indicator.
GCMS-TQ8040 with Smart MRM	Shimadzu	Shimadzu Scientific Instruments launched the GCMS-TQ8040 with Smart MRM. The instrument features the company's Smart Productivity for analysis of 400+ compounds in a single MRM run, Smart Operation MRM Optimization Tool that automatically determines optimum transitions and collision energies for all compounds in a single sequence for rapid method development, and Smart Performance in the ion source and collision cell to provide low detection limits. The patented ion source's design and uniform temperature prevent active spots and boost sensitivity during analysis. Off-axis ion optics eliminate chemical noise.
Lumin purge-and-trap concentrator	Teledyne Tekmar	Lumin is Teledyne Tekmar's newest stand-alone purge-and-trap concentrator, incorporates an electronic mass flow controller (MFC) for either helium or nitrogen that delivers extraction gas to the sample,strips the volatile organic compounds (VOC), and delivers them to a sorbent trap. The trap is then heated and back-flushed to a GC system for separation and subsequent detection. The system automatically performs a cleanup step to prepare for the next sample analysis. The system also provides water management and a proprietary trapping material, automated leak checking, sample logging, foam detection and prevention options, and software control for built-in diagnostics and self-testing.
Q Exactive GC hybrid quadrupole-Orbitrap GC–MS-MS	Thermo Scientific	Thermo Scientific's Q Exactive GC hybrid quadrupole-Orbitrap GC–MS-MS instrument combines gas chromatography with high-resolution accurate-mass (HRAM) Orbitrap mass spectrometry. The system is designed to provide comprehensive characterization of samples in a single analysis for increased performance in compound discovery, identification, and quantification. The system brings Orbitrap MS from liquid chromatography (LC) applications into the GC realm. The system builds upon the company's modular TRACE 1300 Series GC system with user-exchangeable injectors and detectors. The Q Exactive GC system is suitable for untargeted profiling experiments and can add capabilities to screening in food safety, forensic toxicology, and anti-doping.
TSQ 8000 Evo triple-quadrupole GC–MS	Thermo Scientific	The Thermo Scientific TSQ 8000 Evo system improves on the features of its predecessor, the TSQ 8000, with EvoCell technology as demonstrated in company-run experiments using triple selected reaction monitoring (SRM) transition rates without compromising sensitivity. Timed-SRM software for optimizing selected reaction monitoring schedules is included. Company-run experiments also showed that the EvoCell can yield triple the sensitivity at the same scan speed, which allows users to screen and quantify more than 1000 compounds in a single run at low limits of detection. Thermo's AutoSRM software automates method development and management with enhanced selected reaction monitoring (SRM) experiments. The instrument's ExtractaBrite ion source is designed for high matrix tolerance to minimize sample preparation and cleaning. When the source does need maintenance, it can be removed without breaking vacuum.
VUV PIONA+	VUV Analytics	VUV Analytics released its VUV PIONA+ application, which is built upon the VUV Analyze software. The application provides detailed and bulk classification analysis of petroleum-based fuels. It demonstrates the potential for GC–VUV (vacuum ultraviolet detection) to significantly reduce complexity and run times compared to existing ASTM methods for fuel analysis, as well as the potential to combine information currently obtained using multiple methods. The VUV PIONA+ method results in a per-measurement information set that would typically require implementation of multiple ASTM methods such as D5769, D5580, D1319, D6550, D3606, D4815, D5599, or D584. The VUV PIONA+ method uses relatively simple instrumentation: a gas chromatograph, a standard 30-m nonpolar column, and the company's VGA-100 vacuum ultraviolet detector. Bulk concentrations of paraffin, isoparaffin, olefin, naphthene, and aromatic hydrocarbon classes are determined. Specific analytes can also be singled out for further characterization, for example individual oxygenates or aromatics belonging to the BTEX complex. The setup procedure requires no precolumn tuning or valve timing adjustments. Additionally, analyses are faster since the method can handle coelution among various species and hydrocarbon classes.

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Table III: New GC accessories

Product	Company	Description
8355 sulfur chemiluminescence detector and 8255 nitrogen chemiluminescence detector	Agilent Technologies	Agilent's 8355 sulfur chemiluminescence detector and 8255 nitrogen chemiluminescence detector are fully integrated with the Agilent 7890B gas chromatograph. They are also available as stand-alone units that can be connected to any gas chromatograph. The 8255 nitrogen chemiluminescence detection (NCD) system produces a linear and equimolar response to nitrogen compounds. It uses a stainless steel burner to achieve high-temperature combustion of nitrogen-containing compounds to form nitric oxide (NO). A photomultiplier tube detects the light produced by the subsequent chemiluminescent reaction of NO with ozone. Because of the specificity of the reaction, complex sample matrices can be analyzed with little or no interference. The detector has picogram-level detection limits, no hydrocarbon quenching, responds to ammonia, hydrazine, hydrogen cyanide, and NOX, and also has a nitrosamines-specific configured option. The sulfur chemiluminescence detection (SCD) system comprises a simplified burner design with 50% fewer components. The SCD system employs a dual plasma burner to achieve high temperature combustion of sulfur-containing compounds to form sulfur monoxide (SO). The chemiluminescent reaction of SO with ozone is detected with a photomulitplier. The SCD system is suitable for GC or supercritical-fluid chromatography (SFC). In addition to the main features of the NCD system, the SCD system is ASTM methods compatible, capable of tandem SCD and flame ionization detection (FID) operation, and has approximately 40% reduction in burner components. The latter feature results in the reduction of potential leak points, and permits inner ceramic tube replacement in a 10 min activity.
Column installation pre-swaging tool	Agilent Technologies	The Agilent pre-swaging tool makes swaging ferrules simple, easy, and ensures a proper length of column penetration into the fittings. The product is designed for the pre-swaging of graphite and UltiMetal Plus flexible metal ferrules.
Self Tightening column nut	Agilent Technologies	Agilent's Self Tightening column nut requires no modifications or special adapters to the instruments inlets or detectors. It requires no wrenches and eliminates the need to retighten the fitting after thermal cycling of the GC oven. The column nut allows analysts to use the same short ferrule on the inlet and detector, and it eliminates the need to have multiple ferrules on hand. The column nut is available for GC systems from Shimadzu, Thermo, PerkinElmer, and Varian/Bruker.
Ultra Inert Direct Connect liners	Agilent Technologies	Agilent's Ultra Inert liners are designed specifically for trace analysis with active analytes or sensitive compounds. The liners are delivered in the company's touchless packaging with a preinstalled, cleaned, conditioned, and nonstick plasma treated O-ring. Touchless packaging aids in removal of the old liner, and easy installation of the new, clean, preconditioned liner—without the risk of contamination from touching. The liners are compatible with Agilent's split–splitless and MMI inlets.
Ultra Inert Gold Plated inlet seals	Agilent Technologies	Agilent Ultra Inert Gold Plated inlet seals combine robust mechanical sealing with an inert surface. Agilent's Ultra Inert chemistry is applied on top of the gold plating for a leak-free seal that also reduces active analyte adsorption.
Point-of-Use Purifiers, Nova Series, and Pro-Panel Series	ARM, Inc.	ARM introduced its line of point-of-use purifiers, including the Nova Series and Pro-Panel Series. These ultrahigh-purity gas purifiers are used in applications where trace-level gas impurities or particulates can cause false results or reduce yields. This purifier family offers sub parts-per-billion (ppb, 10^{-9}) purity capability and are ideal for argon, nitrogen, oxygen, clean dry air, hydrogen, and numerous other gases. They are intended for use in analytical equipment with nearly any application requiring high-purity gas at operating pressures up to 250 psi and flow rates up to 300 slpm. The point-of-use purifiers are available as a vessel only, or in the Nova Series and Pro-Panel Series the purifiers house the purifier and valves in a wall mountable metal enclosure, with a heater jacket, thermocouple, factory set temperature controller, and status lights. Inlet–outlet connections are made via metal gasket face seal fittings or optional tube compression fittings. Electrical hookup is via an included cord and plug for connection to optional 120–240 VAC 50–60 Hz input power. Purity is ensured with use of electro-polished 316 L stainless steel for all wetted surfaces, high-purity full-penetration orbital welding, and factory-installed seal caps on the inlet–outlet connection. All ARM point-of-use and small area purifiers are offered with a 0.5-µm integral filter, or for more stringent requirements, an optional 0.003-µm integral filter. All purifiers are activated at the factory and are shipped ready for installation and operation out of the box.
GasTrap	Quadrex Corporation	These self-regenerating helium, hydrogen, nitrogen, and air purifiers can extend the life of disposable filters and enable the use of less expensive lower grade feed gas to achieve ultrahigh purity. The gas purifiers use mini pressure-swing adsorption (PSA) technology to purify hydrogen, helium, nitrogen, argon, and air, at flow rates up to 300 mL/min. They yield purities of to 99.999%+, or less than 2 ppm total impurities. Where applicable, the purifiers remove carbon dioxide, oxygen, moisture, and hydrocarbons. They are suitable for application to GC–MS with helium or hydrogen carrier gas, electron-capture detection (ECD), or for purifying nitrogen in LC–MS.
HDHT headspace syringe	Hamilton Company	Hamilton's headspace syringe is designed for use with CTC PAL Combi-xt Headspace autosamplers. It features a cement-free needle attachment that eliminates detached needles due to contact with organic and chlorinated solvents as well as minimizing ghost peaks. The syringe is temperature stable up to 200 °C so that a wider range of sample components that can be analyzed. A spring-in-plunger design creates a dynamic seal between the plunger tip and the inside of the glass barrel for leak-free operation. The syringe is intended for analysis of alcohols in blood and residual solvents in pharmaceutical products, volatile and semivolatile organics in solid, liquid, and gas samples, industrial analysis of monomers in polymers and plastic, flavor compounds in beverages and food products, as well as fragrances in perfumes and cosmetics.

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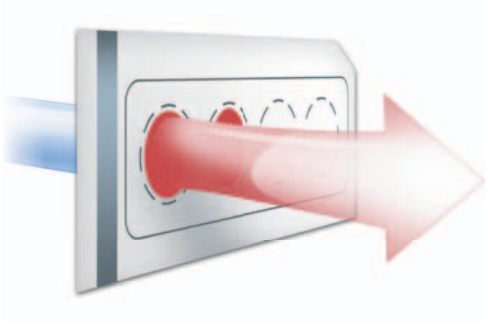

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Table III: New GC accessories (continued)

Product	Company	Description
Photoionization MS source	JEOL	JEOL introduced a combination electron ionization–photoionization (EI–PI) source for its fourth-generation AccuTOF-GCX high-resolution time-of-flight mass spectrometer. The EI–PI source complements the dedicated EI, positive–negative chemical ionization (CI), field desorption–field ionization (FD–FI), and combination EI–FI–FD ion sources and direct probes of the GCX system. Much like field ionization, photoionization is a soft ionization method that provides molecular weight information with minimal fragmentation. The PI lamp is installed on the EI ion source, which makes two ionization methods available without changing the hardware. Photoionization is very sensitive for certain environmentally important compound classes such as polycyclic aromatic hydrocarbons (PAHs). The PI source is compatible with both standard gas chromatography (GC) and comprehensive two-dimensional gas chromatography (GC×GC).
TC-20 TAG	Markes International	The TC-20 TAG tube conditioner is designed for use with thermal desorption sorbent sampling tubes. Tube conditioning is used by thermal desorption–GC analysts who want to avoid using instrument time for conditioning sorbent tubes. The tube conditioner simultaneously conditions or removes excess moisture trapped during sampling from up to 20 sorbent tubes with or without RFID tube tracking tags. The tube conditioner is especially useful for those working to the US EPA Method 325 regulation and is suitable for industry standard-size tubes (3.5 in. long × ¼ in. o.d.)
PAH tube	Markes International	The Markes PAH adsorption tube is the outcome of a detailed optimization process. According to Markes, existing solvent-based techniques for analysis of PAHs in air are labor-intensive and prone to loss of analytes. The PAH thermal desorption tube brings full automation to this application, as well as the high sensitivity inherent to thermal desorption. The company says that the PAH tubes ensure the transfer of even the heaviest PAHs from the sorbent sampling tube into the GC. Used with the company's UNITY or TD-100 instruments, the PAH tubes give negligible carryover and excellent reproducibility. Also, the tubes use significantly lower sampling volumes than do solvent-based techniques.
5383 pulsed flame photometric detector	OI Analytical	OI Analytical's second-generation 5383 pulsed flame photometric detection (PFPD) system is well suited to organophosphate (OP) pesticide detection, as well as petrochemical, environmental, and food and beverage applications. The PFPD system gives chemists the ability to specifically determine and selectively analyze low levels of sulfur, phosphorus, and 26 other analytes of interest. The detector features a modular design with separate electronics and flow modules, and it uses less gas and requires less maintenance than SCD systems or flame photometric detection systems.
Monolithic reflectron lens	Photonis	Reflectron lenses are used in time-of-flight (TOF) mass spectrometers to create an electrostatic field. Photonis' Monolithic reflectron lenses replace conventional multipiece stacked ring assembly lenses and eliminate an otherwise complex assembly and cleaning process. Monolithic reflectron lenses are made with resistive glass, which preferentially attracts ions for a larger sample size and better analysis.
Polyarc Reactor	Activated Research Company	The Polyarc catalytic microreactor converts all carbon-containing species to methane through a series of catalytic reactions before they are detected by an FID system. The Polyarc is a 3D-printed microreactor that is sulfur-compatible and enables quantification of compounds such as carbon monoxide, carbon dioxide, and formic acid that are otherwise nonresponsive in an FID system. It can be used with packed or capillary GC columns. According to the manufacturer, calibration of compounds that don't have commercial standards becomes possible through measurement of their carbon content.
Electronic Maintenance Indicator for gas traps and purifiers	Scientific Glass Technology Singapore Pte. Ltd.	SGT's Electronic Maintenance Indicator helps prevent breakthrough of collected contaminants in gas purifiers by providing advanced indication of a maintenance interval expiration. The device can be programmed for custom maintenance schedules or with a default interval of 12 months.
ECD-2010 Exceed	Shimadzu	Shimadzu Scientific Instruments announced the release of its ECD-2010 Exceed ECD system. The detector features a newly designed capillary ECD cell that uses the company's contact-free technology to reduce the effect that a dirty sample matrix can have on the detector's radioactive source. Contact-free technology is made possible by a unique flow design that uses a sweep gas to minimize contact of the sample with the detector source while at the same time facilitating the detection process. According to Shimadzu, the result is a longer lasting ECD system that will increase productivity by increasing uptime between maintenance and cleaning operations. The sensitivity of the detector has also been enhanced by this design, with a specification of 4 fg per s and a dynamic range of 1×10^5 for gamma-BHC.

is all of the submitted information necessarily included here because of the limited available space and the editors' judgment as to its suitability.

Gas Chromatography: 2015–2016

The number and type of new product introductions that were gathered for this review are an indication of the continuing viability of gas chromatography in the face of more recent major developments in small molecule analysis. GC is still the "go-to" standard for volatile and semivolatile compounds. Although new mini- or micro-GC systems were not in abundance in this year's crop, introductions in selective detectors, including mass spectrometry (MS) detectors and otherwise, method-specific autosamplers and accessories, and highly selective column stationary phases certainly were.

Thermo Scientific, for example, introduced two major advances in their MS detector product line: the Q Exactive GC hybrid quadrupole-Orbitrap GC–MS-MS system, and the TSQ 8000 Evo triple-quadrupole GC–MS system. Shimadzu also advanced their MS detector offerings with the GCMS-TQ8040 system with Smart multiple reaction monitoring (MRM). From Agilent Technologies, the 5977B GC–MSD system with the company's High Efficiency Source extends

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Table IV: New GC columns

Product	Company	Description
DB-WAX Ultra Inert	Agilent Technologies	Agilent's DB-WAX Ultra Inert GC columns have a Durabond polyethylene glycol stationary phase with the same selectivity as DB-WAX GC columns. They are compatible with organic acids without the need to run a separate method using an acid-treated FFAP-type column. The stationary phase is available in columns 10–60 m in length, 0.1–0.53 mm in inner diameter, and with film thicknesses of 0.1–1.0 µm, although not all combinations are possible.
Zebron ZB-5MSPLUS	Phenomenex	Designed with rigorous fused-silica deactivation and quality control processes, the Zebron ZB-5MSPLUS column substantially eliminates active sites on the column's surface that could negatively affect peak shapes for challenging compounds. Phenomenex states that gas chromatographers who are accustomed to using a 5% phenylarylene phase can switch to the inert column and achieve higher responses and lower detection limits, without redeveloping GC methods. Available in a wide range of length and diameter; the film thickness ranges from 0.10 to 3.00 µm, and operating temperature ranges are from -60 to 325–350 °C (isothermal and programmed temperature).
Rxi-1301Sil MS	Restek	Restek designed the Rxi-1301Sil MS column for the separations of solvents, glycols, and polar compounds with MS detection. The columns are highly inert for a broad range of compounds, including acids and bases. They are available in 15–60 m lengths, with 0.25-, 0.32-, or 0.53-mm inner diameters and film thicknesses of 0.25–3.0 µm. Their operating temperature range is 60–320 °C.
200-m versions of SP 2560 and SLB IL111	Supelco, part of MilliporeSigma	Supelco's 200-m columns are specifically designed and specialty tested for the highly detailed analysis of cis–trans fatty acid methyl ester (FAME) isomers. The SP 2560–SLB IL111 pairing allows comprehensive fatty acid composition analysis that is able to provide accurate results, both qualitative and quantitative, for both saturated and trans fatty acids. Supelco's observations include elution of analytes from SLB IL111 at a lower oven temperature; SLB IL111 provides resolution of C18:1Δ9c from all trans FAMEs; SP 2560 provides better resolution of saturated FAME isomers; SLB-IL111 provides increased retention of unsaturated FAME isomers; and highly detailed analysis of cis–trans FAME isomers. The SP 2560 column has dimensions of 200 m × 0.25 mm with a 0.20-µm film and a temperature range from subambient to 250 °C. The SLB IL111 column has the same dimensions and a 270 °C maximum temperature.
SLB ILD3606	Supelco, part of MilliporeSigma	Supelco's SLB ILD3606 column is designed for the determination of benzene in gasoline. It is able to resolve benzene, other aromatics, and oxygenates in reformulated gasoline using a one-column setup that is much simpler than the existing two-column method. The column is available in 30- or 60-m lengths with a 0.25-mm inner diameter and a 0.20-µm film thickness.
SLB IL (i-series): SLB IL60i, SLB IL76i, and SLB IL111i	Supelco, part of MilliporeSigma	According to Supelco, improved inertness for polar analytes was the inspiration for development of the SLB IL (i-series) ionic liquid capillary GC columns. They maintain inertness while offering a range of selectivity for polar analytes. The columns target high selectivity and high inertness toward polar analytes. A range of i-series columns was developed, classified as polar (SLB IL60i), highly polar (SLB IL76i), and extremely polar (SLB IL111i). The selectivity of SLB IL60i is more polar than PEG/wax phases, resulting in unique elution patterns. It has a higher maximum temperature of 280 °C, higher than most PEG/wax columns at 260–270 °C. It also is a good GC×GC column choice. Its temperature range is 35 °C to 280 °C , and it is available in combinations of 20–60 m lengths, 0.18–0,32 mm inner diameters, and 0.14–0.26 µm films. The SLB IL76i phase structure is engineered with numerous interaction mechanisms that result in selectivity differences even when compared to columns with similar GC polarity scale values. It also is another good GC×GC column choice. It is available in a single 30 m × 0.25 mm, 0.20-µm d_f size and has a maximum temperature of 270 °C. The selectivity of the SLB IL111i phase is most orthogonal to nonpolar and intermediate polar phases, resulting in very unique elution patterns. It is a great choice for separation of polarizable analytes, those that contain double or triple carbon–carbon bonds, from neutral analytes. It is available in 30- and 60-m lengths with a 0.25-mm inner diameter and a 0.20-µm film thickness; maximum temperature is 260 °C.
Watercol 1910	Supelco, part of MilliporeSigma	The Watercol 1910 column from Supelco targets the measurement of water. It can also be used to analyze small polar analytes in water, because the water peak does not tail and does not interfere chromatographically with other analytes. Watercol capillary GC columns contain ionic liquid stationary phases that produce a sharp peak shape for water, allowing the convenient measurement of water by GC. Narrow peak widths and optimal peak heights are also produced for many other small polar analytes. The sharp water peak shape produces both a linear response plus high sensitivity and reproducibility. This column has a temperature range of 30–180 °C (isothermal or programmed) and is available in 30-m lengths with 0.18–0.32 mm inner diameters and 0.14–0.26 µm film thicknesses.

the lower detection limit of the company's single-quadrupole detector. The BenchTOF-Evolve system from Markes is a new time-of-flight mass spectrometer for GC or GC×GC. All five MS offerings represent significant advances in MS capabilities for the gas chromatographer in a variety of applications.

VUV Analytics released its new VUV PIONA+ application, which is built on the company's vacuum-ultraviolet (UV) detector, and provides group-type selectivity for the targeted paraffin, isoparaffin, olefin, naphthene, and aromatic hydrocarbon classes. OI Analytical and Teledyne Tekmar both introduced new purge-and-trap concentrators that provide enhanced and advanced capabilities for this venerable application.

There were numerous new offerings in the GC detector realm. From Agilent,

two chemiluminescent detectors, the 8355 sulfur chemiluminescence detector and 8255 nitrogen chemiluminescence detector, offer enhanced sensitivity, compatibility with Agilent's latest laboratory GC systems, and simplified designs with fewer parts. OI Analytical came out with a second-generation pulsed flame photometric detector (PFPD) that features a modular design and reduced gas-flow requirements. Shimadzu introduced its contact-free ECD-2100 detector, which uses a sweep gas to minimize contact of the sample with the detector source and produces lower detection limits and longer maintenance intervals. Activated Research Company introduced its Polyarc catalytic post-column reactor that converts all carbon-containing compounds exiting the column into methane, which simplifies many calibration problems as well as responding to carbon monoxide, carbon dioxide, and formic acid.

The GC accessories were rounded out by an interesting assortment of tools, inert liners, gas purifiers, thermal desorption tubes and conditioners, and syringes.

In GC columns, Supelco, part of MilliporeSigma, continues to expand its ionic-liquid (IL) column offerings with additional unique selectivities as well as a water-specific phase that is claimed to deliver a symmetrical water peak. Agilent, Phenomenex, and Restek all showed their latest columns with improved inertness and selectivity, plus in some cases higher temperature ranges.

All-in-all it was a healthy year for GC with the large number of varied offerings. I look forward to Pittcon 2017 and the opportunity to meet next years's new class of GC products and technologies.

Acknowledgments

I would like to thank the manufacturers and distributors that kindly furnished the requested information, which allowed a timely report on new product introductions over the past year. For those manufacturers who did not receive a "New Products" questionnaire this year and would like to receive one and be considered for early inclusion into the 2017 new GC and related product introductions review, as well as the other related review articles to be published in *LCGC North America*, please send the name of the primary company contact, the mailing address, fax number, and e-mail address to Laura Bush, Editorial Director, *LCGC* and *Spectroscopy*, Advanstar Communications, 485 Rte. 1 South, Bldg. F, Suite 210, Iselin, NJ 08830, Attn: 2017 New Chromatography Products. The questionnaire will be sent out in late 2016.

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John V. Hinshaw

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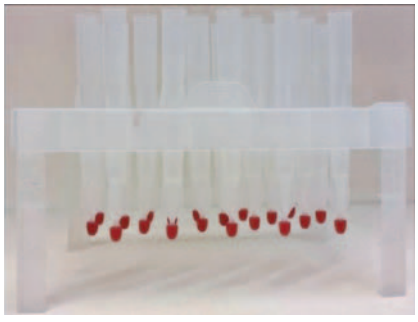
Volumetric Absorptive Microsampling for Hepcidin Peptide Extraction from Whole Blood



Whole blood analysis is an emerging trend in the field of bioanalysis. We developed a fast and simple protocol to extract and analyze a peptide, hepcidin, from whole blood. Sampling and extraction were carried out using volumetric absorptive microsampling (VAMS), a novel blood collection method that allows the sampling of a known blood volume independently from hematocrit. The composition of the extraction medium was optimized using an experimental design to get the most intense signal of hepcidin, considering different organic solvents and acidic additives.

V. Houbart, G. Cobraiville, G. Nys, A.-C. Servais, and M. Fillet

Blood is a commonly used biological matrix to perform varied analyses as routine checkup, disease diagnosis, or medical treatment monitoring. However, the major part of blood analysis is not performed on whole blood, but on plasma or serum obtained from blood after centrifugation. This procedure, even if not complex, requires material, sample manipulation, and time.

Dried blood analysis has been known for decades (Robert Guthrie developed his well-known phenylketonuria test on dried blood spots in the late 1950s), but it has regained interest in the last few years (1,2). It offers an easy way of collecting, shipping, and storing samples at room temperature, unlike plasma or serum samples that require freezing for shipping and conservation.

Blood is commonly collected by venipuncture, but alternative (self-) sampling techniques are available, such as finger or heel pricks that allow painless and less-invasive collection of a small volume (a few drops) of capillary blood. In practice, the pain-free collection of a few blood drops to perform one or more analyses has several advantages. These advantages are well-known in the context of newborn screening tests, but at-home blood sampling in the context of personalized medicine, or studies in small laboratory animals represent other promising applications. For example, in the context of the three R's (refine, reduce, replace), performing a complete pharmacokinetic (PK) study on the same animals all along during the experiment is valuable, not only from the ethical and economical point of view, but also for the significance of the observed results (3,4).

Whole blood analysis has a well-described challenge: the complexity of the matrix. Blood is composed of cells and plasma, with the latter containing a huge variety of components, such as proteins, lipids, sugars, amino acids, salts, hormones, and metabolites. The analytical challenge consists of specifically detecting the compounds of interest without being affected by the other compounds of the matrix in an uncharacterized manner (5).

Manufacturers are putting effort into the development of new collection supports that allow easy sample collection (feasible by the patient itself at home, for example), and fast and reliable subse-

quent analysis in the laboratory. Dried blood spot (DBS) sampling is the most common dried blood analysis sampling technique: a blood drop is collected on an appropriate filter paper and allowed to dry before further handling (1,6). Several kinds of filter papers have been commercialized, based on cellulose or a polymeric support, with or without pretreatment. One of the well-described difficulties of DBS is the difference in sample diffusion on the filter paper from one sample to another. The sample diffusion is affected by blood viscosity that strongly depends on hematocrit (the ratio of the volume of red blood cells to the total volume of blood). Since the hematocrit varies between individuals, it represents a source of bias that is problematic for quantitation. This limitation could be avoided by spotting a precise blood volume (for example, by means of a glass capillary) and cutting the whole blood spot, whatever its size (6).

Aside from the DBS filter paper collection technique, some alternate methods have been proposed. Volumetric absorptive microsampling (VAMS) is a novel collection approach that allows the collection of a defined volume of blood independently of hematocrit (7,8). The sampling device consists of a polymeric porous and absorbent tip (hydrophilic porous material) located at one extremity of a plastic body. The tip is placed at the surface of the blood sample. When fully red, it is then allowed to dry for at least 2 h at room temperature. Each tip has a constant, highly reproducible internal porous volume, which has been designed for accurate and precise wicking volume (7,8). The extraction is performed in a 96-well plate with an appropriate extraction solvent, then filtrate. This step of the procedure has to be carefully optimized (12). It requires low sample volume (10 mL), which is interesting when the availability of the sample is limited, for example. This is the case for pediatric applications, but also for studies on small laboratory animals (9,10).

In this work, the VAMS technique was used to collect human blood to extract hepcidin, a peptide hormone, for further analysis using microfluidic liquid chromatography coupled to tandem mass spectrometry (LC–MS-MS). Experimental design was used to find the most appropriate extraction medium composition to maximize analyte signal.

Experimental

Chemicals

Human synthetic hepcidin (DTHFPICIFCCGCCHRSKCGMCCKT) was synthesized by the Peptide Institute Inc. Whole blood was collected in MiniCollect EDTA tubes (Greiner Bio-One) from a healthy human control subject by finger prick. The ethical committee of the university hospital (CHU-Liege, Belgium) approved this study and the healthy control gave written informed consent.

Instruments and Materials

Chromatographic separation was achieved on an Agilent 1200 series LC-chip system consisting of a nanoflow pump, a capillary pump, a well-plate sampler and an LC-chip–MS interface. Mass spectrometric detection was performed using a 6340 ion-trap MS system equipped with a nanoelectrospray ionization source operating in positive mode (Agilent Technologies). ChemSta-

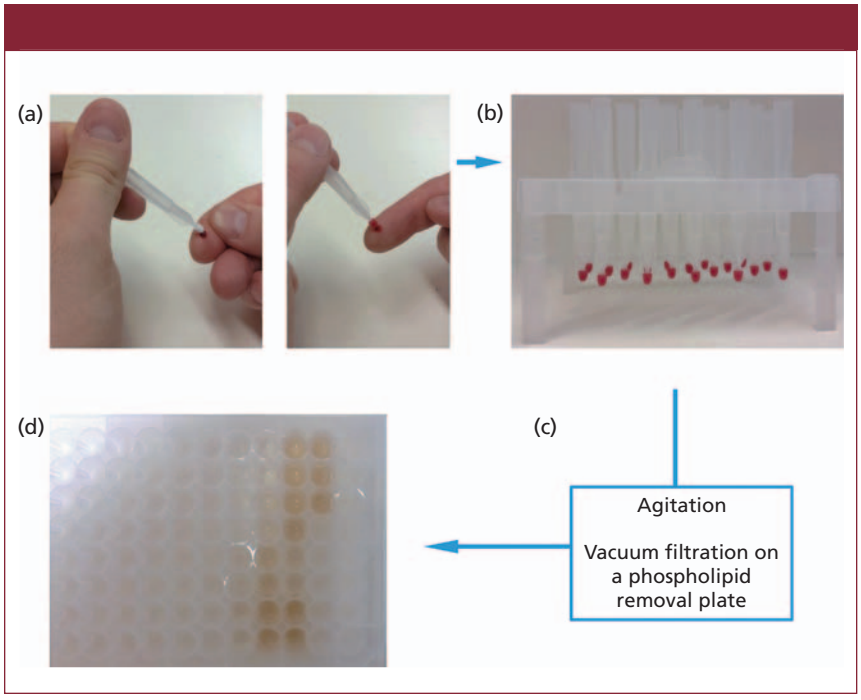


Figure 1: VAMS sampling and extraction procedure: (a) blood drop absorption on the porous tip of the VAMS; (b) drying of the VAMS devices (>2 h at room temperature); (c) extraction in the optimized solvent on a phospholipid removal plate, then phospholipid removal from the extracted solution on a vacuum manifold; (d) final extracted solutions (different in extract colors are due to the different extraction mixtures that were used (see Figures 3 and 5).

tion software (Agilent Technologies) was used for instrument control. MS detection parameters were set by TrapControl software (Bruker Daltonik GmbH). Raw MS data were processed using DataAnalysis software (Bruker Daltonik GmbH).

Mitra volumetric absorptive microsamplers were obtained from Neoteryx. Sample evaporation was performed on a vacuum concentrator (Labconco).

Sample Preparation

Hepcidin was dissolved in a mixture of 80:20:0.1 (v/v/v) water–acetonitrile–formic acid to reach a concentration of 1 µg/mL. The solution was then separated in aliquots and stored at -80 °C.

For each sample realized in duplicate, 22.5 µL of fresh blood was spiked with 2.5 µL of hepcidin solution at the appropriate concentration to obtain 25 µL of spiked matrix at 50 ng/mL in a Protein LoBind tube (Eppendorf). After that, the microsampler was dipped into the matrix sample so that the tip just touched the surface of the sample. After the tip was fully colored, an additional

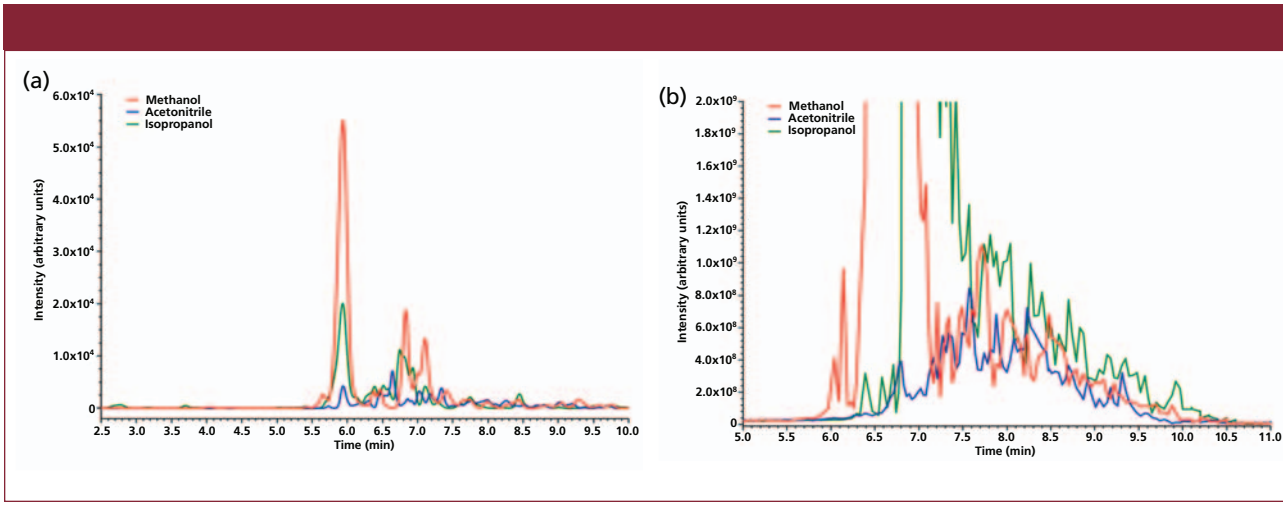


Figure 2: Influence of the nature of the organic solvent for hepcidin extraction (methanol, acetonitrile, and isopropanol). The extraction mixture was 80% organic solvent, 20% water, and 0.1% formic acid. (a) EIC for hepcidin and (b) TIC full scan.

contact of 2 s was observed. The microsampler was then allowed to dry for at least 2 h at room temperature. The microsamplers were placed on a 96-well Ostro sample preparation plate (Waters Inc.) and allowed to shake in 200 µL of the appropriate extraction solvent for 5 min at 600 rpm (20 °C) in a Thermomixer mixer and incubation device (Eppendorf). The plate was then placed on a vacuum manifold (Waters Inc.) and the samples were collected in an Eppendorf Protein LoBind 96-well plate before evaporation and reconstitution in 100 µL of 33.8:66.2:0.1 (v/v/v) acetonitrile–water–trifluoroacetic acid (11,12). This collection and extraction scheme is illustrated in Figure 1.

LC–MS-MS Analysis

Chromatographic separation was performed on a ProtID-chip including a 40-nL trapping column and a 43 mm × 75 µm analytical column, both packed with Zorbax 300SB 5-µm C18 phase (Agilent Technologies). Mobile-phase A was 100:0.1(v/v) water–formic acid, and mobile-phase B was 90:10:0.1 acetonitrile–water–formic acid, both degassed by ultrasonication for 15 min before use. The analytical process was performed in two steps: First, the sample was loaded on the trapping column during an isocratic enrichment phase using the capillary pump delivering a mobile phase in isocratic mode composed of 15% B at a flow rate of 4 µL/min. A flush volume of 6 µL was used to remove unretained components. Then, after valve switching, a gradient elution in backflush mode was performed through the enrichment and analytical columns using the nanopump. The gradient started at 15% B and linearly ramped up to 95% B in 5 min. This proportion was maintained for a further 5 min before it was returned to 15% B. Before the next injection, 10 column volumes were used for reequilibration. All of the experiments were carried out with a 1-µL sample injection volume. For MS-MS detection, the $[M+4H]^{4+}$ parent ions were used for hepcidin (*m/z* 698.4) detection, and eight fragment ions were extracted for each compound to provide

chromatograms that were used for quantitation, as previously described (11).

Design of Experiments

To optimize the hepcidin extraction, a two-step chemometric procedure was implemented. First, an L18 Chakravarty screening design was used to select the most significant factors to be used in the optimization design. The second step consisted of a face-centered central composite design (CCD) for optimization. These designs and their subsequent statistical evaluation were performed with JMP software (SAS Institute).

Results and Discussion

Preliminary Study

Optimization of all factors relative to the analyte extraction from the biological matrix is of the utmost importance to obtain good sensitivity and robust conditions ensuring reproducible results required for quantification.

In this study, hepcidin was chosen as the model peptide. Hepcidin is a rather challenging peptide to be quantified because of its low concentration in biological fluids, but also because it was found to stick onto the container surface and the different parts of the analytical system (11). This peptide plays a key role in the regulation of iron homeostasis. Its determination is thus useful for the diagnostic or classification of iron disorders, such as hereditary hemochromatosis, anemia from chronic disease, or iron deficiency anemia. More recently, it was also described as an interesting biomarker for some inflammatory diseases and cancers (13,14).

Out of the number of peptides potentially relevant for clinical applications, hepcidin is present at a low abundance in the biological fluids; therefore, the signal intensity (that is, hepcidin peak area) of hepcidin will be the decisional criterion for the present study. MS detection parameters were optimized by infusion using the electrospray interface in positive ionization mode. Among the different peaks related to hepcidin, *m/z* 698.4 corresponding to the four times charged species, was selected. A fragmentation amplitude value of 0.9 V was found to be optimal, as abundant doubly, triply, and quadruply charged product ions were obtained, including $(y_{23})^{4+}$, $(y_{24})^{4+}$, $(y_{19})^{3+}$, $(y_{21})^{3+}$, $(y_{22})^{3+}$, $(y_{23})^{3+}$, $(y_{19})^{2+}$, and $(y_{21})^{2+}$.

We previously worked on the quantification of this peptide in human plasma using a solid-phase extraction (SPE) approach for sample preparation (11) and in human whole blood using VAMS, but without carefully optimizing the nature of the organic solvent and the acidic additive present in the extraction medium (12). In this previous study, we demonstrated that the extraction time duration is an important parameter to be considered. Indeed, if the extraction duration is too long, the matrix effect and the ion suppression become very important, resulting in the complete loss of the hepcidin signal. After careful optimization of this parameter (12), the optimal extraction duration was settled at 5 min.

In this work, preliminary experiments were performed to investigate several potentially interesting organic solvents for hepcidin extraction from the dry blood matrix. As can be seen in Figure 2a, methanol, isopropanol, and acetoni-

trile were tested at 80% in the extraction mixture and were found to provide significant differences in the hepcidin extraction yield. Figure 2b illustrates the overall extraction of endogenous compounds of the blood matrix. As can be seen in this figure, acetonitrile provided the lowest total ion chromatogram (TIC) signal, probably because of the fact that most of the matrix proteins are precipitated and remained embedded in the tip leading to low hepcidin extraction. On the contrary, TIC signals with methanol and isopropanol were found to be more intense meaning that more molecules, not only hepcidin but also potentially interfering compounds, were extracted.

From these results, only methanol and isopropanol were kept for further investigations.

Design of Experiment Approach

To efficiently optimize the extraction procedure of hepcidin from VAMS, a chemometric approach using design of experiments was performed. First, a screening design narrowed down a list of several inputs to a more manageable range. After that, a response surface design was used to investigate the main effects, but also potential quadratic effects and interactions between the selected factors.

Screening Design

The experimental factors were the nature of the organic modifier and the volatile acid present in the extraction medium. Two organic modifiers were studied: methanol and isopropanol as well as three volatile acids: formic acid, trifluoroacetic acid, and trichloroacetic acid. The tested ranges were from 50% to 90% of organic modifier, and from 0.01% to 0.5% for volatile acid. The peak area was chosen as response because it reflects the combination of extraction yield from the VAMS device as well as matrix effect caused by the coextraction of endogenous compounds from blood.

An L18 Chakravarty screening design was implemented to determine the significant factors that would be further investigated in the second step. This screening design allowed the management of all the considered factors, including both three-level categorical factors. Finally, 18 experimental conditions were defined and performed in duplicate, leading to a total of 36 experiments.

Because of the non-normal distribution of the results, a Box-Cox transformation was applied ($\lambda = 0.4$). The model built by the software had an excellent prediction since the adjusted r^2 of the model was 0.9772, roughly meaning that the model explains more than 97% of the peak area variability of (see Figure 3a). The main effects of all the considered factors were found to be significant (p-value < 0.0001) on peak area.

For the qualitative factors, the nature of organic modifier had a significant impact on the intensity of the hepcidin signal (see Figure 3c). As maximization of the hepcidin signal was required in this screening design, isopropanol was chosen for subsequent optimization. Moreover, as shown in Figure 3b, the extracts were cleaner when extracted with isopropanol (wells D5 to G6) than methanol (wells H6 to E8), which means that smaller amounts of blood components were coextracted. It is noteworthy that the isopropanol extraction was also more repeatable than methanol extraction: the variability on methanol extraction is noticeable by the variation of sample color

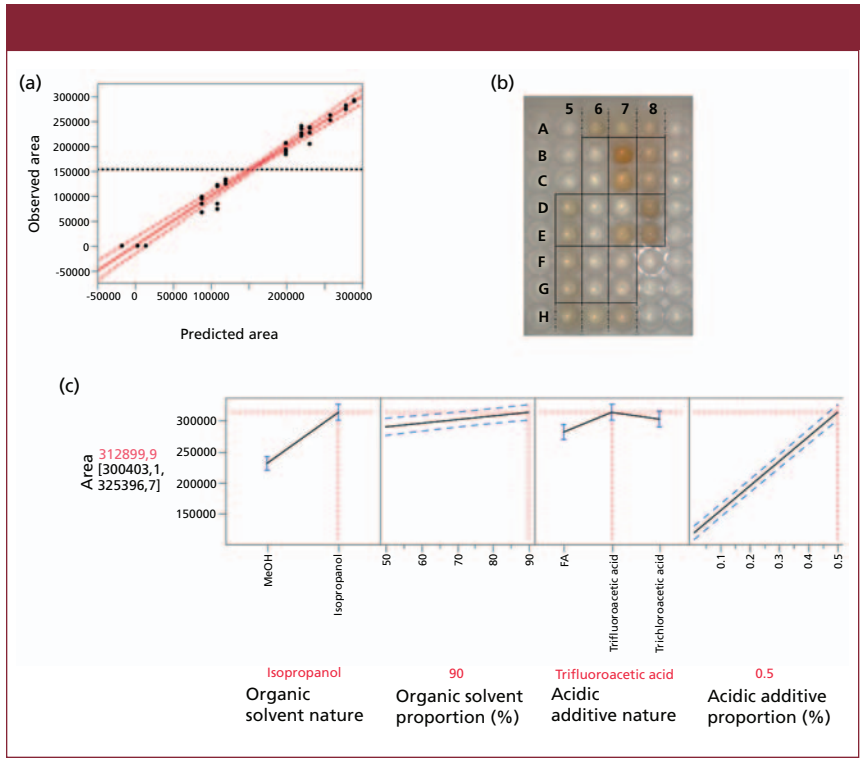


Figure 3: Optimization of the extraction conditions for volumetric absorptive microsampling by experimental design (L18 screening design). (a) Agreement between the observed and the predicted results (adjusted r^2: 0.9772). (b) Visual aspect of the extracts depending on the extraction medium composition. Duplicate of different conditions (v/v/v proportions): D5-E5: 50:50:0.5 isopropanol–water–formic acid; F5-G5: 50:50:0.5 isopropanol–water–trichloroacetic acid; H5-A6: 50:50:0.5 isopropanol–water–trifluoroacetic acid; B6-C6: 90:10:0.01 isopropanol–water–formic acid; D6-E6: 90:10:0.01 isopropanol–water–trichloroacetic acid; F6-G6: 90:10:0.01 isopropanol–water–trifluoroacetic acid; H6-A7: 50:50:0.01 methanol–water–formic acid; B7-C7: 50:50:0.01 methanol–water–trichloroacetic acid; D7-E7: 50:50:0.01 methanol–water–trifluoroacetic acid; F7-G7: 70:30:0.225 methanol–water–formic acid; H7-A8: 90:10:0.5 methanol–water–formic acid; B8-C8: 90:10:0.5 methanol–water–trichloroacetic acid; D8-E8: 90:10:0.5 methanol–water–trifluoroacetic acid. (c) Prediction profile of the screening design results: influence of the four selected factors on the response. Dashed blue lines represent the 95% confidence intervals.

between duplicates on Figure 3b. For the nature of the volatile acid, trifluoroacetic acid provided the highest intensity as shown in Figure 3b.

The influence on the proportion of these selected parameters (for example, isopropanol and trifluoroacetic acid, Figure 3c) in this screening step were therefore studied further in the optimization design.

Optimization Design

Based on the results of the screening design, isopropanol and trifluoroacetic acid proportions in the extraction medium were thoroughly investigated in the interval ranging from 40% to 80%, and 0.1% to 1%, respectively.

A face-centered CCD was applied to optimize the selected factors. This CCD consisted of 10 experiments performed in a random order in duplicate, with a total of four replicates at the central point. A Box-Cox transformation was applied to normalize the results ($\lambda = -0.6$). A response surface model was used to modelize the hepcidin area. It included six coefficients (the intercept, β_0, the main effects, β_1 and β_2, the interaction term, β_{12}, and the quadratic terms, β_{11} and β_{22}) as indicated in the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \quad [1]$$

where Y is the hepcidin area, X_1 is the isopropanol proportion, X_2 is the trifluoroacetic acid proportion in the extraction medium, and ε is the error term.

The model adjusted r^2 equals 0.9549, indicating that about 95% of the variability is explained by the model. The coefficients of the model and their statistical significance are listed in Table I. The *p*-value is the probability of getting a result as extreme or more extreme than the one observed if the proposed null hypothesis is correct. Considering the application (analysis of peptides in LC–chip-ESI-MS-MS), a *p*-value lower than 0.05 was considered significant.

Table I: Model terms and associated *p*-values of the optimization design

Term	Studied Factors	*p*-Value
X_1	% isopropanol	<0,0001
X_2	% trifluoroacetic acid	<0,0001
$X_1 X_2$	% isopropanol * % trifluoroacetic acid	0,0005
X_1^2	% isopropanol * % isopropanol	<0,0001
X_2^2	% trifluoroacetic acid * % trifluoroacetic acid	<0,0001

The statistical analysis of the model reveals that peak area is significantly affected by the isopropanol and trifluoroacetic acid proportions. The response surface plot shows (Figure 4b) that increasing the proportion of trifluoroacetic acid in the extraction solvent has a major effect on hepcidin peak area. The plot also shows that a decrease in the isopropanol proportion increases peak area. Visually, variations in the color of the extracts can also be seen in Figure 4b: above 60% isopropanol, extracts are more intensely colored, reflecting a large coextraction of endogenous blood components. The optimal extraction medium composition in terms of hepcidin area is a 40:60:1 (v/v/v) mixture of isopropanol–water–trifluoroacetic acid with satisfactory reproducibility (relative standard deviation [RSD] = 14.6%; $n = 4$). It is noteworthy that exact values for the limit of detection (LOD), limit of quantitation (LOQ), trueness, accuracy, or precision can only be given after a full validation of the method.

Conclusion

In this study, we developed a simple protocol to extract a peptide, hepcidin, from

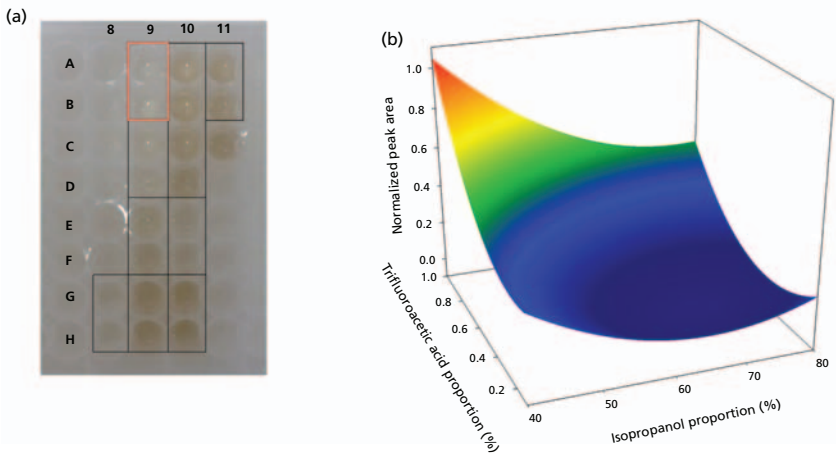


Figure 4: Optimization of the extraction conditions for volumetric absorptive microsampling by experimental design (optimization design). (a) Visual aspect of the extracts depending on the extraction medium composition. Duplicate of different conditions (v/v/v proportions, all with isopropanol–water–trifluoroacetic acid): G8-H8: 40:60:0.1; A9-B9: 40:60:1; C9-D9: 40:60:0.55; E9-F9: 60:40:0.1; G9-H9: 60:40:1; A10-D10: 60:40:0.55; E10-F10: 80:20:0.1; G10-H10: 80:20:1; A11-B11: 80:20:0.55. (b) Response surface plot showing the influence of isopropanol and trifluoroacetic acid proportions on hepcidin peak area after Box-Cox transformation (note: 1 represents the maximal area).

whole blood. Blood sampling and extraction were performed using VAMS with a disposable device that allows the collection of a defined blood volume from a finger prick. Design of experiments was used to optimize the extraction medium composition to get the highest signal intensity for hepcidin. The results confirmed that the choice of the extraction medium has to be made carefully since the optimal conditions are far from the full methanol extraction that is recommended by the manufacturer as a starting point. A compromise always has to be found between extraction yield of the analyte, and coextraction of matrix components that could cause ion suppression. Compared to classical plasma or serum analysis, the developed protocol is much simpler since it does not require venipuncture, blood centrifugation, sample freezing, or a multistep sample preparation procedure.

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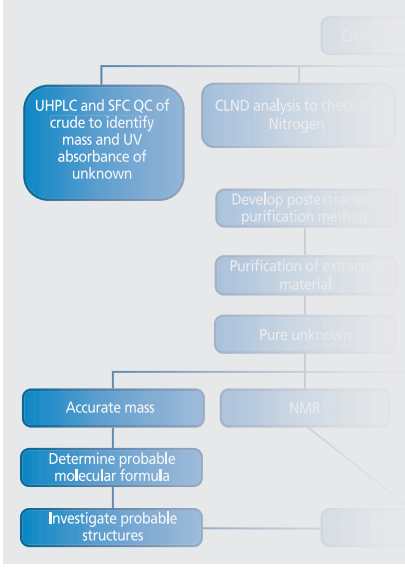
How to Tackle an Unknown: Notes from the Fourth Method Development Olympics at CoSMoS



How would you analyze a bag of gummy bears that showed up on your laboratory bench? This was the challenge taken on by teams of analysts in advance of the Conference on Small Molecule Science (CoSMoS) that was held in August 2015 in San Diego, California. This article shares insights from how the finalists approached the question.

Michael P. Balogh

An increasingly popular event each year at the Conference on Small Molecule Science (CoSMoS) is the Method Development Olympics. The program was initiated in 2011 by two conference committee members, Damian Morrison and Ken Fountain. Since then, it has evolved each year because a changing committee, often involving past winners, has lent its expertise to the next year's challenge. This year's committee consisted of Karen Alsante, Ruchi Mehta, and William Farrell, all of Pfizer, along with Kevin Gauger of Catalent Pharma Solutions, and was led by Jeff Kiplinger of Averica Discovery. The committee devised a sample that met a variety of requirements: It had to be nontoxic, be able to be sent to candidates, and remain stable. And of course, the sample had to be intriguing for those keen to challenge their analytical prowess.

How the Method Development Olympics Work

The process of the Method Development Olympics is simple. A challenge is posted on the CoSMoS website early in the year. Scientists register their interest in participating, and the sample is sent to them. Once they believe they have solved the question, they submit their answer, and the process they followed to get to the answer, to the committee. The committee then chooses three finalists, from among those who submitted correct answers, to present their approaches at CoSMoS in August. Conference attendees then vote, based on the novelty of the solutions, which teams should receive the gold, silver, and bronze medals.

The competition reflects what CoSMoS has been about for the past 12 years. CoSMoS is an analytical conference integrating discussions of tools, separations, chemistry, and experimental design, where practitioners gather to debate the best ways to solve complex mixture analysis problems in fields such as pharmaceuticals and health sciences, petroleum, food, and forensic toxicology. The same analytical tools are used in each industry but often with subtly different approaches. Discussing the differences provides a distinct benefit to everyone, giving them practical takeaway knowledge.

In brief, the instructions for this year's Method Development Olympics stated, "Each participant will be sent a container of gummy bears adulterated with an unknown compound. The goal is to identify and quantitate the unknown compound and discover any other pertinent information." The participants had to figure out that the samples had been coated with indoprofen (2-[4-(1-oxo-1,3-dihydro-2H-isoindol-2-yl) phenyl]propanoic acid). Jeff Kiplinger, this year's committee lead, cleverly included that last phrase of the instructions, "and dis-

The Competitors in the Method Development Olympics

The Gold Medalist
Merck Team
Ryan Cohen
Roy Helmy
Leo Joyce
Alexey Makarov
Amanda Mann
Justin Pennington
Mikhail Reibarkh
Huaming Sheng
Tiebang Wang
Thomas Williamson

The Silver Medalist
Catalent Team
Matt Lochanksy
Wei Pan
Stephen Carino
Pingyun Chen
Kayla Le
Senthil Sukumar
Natalja Tonkiha
Chris Wittum

The Bronze Medalist
Novartis Team
Lucas Westling
Kevin Johnson
Perry Gordon
Mike Gibney
Tom Hollenbeck

cover any other pertinent information," to test participants' analytical skills at yet another level—by including chirality as a less obvious aspect of the adulterant.

All participants had to fit the challenge around their work schedules. The competition is designed to weigh heavily on the participants' abilities to design the experiments and on their skills as analytical scientists. As the competition guidelines say, judging is based on "accuracy of the results, the amount of ancillary information discovered, as well as the creativity of the method development approach."

Each competitor had access to different analytical tools and support. Gold medalist Ryan Cohen of Merck in Rahway, New Jersey, led a team with expertise in high-throughput analysis, mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, metals analysis, and drug product analysis.

Lucas Westling, the bronze medalist, works at the Genomics Institute of the Novartis Research Foundation. For him, a really positive part of the competition was exposure to instruments and techniques that he doesn't normally use in his day-to-day responsibilities. In his case, those were NMR and accurate mass. "I hadn't used NMR for several years and am still quite a beginner, so the initial sample prep and instrument queuing took some time," he said. "The accurate mass instrument is used in our lab for metabolite identification but, luckily, I was able to have its operators sit down with me and quickly run a few injections of my crude sample solution."

Matt Lochansky and colleagues at Catalent Pharma Solutions in Research Triangle Park, North Carolina, entered the competition to showcase their diverse scientific talents and technical capabilities. They also looked forward to the opportunity to work together as a team to tackle an interesting, challenging problem and have fun.

"Because we are a contract develop-

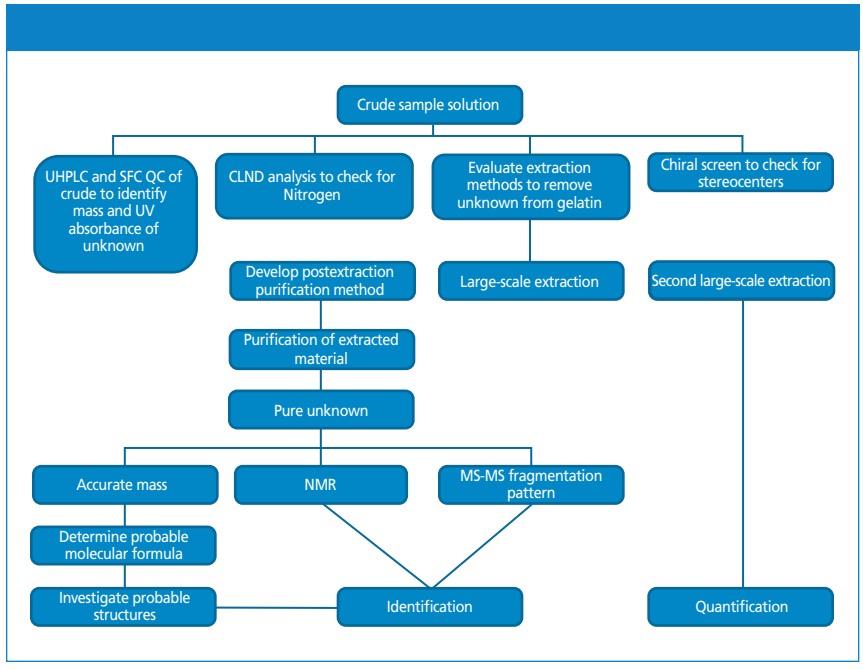


Figure 1: A prospective approach to characterizing an unknown. (Courtesy of L. Westling.)

ment and manufacturing organization, most of our projects are confidential and well-defined in scope, so our scientists rarely have freedom in the selection of techniques and approaches to solve a problem or the opportunity to publish the results," said Lochansky. "The CoSMoS Method Development Olympics provides a unique opportunity for the scientists to fully leverage their expertise and creativity and compete against the best in the industry." When his team learned that this year's challenge was to identify and quantitate an unknown adulterant in gummy bears, they started calling the project "CSI: Catalent."

The Samples Arrive

Rarely do scientists have either the full range of freedom in their jobs or the necessity to investigate samples in such broad scope. More often, there is some context, such as known starting materials, expected outcomes, and even historical data. Investigators engaged in the Method Development Olympics would likely agree the first step with an unknown is to characterize its gross morphology in true forensic style, allowing one's experience as an analytical scientist to dictate the next steps while not prejudging outcomes based on scant initial data.

Westling's response to receiving the samples was typical of the competitors. "There was an initial burst of energy or

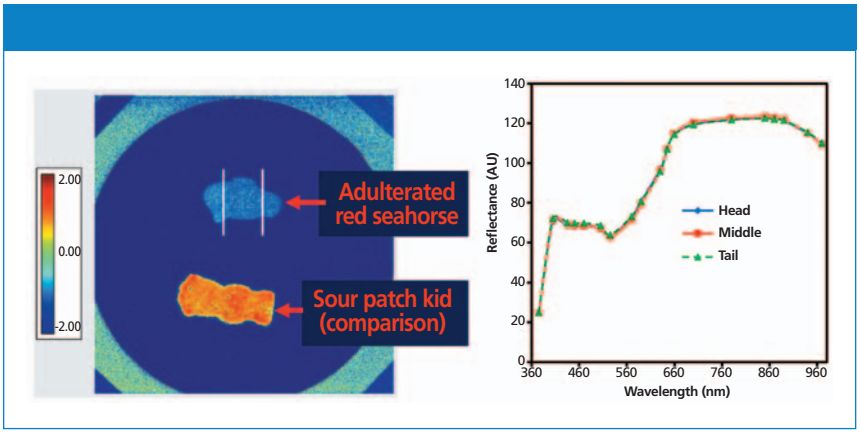


Figure 2: Initial spectral analysis of intact unknown in the gummy bear sample. Left: a heat map showing reflection differences; right: diffuse reflectance of an adulterated red seahorse. (Courtesy of R. Cohen.)

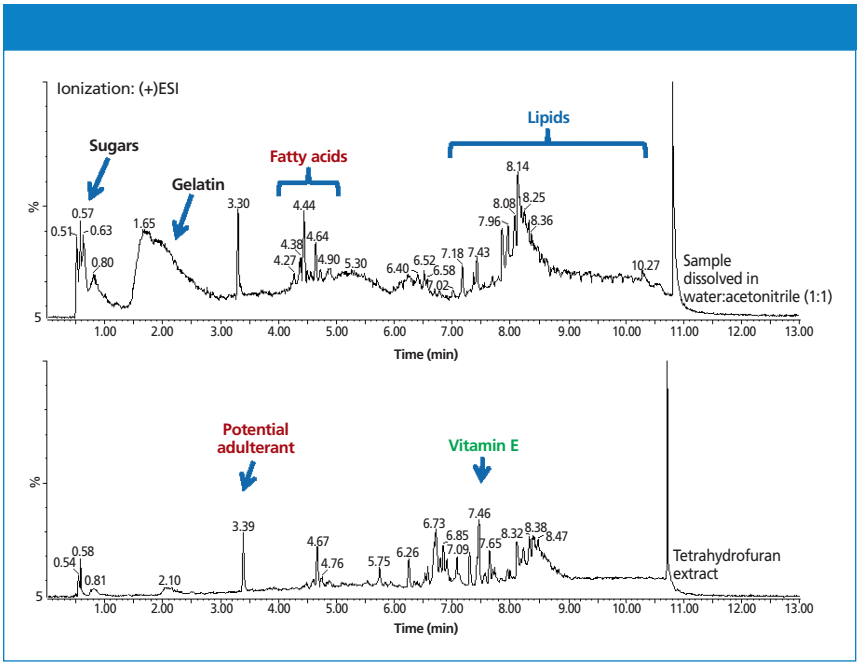


Figure 3: LC–MS and initial method survey by the Merck team. Column: 100 mm X 2.1 mm, 1.7-µm d_p BEH-C4-300 Å; mobile-phase A: 0.1% trifluoroacetic acid in water; mobile-phase B: 0.1% trifluoroacetic acid in acetonitrile; flow rate: 0.7 mL/min; gradient: 5–95% B in 8 min, then hold for 2 min; detection: UV absorbance at 210 nm; temperature: 45 °C; injection volume: 3 µL. (Courtesy of R. Cohen.)

urgency to get started," he said. "I had a map in my head of how I was going to proceed, so within several days most of the initial qualitative analyses were finished." (See Figure 1.)

The Merck team employed spectral imaging to compare the sample with a putative control (Figure 2) based on the information readily at hand and appearance of the sample. The Merck team examined 19 wavelengths using a videometer (Videometer A/S, Hørsholm, Denmark) at a spatial resolution of 45 µm. They noted that no regional differences were seen on the adulterated red seahorse-shaped gummy bear sample. Therefore, at least lengthwise, they were dealing with a homogenous sample.

Participants received only five gummy bears, so sample conservation was a big concern, particularly during the initial extraction and quantitation portion of the competition. "I wanted at least two full gummies (ideally three) for the final quantitation," noted Westling.

The Catalent team was also careful to preserve sample. To do so, they carefully examined the gummy bears under

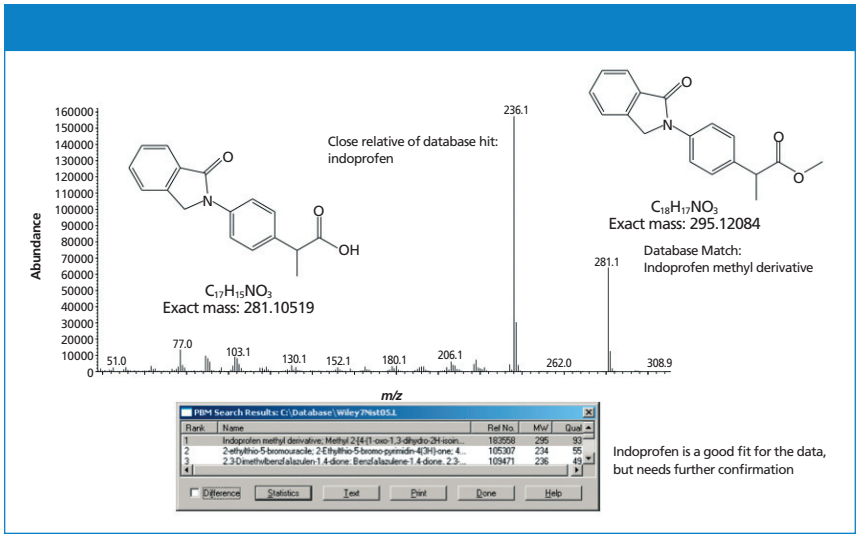


Figure 4: GC–MS yielded a direction but needed further corroborative effort. (Courtesy of M. Lochansky.)

a microscope to look for potential sites where the adulterant could have been injected. Then they carefully dissected and examined the first gummy bear. "We did not find any evidence of injection sites on the surface or the cross sections of the dissected gummy bear," said Lochansky.

The Initial Analytical Attempts

The initial visual examinations, hypotheses, and suppositions must lead to testing, of course. The results from broad-spectrum analytical tools used in the first phase were typically supplemented by correlative, orthogonal approaches.

Merck developed a series of reversed-phase liquid chromatography–mass spectrometry (LC–MS) analyses to rapidly assess sample composition. Their first step was an achiral ultrahigh-pressure LC (UHPLC) screen. They used six columns: Eclipse Plus C-18, SB-CN, SB-C8, and SB-Phenyl (all from Agilent) and Gold AQ and Gold PFP (from Thermo Scientific), along with six mobile phases. The weak eluents were 0.1% phosphoric acid, 0.1% phosphoric acid + 35 mM potassium hexafluorophosphate, 0.02% perchloric acid with 150 mM sodium perchlorate, and 5 mM ammonium phosphate (dibasic). The strong eluents were acetonitrile and methanol.

They followed with a large-molecule screening step. They used six columns (BEH-C18-135A, C-18-300A, and C4-300A [from Waters Corporation]; SEC125, SEC200-Unix [from Bio-Rad Laboratories]; and SEC300-Zenix [from Sepax]) and six mobile phases. The weak eluents were 0.1% trifluoroacetic acid (aq), 50 mM ammonium acetate, and 50 mM ammonium formate. The strong eluent was 0.1% trifluoroacetic acid in acetonitrile.

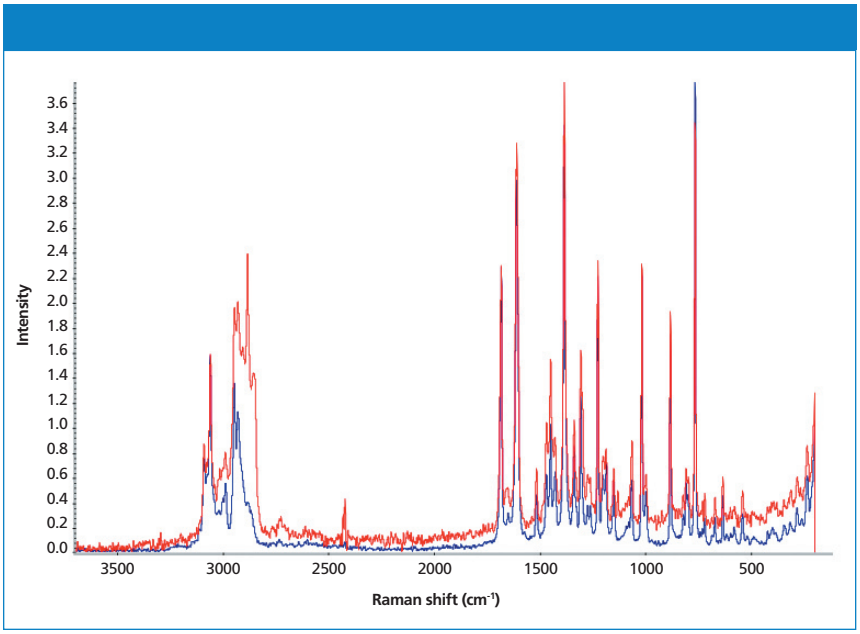


Figure 5: Overlay of outer surface Raman spectrum extracted from CH_2Cl_2 (red trace) and reference spectrum of suspected adulterant indoprofen (blue trace). (Courtesy of M. Lochansky.)

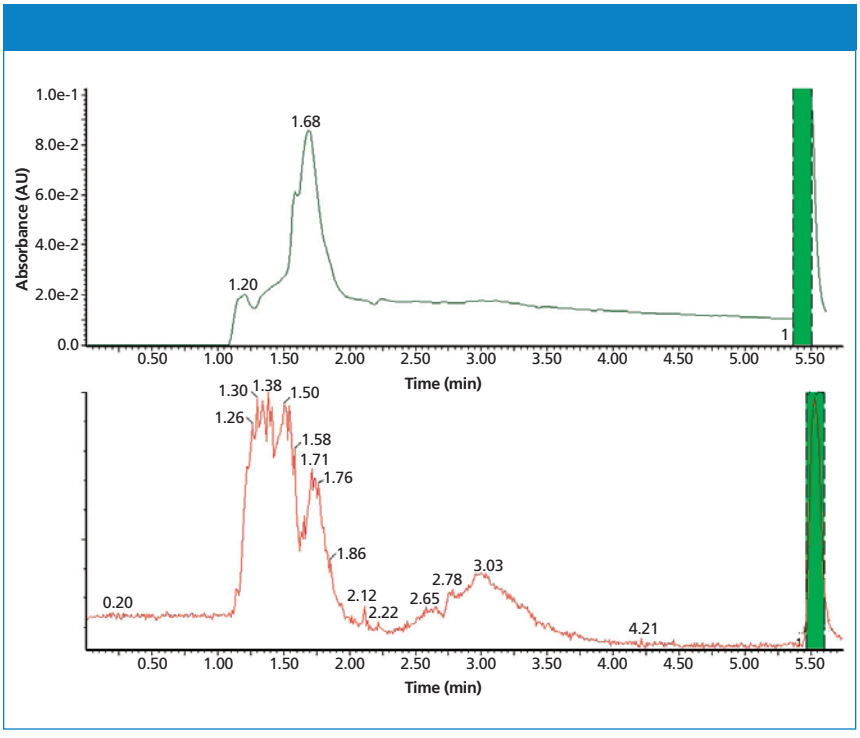


Figure 6: Isolation of unknown post extraction via LC–UV (top) and LC–MS (bottom) purification. (Courtesy of L. Westling.)

According to Lochansky's colleague and Catalent team captain Wei Pan, who is the director of Strategy and Analytical CMC, the team brainstormed a hypothesis after receiving the description of the challenge. "We thought that the most likely approach to adulterate the candy with a drug is by injecting the drug," Pan said. The team admitted that their thinking was influenced by the urban legend of adulterated Halloween candy in the Unites States. The team decided to dissolve the gummy bear in water. They also thought it was critical to remove the gelatin to avoid clogging the MS detector (which would result in the team's being banned from access to the MS instrument in the future). A centrifu-

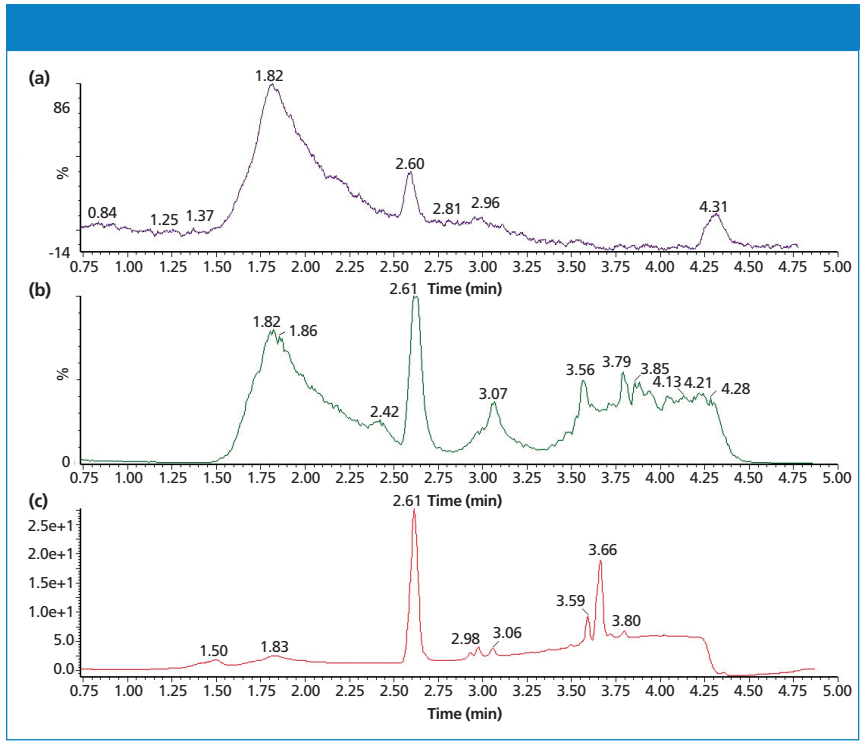


Figure 7: UHPLC-facilitated CLND (top), MS (middle), and diode-array UV (bottom) analysis of the sample as a crude extracted unknown. (Courtesy of L. Westling.)

gal molecular membrane filter was proposed as an easy solution, assuming the adulterant was a small molecule (a safe assumption considering the host confer-

ence). Gummy Bear brand candies were purchased from a local grocery store to test the procedure and the resulting solution was analyzed by both gas chromatography–mass spectrometry (GC–MS) and LC–MS. No peaks were observed in the profile. "At this point, we thought we were ready for the real sample," said Pan.

When the real sample gummy bear was placed in cold water, a white precipitate was observed immediately. "This observation was very different from that of the purchased gummy bear," said Pan. Next, the gummy bear was removed from the suspension, rinsed, and placed into another beaker and dissolved with heated water. The resulting solution was filtered through the centrifugal molecular filter, divided into three portions, and extracted with methylene chloride at neutral, acidic, and basic pH levels. They then analyzed the three portions by GC–MS and LC–MS. "The extract at neutral pH showed vitamin E and fatty acids esters by GC–MS," the team noted. "At this point, we thought vitamin E was the adulterant and we ordered a vitamin E standard for confirmation by Fourier

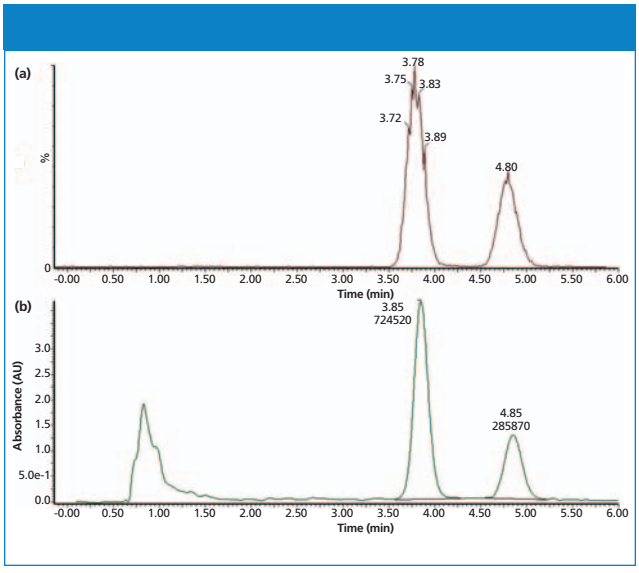


Figure 8: Many teams used SFC to characterize the "bonus" hidden chirality attribute embedded in the samples. Top: MS analysis; bottom: diode-array UV analysis. Column: 100 mm X 4.6 mm, 5-µm d_p ChiralPak AS; mobile phase: 70:30 carbon dioxide–methanol; flow rate: 2 mL/min; pressure: 125 bar. (Courtesy of L. Westling.)

transform infrared (FT-IR) and Raman spectroscopy."

After the FT-IR and Raman spectra showed only a trace amount of vitamin E and an overwhelming amount of fatty acid, the team became less confident that vitamin E was the adulterant, especially knowing there are vitamin gummy bears available on the market. "This led us to examine the white precipitate," said Pan.

The amount of precipitate was insufficient for filtering, so an aliquot of the suspension was extracted with methylene chloride and directly injected into a GC–MS instrument. A large peak was present in the profile and was later correctly identified as indoprofen. This result was confirmed by FT-IR and Raman spectral analyses of the surface of a second gummy bear, of reference indoprofen (Sigma-Aldrich), and of a sugar sample from the company break room. These results confirmed that indoprofen was present on the surface.

Even though most teams used NMR to confirm the chemical identity of the adulterant, the Catalent team chose FT-IR and Raman spectroscopy because these are the most commonly used nondestructive techniques in forensic laboratories and law enforcement. "In addition to the confirmation of the chemical identity of the extracted material, we also used Raman microscopy to confirm that the adulteration was indeed on the surface of the gummy bear, not in the interior as hypothesized initially."

At Novartis, Westling used high-resolution MS as well as NMR to achieve a finely detailed characterization of the sample. In addition to using supercritical fluid chromatography (SFC) as an orthogonal method, and taking the novel approach of using chromatographic anion analysis to check for post-purification salt formation, Westling used evaporative light scattering detection (ELSD) and chemiluminescence nitrogen detection (CLND). Westling also invested in preparative chromatography work (Figure 6) using a mass-triggered preparative LC–MS system to develop scaled-up extracted material in view of possible matrix complexities and to compensate for having so little information about the sample at the outset.

The team at Merck conducted a thorough and detailed examination of the samples, displaying their skills with the range of equipment they had access to. High-resolution MS-MS and chiral techniques were used for aspects of characterization in addition to NMR, which is classically used to confirm what might be found by other techniques. The Merck team's results (Figure 9) further indicated that the indoprofen was not observed in the sample core but only on the surface.

Further characterization by the group at Merck displayed a rather pleasing cyclical conclusion: They refined the morphological characterization they did at the beginning of the exercise by using scanning electron microscopy (SEM), as shown in Figure 10.

Because the scope of this narrative is necessarily limited, much of the elegant work done by the teams has been omitted here. Details about the work the teams did using conformational analysis and electronic circular dichroism (ECD) calculations, characterization of additional unknowns in the samples, spectral deconvolution of proton NMR using a complete reduction to amplitude-frequency table (CRAFT), quantitative summaries, and more, can be reviewed on the CoSMoS website (www.CoSMoScience.org). Links to each team's presentation can be found in the right-most panel of the home page.

At the conclusion of this year's Method Development Olym-

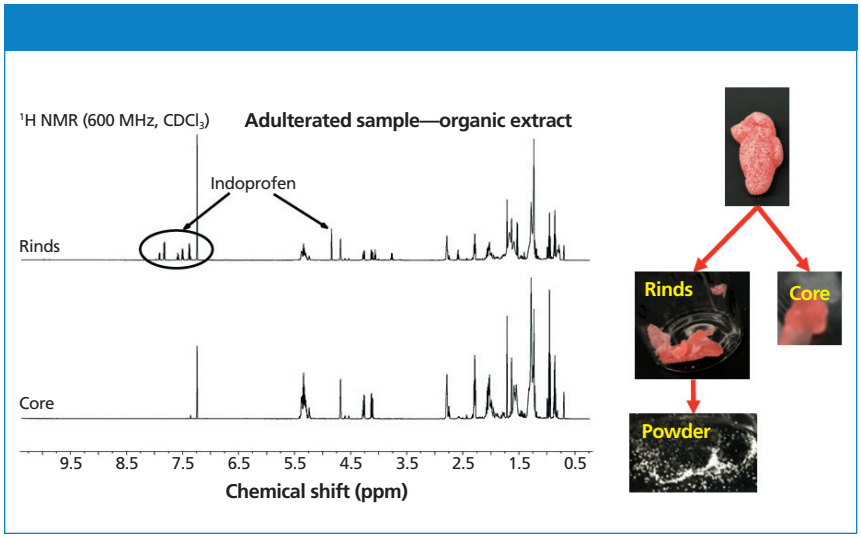


Figure 9: NMR confirmation that adulteration was only present on the sample surface. (Courtesy of R. Cohen.)

pics, it was clear from the enthusiasm of the attendees, the range of questions, and the engagement, that these three teams represented the best of the best, making it hard to rank them. They all brought valuable experience to share with their colleagues. In the end, the bronze medal went to the Novartis team, the silver to the Catalent team, and the gold to the Merck team.

Conclusion: Big Benefits for Participants

It is clear from the comments made by the participants that they found the experience rewarding in many ways. Lucas Westling of the Novartis team noted that being chosen as a finalist offered an opportunity to present his team's work to an audience in a relatively stress-free setting. "I say 'relatively stress-free,' because I am terrified of speaking in front of groups," he admitted. "The CoSMoS conference offers a modestly sized, interested, and engaged audience that may help one feel more comfortable in a presentation setting."

Wei Pan noted that his team's silver medal has been viewed as a huge success within Catalent, and the story made headline news on the Catalent intranet. The team then held a special Lunch-and-Learn session about their experience. "The exercise demonstrated that when people with different training and skills work together as a team, amazing things can happen and science can be fun," said Pan. After the news story ran on the company's intranet, he and his teammates received questions from scientists at other Catalent sites about how to participate in the next competition. "There may be multiple Catalent teams competing in the CoSMoS Method

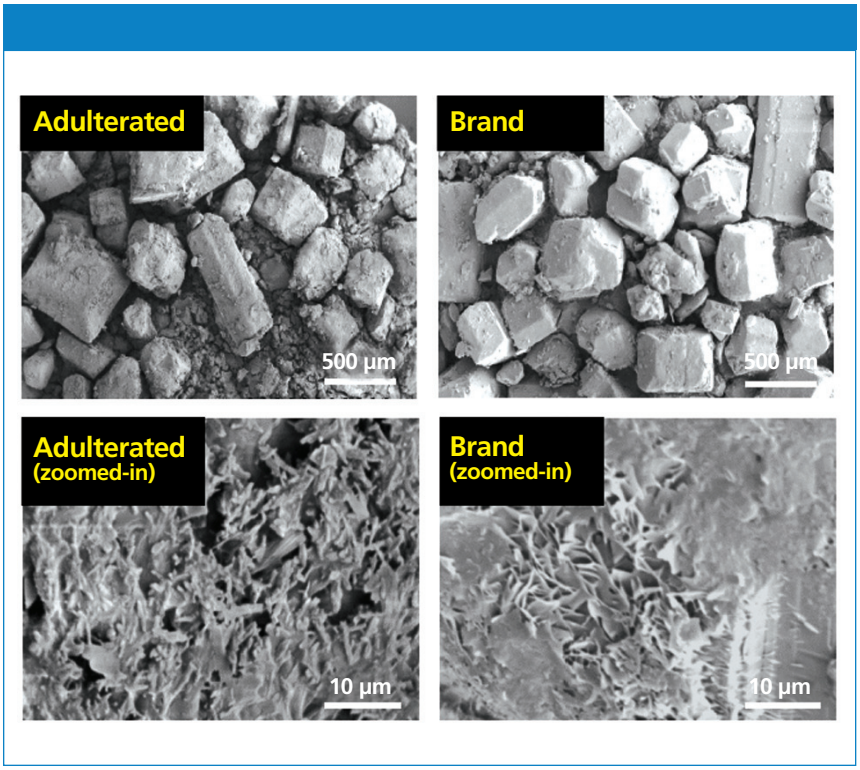


Figure 10: Refined scanning electron microscopy (SEM) of the adulterated samples and a control indicated changes in the surface were present. (Courtesy of R. Cohen.)

Development Olympics next year," he said.

The benefits of teamwork that Pan mentioned were also important to the Merck team. "The main reason Merck became involved was that it would foster better interaction between the specialty groups (NMR, MS, and high-throughput analysis) and the drug product analytical group," said Ryan Cohen. "It's being looked at as a huge success story internally, as a very good example of collaboration. I'm really glad that you guys are running this and we could be a part of it."

Michael Balogh *is a strategic technology development consultant. A former consulting principal scientist at Waters Corporation and Director of Strategic Relations, he has held the position of adjunct professor and visiting scientist at Roger Williams University and has been a reviewer for grant proposals for the National Science Foundation (NSF). Direct correspondence to: mpbalogh@gmail.com* ■

Highlights of SFC

Highlights from the oral and poster programs of the 9th International Symposium on Packed Column SFC (SFC 2015) are reviewed in this synopsis.

Larry Taylor

The 9th International Symposium on Packed Column Supercritical Fluid Chromatography (SFC 2015) was held in Philadelphia, Pennsylvania, on July 22–24, 2015. Selected highlights of both the oral and poster SFC 2015 programs are reviewed in this synopsis.

Scale-Up

SFC has long been used for chiral analysis in support of pharmaceutical development, but implementation of the technology in a regulated good manufacturing practice (GMP) production has its challenges. Daniel Markowitz from Johnson Matthey Pharmaceutical Material and Services presented benefits of this green alternative to conventional solvent-based crystallizations and low-pressure chromatography. The effects of injection loops, stacked injections, UV scaling, resin selection as well as solvent and CO_2 recycle at the plant scale were discussed. A new system was described that contained two 20-cm columns in parallel.

Jeffrey Kiplinger from Averica Discovery Services reminded the audience that the only important criteria for adoption of a technology are economic. In this regard, batch-to-batch cross contamination and system cleanout protocols and alternative fractionation or collection design were considered. Direct measurement of economic impact can result in better instrument design and better planning for new technology implementation.

Theory

Interest concerning various phenomena taking place in a column used for packed-column SFC separations continues to be relatively high. Don Poe of the University of Minnesota discussed the Joule–Thompson coefficient as a criterion for efficient operating conditions in SFC using porous and superficially porous packings in a convective air environment. The efficiency for elution of n-alkylbenzenes on 250 mm × 4.6 mm columns packed with 5-µm fully porous and superficially porous particles at optimum flow rates in a convective oven at 20 °C to 60 °C to 80 °C and pressures from 90 bar to 250 bar, with CO_2 mobile phase containing 5%, 10%, and 20% methanol (v/v) was measured.

In a separate study, Ruben De Pauw from the Vrije Universiteit Brussel identified and quantified the different contributions to extra-column band broadening in packed-column SFC, such as the influence of sample solvent, injection volume, extracolumn volumes, and detector cell volume or design. Abhijit Tarafder from Waters Inc., investigated how density gradient along a packed SFC column affects the overload band profiles of preparative SFC. A computer program that simulates changes in overloaded peak shapes as a function of density gradient at a given operating condition was used. Tarafder considered if having a steep density gradient in preparative SFC is always deleterious as in analyti-

cal situations, or if there could be some advantage in having density gradients in prep-SFC.

Both Poe and Tarafder preceded their technological presentations with a personal tribute to Professor Georges Guiochon, formerly of the University of Tennessee, with whom many manuscripts concerning packed-column SFC had been coauthored within the past five years.

Hyphenated Methods

The increasing demand for shortening development time lines in the pharmaceutical industry has made throughput analysis techniques very popular. Mohammad Al-Sayah from Genentech Inc., described the development of an on-line two-dimensional (2D) chromatographic system utilizing reversed-phase liquid chromatography (LC) in the first dimension and SFC in the second dimension. The 2D LC–SFC system could achieve simultaneous achiral and chiral analysis of pharmaceutical compounds. The peaks of interest from the first reversed-phase LC dimension column were effectively focused as sharp concentration pulses on a small-volume C18 trapping column and then injected onto the second-dimension chiral packed-column SFC column. Full automation of the system was achieved.

Christine Aurigemma from Pfizer Inc., discussed early efforts to implement open-access analytical packed-column SFC–LC in the medicinal chemistry laboratory that would allow chemists to work more efficiently. Adapting the technology to align with the work habits of chemists will be a key factor in facilitating the adoption of SFC to boost chemist productivity and efficiency.

Stationary Phases

Daniel Armstrong of the University of Texas at Arlington provided a lucid discussion of recent directions in packed-column SFC concerning chiral separations including new chiral selectors and small-particle-diameter column packings that will have a significant impact on the field. Another presentation by Vincent Desfontaine of the University of Geneva focused on the evaluation of new stationary-phase chemistries in sub-2-µm and core–shell material for the analysis of basic compounds in SFC. Various representative sets of basic drugs were injected such as mixes of active pharmaceutical ingredients with their respective pharmacopeia impurities. Different analytical conditions were also compared such as absence of additive, additive in the mobile phase, and additive in the injection solvent.

A lecture by Oleg Pokrovsky from the Kurnakov Institute of General and Inorganic Chemistry concentrated on stationary phases where the separation of closely related compounds was not based on hydrogen bonding. In these instances, the separation required involvement of other types of interaction such as electrostatic, dipole-induced dipole, or dispersion. A survey of several cases of non-hydrogen-bonding-driven separations of closely related compounds was considered such as xylenes, dichloroanilines, and methoxy-derivatives of psoralen. All ortho-substituted compounds were eluted earlier than "ortho-free" isomers. A distinctly stronger retention of 3,4-dichloroaniline compared with 3,5- and other isomers confirmed the significance of dipole–dipole and other electrostatic intermolecular interactions in the separation of this model compound. Standard column screening revealed that no achiral column was able to separate meta- and para-xylene in packed-column SFC except porous graphitic carbon (PGC), which suggested that PGC differs substantially from both hydrogen bonding and non-hydrogen bonding silica-based phases in the elution order of dichloroanilines.

Polysaccharide-based chiral stationary phases (CSPs) are well recognized as a powerful tool in chiral separation. To clarify the potential and selectivity feature of these phases in achiral separations, Tohru Shibata and associates from Daicel Corp., have systematically studied achiral isomer separations with polysaccharide-based phases under both high performance liquid chromatography (HPLC) and SFC conditions. The retention under both conditions roughly correlated, but inversion of elution order between isomers was sometimes observed. Nevertheless, these CSPs exhibited excellent potential for achiral separations and analysis in packed-column SFC as well as HPLC.

Applications: Cannabinoids

The cannabis industry is one of the fastest growing industries in the United States. Christopher Hudalla from ProVerde Laboratories Inc., presented the development of a workflow based on supercritical fluid technologies for the analysis, extraction, and purification of cannabinoids for the preparation of cannabis-based therapeutics. Complementing this presentation was the lecture of Ira Lurie from George Washington University on ultrahigh performance SFC for the analysis of synthetic cannabinoids. Presently, there are more than 20 synthetic cannabinoids under permanent or temporary federal control in the U.S. The effects of cosolvents, additives, pressure, temperature, and gradient slope on optimizing the separation were presented.

Applications: Medicinal

Productivity of modern medicinal chemistry requires instrumentation for automated synthesis and high throughput purification that can process a large number of samples within a meaningful timeframe. Gerard Rosse from Dart Neuroscience LLC discussed the decision-making process for selecting packed-column SFC coupled to mass spectrometry (MS) as the prevailing method for compound purification. Instrumentation for analytical and preparative SFC–MS, infrastructure, logistics, workflows, and robotics to support the purification of >10,000 compounds each month was presented.

The application of SFC for chiral metabolite separations in a DMPK environment was discussed by Hermes Licea Perez from GlaxoSmithKline. A complex mixture of 14 stereoisomeric metabolites provided important data on which species circulate in the human body. Packed-column SFC in combination with chemical derivatization was proven superior for the separation of four diastereoisomeric species of another drug development compound. The method was fully validated and applied to evaluate potential in vivo

chiral conversion in pooled clinical and preclinical samples.

Implementation of SFC in regulated GMP laboratories has been somewhat slow, owing to limitations in instrument sensitivity, reproducibility, accuracy, and robustness. Michael Hicks from Merck Inc., reported on an investigation into the use of modern packed-column SFC for enantiopurity analysis of several pharmaceutical intermediates and compared the results with conventional HPLC approaches historically used for analysis in a GMP setting. The findings clearly illustrated that modern packed-column SFC now exhibits a degree of precision, reproducibility, accuracy, and robustness comparable to that of HPLC.

Alexandre Grand-Guillaume Perenoud of the University of Geneva focused on the evaluation of a modern packed-column SFC–HRMS (quadrupole time-of-flight [QTOF]) platform as a potential key analytical tool to support bioactive identification. The preliminary screening step involved 15 different state-of-the-art stationary phases and over 100 natural compound standards. The author highlighted the applicability of SFC to the natural compound space, which included highly polar and very apolar molecules. In parallel, selected column chemistries have been identified as particularly well suited for the analysis of specific compound subclasses. Finally, optimized analytical conditions were applied for the full characterization of several plant extracts.

Applications: General

Edgar Naegele from Agilent Technologies lectured on the quantitative determination of multipesticide residues in vegetables by packed-column SFC coupled to triple-quadrupole MS. The final optimized method was performed on an amino column at a flow rate of 3 mL/min in a gradient with methanol as the organic modifier. The multipesticide sample comprised 17 pesticide compounds. For all compounds, the achieved linearity was better than 0.999, limits of quantification (LOQs) were typically below 2.9 ppm, retention time relative standard deviations (RSDs) were below 0.4%, and area RSDs were below 4%. Matrix effects were found in the recovery range of 70–120% and LOQs were at 10 ppb, which met typical requirements for quantitative determination of pesticides in a food matrix.

To minimize internal corrosion of carbon steel, chemical corrosion inhibitors are most frequently used. These materials are amine-based and as such exhibit high toxicity to aquatic organisms, hence stricter regulations have been imposed regarding the use and subsequent discharge of such chemicals in the environment through produced water. John Langley of the University of Southampton reported on the preparation of a model corrosion inhibitor comprising quaternary amines, imidazolines, and imazolines for qualitative analysis using HPLC–MS and ultrahigh-performance SFC–MS (UHPSFC–MS). The use of modern packed-column SFC as the chromatographic separation decreased analysis times by eliminating the sample preparation step before analysis, especially in the case of crude oil, and reduced elution times by a factor of four when compared to HPLC.

Robert Campbell from Dow Chemical Company noted further applications in a study of the composition of co-polymeric surfactant materials via packed-column SFC–MS with electrospray ionization. Frequently, the spectra and chromatograms are too complex for interpretation with acceptable degrees of confidence. Software-assisted characterization has been used for deconvolution of the complex data sets. Factors such as adduct formation, multiple charging, and the degree of ionization were shown to complicate spectral interpretation. The resulting data analysis made the elucidation of detailed structural information possible with a high degree of confidence.

Supercritical Fluid Extraction

The U.S. Food and Drug Administration (FDA) requires that at least 97% of caffeine be removed in order to call the product decaffeinated coffee. Supercritical carbon dioxide is an ideal fluid to apply to this process. Extraction is always performed on the green beans. Dried green beans are quite hard and dense so a wetting process, causing some swelling of the beans, is necessary before supercritical fluid extraction (SFE) can be performed. John Langley from Waters Corp., demonstrated various extraction techniques as well as a method for the rapid and convenient determination of residual caffeine levels using SFC.

Best Posters

The subject of this year's winning poster dealt with SFE. Jacquelyn Runco from Waters Inc., demonstrated an SFE–SFC workflow to isolate target flavor compounds from vanilla beans and ground cinnamon. The use of automated processes such as solvent selection, mobile-phase composition, and vessel switching allowed for quick extraction method screening. For both samples, multiple extraction parameters were evaluated to determine optimal yield and extract complexity.

The award for second place best poster presentation went to Takato Uchikata from Shimadzu Corp., for extraction and analysis using on-line SFC–SFE–MS. The hyphenated system was capable of simultaneous multicomponent analysis with on-line automation of everything from sample pretreatment to separation and analysis. For example, in the analysis of pesticides in food products, the system took only 5 min for a complete analysis involving sample pretreatment when compared to at least 35 min for conventional systems. Additional applications included the analysis of biomarkers from dried blood spots and extraction of trace additives in polymers.

SFC 2016

A one-day SFC conference will be held in San Diego, California on May 23, 2016. SFC 2016 will be held in Vienna, Austria, on October 5–7, 2016. Oral and poster presentations will be solicited in the areas of SFC and SFE in the spring of this year. Information on both conferences can be found at www.greenchemistrygroup.org.

Larry Taylor *is an emeritus professor of chemistry at Virginia Tech, in Blacksburg, Virginia, program co-chair for SFC 2015–2016, and a member of the Green Chemistry Group.* ■

Debby Mangelings, *LCGC*'s 2016 Emerging Leader Award Winner, Focuses on Chiral Separations

Debby Mangelings, the winner of the 2016 *LCGC* Emerging Leader in Chromatography award, is an associate professor in the Department of Analytical Chemistry and Pharmaceutical Technology at the Vrije Universiteit Brussel, in Brussels, Belgium. Mangelings's work has focused primarily on chiral separations. Mangelings recently spoke to *LCGC* about her scientific background, interests, and recent work.

Where or how did your interest in analytical chemistry and chemistry begin?
When I was studying pharmacy, the theoretical courses and the practical exercises of analytical chemistry were always appealing to me. Though these techniques seemed complicated for a student, I enjoyed learning what one can do with them. The same applies for chirality: as a first year student, I became fascinated by the fact that mirror images of molecules exist, and that they display different activities in the human body. Later, I had the opportunity to do my master's degree thesis on chiral separations with reversed-phase liquid chromatography. This was the perfect subject!

You have done some significant research in chiral separations. How did the project on defining and updating chiral separation strategies for several separation techniques such as high performance liquid chromatography (HPLC), capillary electrophoresis (CE), capillary electrochromatography (CEC), and supercritical fluid chromatography (SFC) get started? What were the biggest challenges in that project? What benefits does it bring to the field?
The chiral project in our department already started a few years before I began my PhD work. It originated from a cooperation with Sanofi, who wanted us to develop software (a knowledge-based system) that guided the analyst through chiral method development without prior knowledge of compound structures. This was the basis of developing strategies: They had to be generic, so applicable to any new drug compound, regardless of its structure. To develop such software, the strategies were constructed as decision trees, providing information on what to do next based on the outcome of the previous experiment. In all of our strategies some method optimization steps are also included, while most others just stop after the screening step. We always used polysaccharide-based chiral phases in our strategies. In the initial studies, the selection of the chiral phases for the screening step was quite straightforward. However, after the cooperation with Sanofi was finished, new chiral stationary phases (CSPs) were marketed with chlorinated polysaccharide selectors, which provided possibly better separation rates than those with non-chlorinated selectors. Therefore, in the next project we initiated the update of existing strategies in the HPLC modes and also in CEC. At the same time, we also started research in SFC (earlier work was done at Sanofi), and defined a strategy for that technique too.

The benefits of the strategies are quite obvious: Anyone can use them for chiral method development for their compounds of interest, as they are applicable to any small molecule, independent of its structure.

The biggest challenge for me personally in the chiral project was the introduction of CEC in the lab, and the definition of a chiral separation strategy with this technique, which was the subject of my PhD thesis. CEC is a combination technique of capillary electrophoresis and capillary liquid chromatography, which is still mostly used at the academic level because it has too many disadvantages at this moment to be used in an industrial environment. One also needs some skills to pack CEC columns, but once you have the knowledge, you can easily work with the technique.

In addition, the introduction of SFC in our lab for chiral separations was also a very exciting period: I was really impressed by how fast this technique is and how easy it is to tune the separations.

What prompted your research into the chemometric data analysis of chiral separation data to study systems with similar or dissimilar enantioselectivity? What have your results shown so far?
When selecting the best chiral phases to be included in a screening step, we always counted the number of separations obtained from a test set manually and then we selected a number of

complementary phases to be included in the screening. This work tends to be very time consuming, which is the reason why we tried to use chemometric algorithms to do this selection automatically. It would facilitate the work of updating existing strategies with new phases. If there is a new phase, one just needs to analyze it at the screening conditions of the strategies using a fixed test set of compounds, and then this phase would be characterized by the obtained resolutions or selectivities. Chemometric techniques then allow us to see whether the phase adds something new to the earlier tested ones and then select the most dissimilar (most complementary) phases. Our research indicated the usefulness of chemometrics in the comparison of phases, but also revealed that visualization of the selected results is always needed, because a phase that does not separate any compound is also very dissimilar to a phase that is able to separate many compounds. Of course, it is obvious that the former phase is not useful in a screening step.

Can you tell us about your research in the evaluation of new stationary phases for CEC, such as those with smaller particle diameters or with core–shell particles?

The research on core–shell particles in CEC is challenging, especially at the level of column packing. We are still in the beginning of this research so conclusive results still need to be generated. We also have not yet begun to use very small particles for chiral separations.

You were also recently involved in the successful chiral separation of uncommon compounds as the boron cluster species. Can you tell us about that work and what it entailed?

Boron cluster species composed a completely new set of test compounds to me, so it was a challenge to know whether the screening conditions of our chiral strategies in normal-phase LC and POSC were applicable for them. Two types of boron clusters were considered, that is, zwitterions and anions. For the first class, our normal-phase LC and POSC screenings were applicable. However, the anions usually could not be eluted in normal-phase LC, probably because of precipitation, and in polar organic solvent chromatography (POSC) they were not separated in the majority of the tested conditions. This study showed that the chiral discrimination potential of polysaccharide selectors is meaningful to analyze structurally chiral boron cluster species, but needs further systematic research, in which recognition mechanisms should be explored.

What research are you the most proud of thus far?

I believe that the PhD research one performs always remains special. Given the challenge to introduce capillary electrochromatography in the lab and the results I was able to generate with this technique, I am proud of the work I performed with CEC.

Also, our work in SFC was impressive to me: We introduced the technique in our lab and defined a complete chiral separation strategy in a rather short time.

What kind of research is your group currently involved in?

For chiral separations, we currently have two research projects: The first one investigates new types of chiral stationary phases in CEC mode, such as core–shell phases, and the second focuses on developing a methodology for the enantioseparation of peptides. In all of our research, we try to implement the use of experimental designs in method optimization to gain knowledge about the entire experimental domain.

Besides these projects, we are working on precision improvement and transferability of capillary electrophoresis methods between different instruments and laboratories. Also here, we are using chiral separations as test cases.

In SFC, we have a project on the development of drug impurity profiles. We finished the characterization of an extended set of stationary phases and the selection of the most dissimilar stationary phases. Now we are investigating the parameters that can be used for optimization of the obtained separations, which will be followed by the definition and validation of the complete methodology.

A final topic is the analysis of herbal extracts, on which we have several ongoing projects. Herein we try to characterize given plants with medicinal properties through the development of fingerprint chromatograms, occasionally followed by liquid chromatography–mass spectrometry (LC–MS) to identify important peaks. The resulting fingerprint chromatograms are analyzed by means of chemometric techniques and allow us to identify samples and perform similarity analysis, classify samples, predict activities (for example, anti-oxidant, toxicity) of the samples from the obtained fingerprints, and indicate peaks in the chromatogram that may be responsible for a given activity.

You have previously supervised six PhD students and currently supervise six more. How do you guide your students to select important or relevant theses and research projects?

The topics our PhD students work on are usually defined by me and my colleague (Yvan Vander Heyden) based on what is provided to us as interesting research subjects from pharmaceutical companies, what we see as a next stage in ongoing research, or what we consider interesting for a new PhD project. We define the initial project, but of course the student develops and executes the experiments and occasionally it is to be modified as a consequence of the obtained results.

Your work has been published extensively (about 70 articles and 11 book chapters) and you have given or coauthored 61 oral and 83 poster presentations at national and international congresses and symposia. How do you balance working on new or cutting-edge research and the demands of teaching, supervising PhD students, as well as giving lectures and writing papers to share with your peers?

I consider the supervision of PhD students, giving lectures, and writing papers all as part of research. As a professor at a university, one is supposed

to both teach and do research. Finding the balance between teaching and research is not evident: Often, lots of administrative tasks come along with teaching, and they take more time than one may expect. For the moment, I am teaching all my courses in the first semester of the academic year, which only leaves me limited time for research in that semester. On the other hand, I, in principle, have a whole second semester to spend on research! However, reality is in some periods different because of other university obligations. Searching for a good balance between research, teaching, and other university-related tasks is therefore a continuous challenge!

Have you faced any difficulties as a young, female scientist? What advice would you offer to other female scientists just starting out?

I have never experienced any difficulties as female scientist, or not that I know of. The main reason is that we are a pharmaceutical department and the majority of pharmacists are women.

My advice to other female scientists is that finding a good balance between family life and an academic career is not an easy task, but it is possible and worthwhile to try.

What do you plan to focus on next? Is there one big problem in separation science that you really want to tackle?

Further investigating of the possibilities of CEC as a separation technique will be something I will always try to continue. I hope that the technique will be able to deliver what is expected one day. The project on the chiral separation of peptides is also something I am really looking forward to. We will also use chiral phases with other selectors than those we have been studying until now, so it will definitely be an instructive period!

Debby Mangelings's work has focused primarily on chiral separations. She has focused on the definition and updating of chiral separation strategies for various modes of HPLC—including normal-phase, reversed-phase, and POSC—as well as for CE and CEC. She has more recently developed SFC methods for chiral analyses and non-chiral analysis for drug impurity profiling. The synthesis of in-capillary stationary phases, such as monoliths for both chiral and achiral separations in CEC, is another one of her interests. More recently, Mangelings has become involved in the chemometric data analysis of chiral separation data to study systems with similar or dissimilar enantioselectivity. In CEC, she is working on the evaluation of new stationary phases, such as those with smaller particle diameters or with core–shell particles. Finally, she recently was involved in the successful chiral separation of uncommon compounds as the boron cluster species. This interview has been edited for length and clarity. For more information on our 2016 LCGC *award winners, please visit www.chromatographyonline.com/2016-lcgc-awards.* ■

PRODUCTS & RESOURCES

Solid-core analytical columns

Waters' CORTECS C8 and CORTECS Phenyl analytical columns are designed for HPLC and UHPLC separations. According to the company, the columns are available in 1.6- and 2.7-µm particles and are offered in 50 column configurations.
Waters Corporation,
Milford, MA.
www.waters.com/cortecs

Evaporator

Glas-Col's Zip-Vap4 evaporator is designed for the removal of solvent and water in auto-sample vials and various plate configurations. According to the company, gas flow, temperature, and vertical plate movement are programmable and can be stored as a recipe.
Glas-Col,
Terre Haute, IN.
www.glascol.com/zipvap4

GC–TOF-MS brochure

A brochure from LECO discusses how the company's GC–TOF-MS systems and corresponding products can improve laboratory productivity and efficiency. According to the company, the brochure details how acquisition speed drives deconvolution with its ChromaTOF software.
LECO Corporation,
St. Joseph, MI.
www.leco.com

HPLC column

Machery-Nagel's NUCLEODOR C18 Gravity-SB HPLC column is designed for analytical separation of polar compounds such as antibiotics, water-soluble vitamins, and organic acids. According to the company, the column is a monomeric octadecyl modified phase that features hydrophobic and polar selectivity.
Macherey-Nagel Inc., Bethlehem, PA.
www.mn-net.com

GC detector

OI Analytical's 5383 pulsed-flame photometric gas chromatography detector is designed for determining sulfur and sulfur compounds in petrochemicals, beverages, pesticide residues, and flavor and fragrance analysis. According to the company, when mounted in a GC or GC–MS instrument, the detector provides the ability to determine and analyze low levels of sulfur, phosphorus, and 26 other analytes of interest. **OI Analytical, a Xylem brand,** College Station, TX. www.oico.com

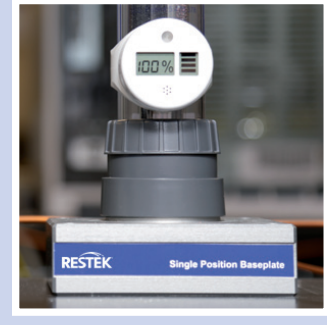
Column-based preparative fractionation system

Polymer Char's PREP C20 automated column-based fractionation system is designed for the fractionation of polyolefins. According to the company, the system is capable of fractionating up to 20 g of polymer—depending on the sample—according to its chemical composition.
Polymer Char,
Valencia. Spain.
www.polymerchar.com/PREP_C20

Gas purifiers

GasTrap purifiers, available from Quadrex, are designed to be self-regenerating, and reportedly can eliminate the need for replacing in-line gas filters. According to the company the purifiers are suitable for GC, GC–MS, and other laboratory applications.
Quadrex Corporation,
Woodbridge, CT.
www.quadrexcorp.com

Electronic maintenance indicator



Restek's electronic maintenance indicator is designed as a monitoring device that warns when planned maintenance for a gas filter is due. According to the company, the indicator can be attached to a consumable and will progressively display real-time filter status, allowing analysts to follow a controlled replacement schedule.
Restek Corporation,
Bellefonte, PA.
www.restek.com

HPLC analyzer

Shimadzu's BioEthanol analyzer is based on the company's Prominence-i integrated HPLC system and is designed for real-time monitoring of the fermentation process in bioethanol production. According to the company, remote monitoring of the analyzer via the i-Series web interface or LabSolutions Direct provides instrument status and chromatogram information from any location, using any smart device or PC. **Shimadzu Scientific Instruments,** Columbia, MD.
www.ssi.shimadzu.com

Bioanalysis application note

An application note from Tosoh Bioscience titled "DAR Analysis of Antibody Drug Conjugates Using a TSKgel HIC Column" reportedly demonstrates the separation of unconjugated and drug conjugated trastuzumab samples with baseline resolution using the company's TSKgel Butyl-NPR column. According to the company, the baseline resolution enabled an integration and quantification of different drug payloads in ADC characterization.
Tosoh Bioscience, LLC,
King of Prussia, PA.
www.tosohbioscience.com

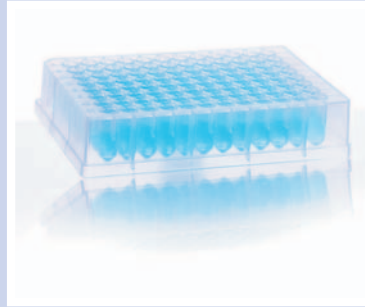
HPLC columns

UCT's Selectra HPLC columns reportedly consist of pure and highly reproducible silanes bonded to a type B spherical silica support. According to the company, 1.8-, 3-, and 5-µm particle sizes are available in HPLC and UHPLC hardware formats.
UCT, LLC,
Bristol, PA.
www.unitedchem.com/sites/default/files/docs/posters-and-papers/hplc_all.pdf

Natural products applications notebook

An applications notebook from Waters Corporation reportedly describes applications with experimental conditions for the LC and LC–MS analysis of samples of botanical, traditional medicine, marine, and bacterial or fungal origins. According to the company, the application notebook is available for download on the company's website.
Waters Corporation,
Milford, MA.
www.waters.com/naturalscience

Microplates



AntiBIND microplates from Wheaton are designed to reduce protein binding and adsorption. According to the company, the surface of polypropylene plates is hydrophilic, resulting in protein recovery increases.
Wheaton,
Millville, NJ.
www.wheaton.com

LC columns

YMC-Triart C18 ExRS columns from YMC America are designed for use with hydrophobic substances and isomers. According to the company, the columns provide high carbon loading (25%) and are chemically stable at pH extremes (pH 1–12), mechanically rugged, and scalable from UHPLC through preparative separations.
YMC America,
Allentown, PA.
www.ymcamerica.com

Elemental analyzer

Thermo Fisher Scientific's FLASH HT Plus EA-IRMS system is designed for the determination of C, N, S, O, and H isotopic signatures in geoscience, ecological, environmental, and food samples. According to the company, the system includes automation for five elements and dedicated features for isotopic determination.
Thermo Fisher Scientific,
San Jose, CA.
www.thermoscientific.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

Autosampler syringes

Autosampler syringes for liquid and gas chromatography from Hamilton are designed specifically for CTC PAL liquid chromatography autosampler systems. According to the company, the S-Line syringes complement its existing C-Line and X-Type syringes, and are suitable for everyday use.

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

Active inlet replacement cartridge

The Opti-Max 600 bar active inlet replacement cartridge from Optimize is designed with 316 stainless steel, PEEK, and zirconia for compatibility in 400-bar and 600-bar applications. According to the company, the cartridge's zirconia ball travel is minimized, allowing the cartridge to exhibit low pulsation.

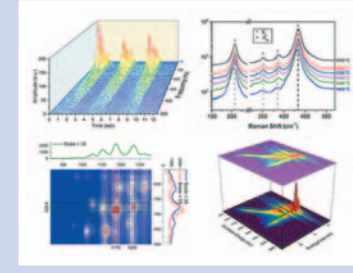
Optimize Technologies, Inc.,
Oregon City, OR.
www.optimizetech.com

Capillary GC columns

Watercol capillary gas chromatography (GC) columns from Supelco, a MilliporeSigma brand, reportedly contain ionic liquid stationary phases that produce a sharp peak shape for water, allowing measurement of water by GC. According to the company, narrow peak widths and optimal peak heights are also produced for other small polar analytes.

Supelco/Sigma-Aldrich,
Bellefonte, PA.
sigma-aldrich.com/watercol

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

Portable GC–MS analyzer

PerkinElmer's portable Torion T-9 gas chromatography–mass spectrometry analyzer is designed to be carried in the field. According to the company, the analyzer enables rapid screening of environmental volatiles and semivolatiles, explosives, chemical threats, and hazardous substances.

PerkinElmer,
Waltham, MA.
www.perkinelmer.com

Postcolumn derivatization system

Pickering's Pinnacle PCX Sigma Series is designed as an optimized HPLC postcolumn derivatization system for the analysis of samples such as amino acids, carbamates, mycotoxins, and antibiotics. According to the company, the system includes an electronic syringe pump and valves, a quick-change reactor cartridge, a column oven, inert flow paths, a liquid crystal display, and control software. The system reportedly works with all HPLC systems.

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

GC–MS system

Thermo Fisher Scientific's DFS GC–magnetic sector high-resolution MS system is designed for the analysis of dioxins and persistent organic pollutants. According to the company, the system provides worldwide full compliance with official dioxin, PCB, and PBDE methods (for example, EPA 1613, 1668, and 1614).

Thermo Fisher Scientific,
San Jose, CA.
www.thermoscientific.com/DFS

Perfluorinated compound SPE system

The TurboTrace PFC Parallel solid-phase extraction (SPE) system from FMS reportedly automates existing manual SPE techniques for perfluorinated compounds (PFCs), which are ubiquitous in the environment. According to the company, the system is closed, and is designed and built to provide low background and deliver reproducible and consistent results.

FMS, Inc.
Watertown, MA.
www.fms-inc.com

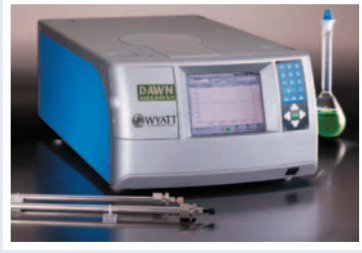
GC–MS system

Shimadzu's GCMS-QP2020 high-sensitivity GC–MS system is designed with a multifunction ion source, high-speed scan control, and an ultrafast turbomolecular pump. According to the company, comprehensive databases and multiple sample introduction devices enable custom configurations for use in environmental, food, and forensics laboratories.
Shimadzu Scientific Instruments, Columbia, MD.
www.ssi.shimadzu.com

UHPLC connection system

The MarvelX UHPLC connection system from IDEX Health and Science, offered by Supelco, is designed for routing throughout an UHPLC instrument and is compatible with 10-32 coned receiving ports and fingertight to 19,000 psi. According to the company, the system is available in stainless steel as well as in biocompatible PEEK-lined stainless steel precision-cut tubing, and comes with removable stainless steel fittings.
Supelco/Sigma Aldrich, Bellefonte, PA.
sigmaldrich.com/hplc-accessories

SEC–MALS detector



The Dawn Heleos-II multiangle light scattering (MALS) detector from Wyatt Technology is designed for absolute molecular weight and size determinations of polymers and biopolymers in solution. According to the company, the detector may be connected in series to any chromatographic system to determine absolute molar masses without the use of reference standards or column calibration.
Wyatt Technology Corp., Santa Barbara, CA.
www.wyatt.com

GC–MS system

The Agilent 5977B high-efficiency source (HES) gas chromatography–mass selective detector (MSD) system is designed as a tandem gas chromatograph and mass spectrometer. According to the company, the detector allows scientists to use smaller sample volumes, spend less time on sample preparation, reduce instrument downtime, minimize solvent usage, and reduce the environmental impact of GC–MS analysis.
Agilent Technologies, Santa Clara, CA. www.agilent.com

Anticorrosion coatings

SilcoTek's coatings for liquid chromatography and gas chromatography applications are designed to make the surfaces of customer-supplied components more suitable for analytical applications. According to the company, the coatings prevent unwanted chemical reactions, corrosion, and particulate sticking within the flow path.
SilcoTek Corporation, Bellefonte, PA.
www.silcotek.com

Gas chromatograph

The Calidus gas chromatograph from Falcon Analytical is designed to use the new ASTM D7798 method to perform simulated distillation nearly six times faster than the equivalent D2887 method. According to the company, the method's speed enables increased throughput, repeatability and reproducibility, tight control parameters, cost reduction, and feedstock conservation.
Falcon Analytical, Lewisburg, WV.
www.falconfast.net/calidus

Reservoir sensor system

The Sonic Reservoir Sensor system from JM Science is designed to measure levels of solvents and liquid waste used in unattended liquid chromatography separations in real time. According to the company, the system automatically sends a signal to stop the pump when solvents get low or to switch to a valve to continue analysis without interruption.
JM Science, Inc., Grand Island, NY.
www.jmscience.com

Robotic sampler

Gerstel's MPS robotic sampler is designed to perform automated liner exchange (ALEX) for routine GC–MS-MS analysis of matrix-containing samples. According to the company, the robotic sampler replaces the GC inlet liner at user-defined intervals, eliminating the need for cleanup steps during sample preparation.
Gerstel GmbH & Co., KG, Linthicum, MD.
www.gerstel.com

CALENDAR

29 May–3 June 2016
40th International Symposium on Capillary Chromatography (ISCC) and 13th GCxGC Symposium
Riva del Garda, Italy
mytus.unime.it/slider.html

5–9 June 2016
64th ASMS Conference on Mass Spectrometry & Allied Topics
San Antonio, TX
www.asms.org/conferences/annual-conference/annual-conference-homepage

7–9 June 2016
IVT's 4th Annual Microbiology Week
Philadelphia, PA
www.cbinet.com/conference/pi16056#.Vl4Ogt-rREI

14 June 2016
5th International Inverse Gas Chromatography (IGC) Symposium 2016
Munich, Germany
inverse-chromatography.com/

19–24 June 2016
44th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2016)
San Francisco, CA
www.hplc2016.org/

21–23 June 2016
14th Annual Product Complaints Congress for Life Sciences
Bethesda, MD
www.ivtnetwork.com/conference/pc16120

3–6 July 2016
18th International Symposium on Advances in Extraction Technologies (ExTech'2016) & 22nd International Symposium on Separation Sciences (ISSS'2016)
Torun, Poland
www.extech-isss2016.pl/

17–20 July 2016
PREP 2016—29th International Symposium on Preparative and Process Chromatography
Philadelphia, PA
www.prepsymposium.org

8–12 August 2016
National Environmental Monitoring Conference (NEMC)
Orange County, CA
nemc.us/index.php

20–26 August 2016
21st International Mass Spectrometry Converence (IMSC 2016)
Toronto, Canada
www.imsc2016.ca/

21–24 August 2016
New Zealand Institute of Chemistry Conference (NZIC-16)
Queenstown, New Zealand
www.nzic16.org/

28 August–1 September 2016
31st International Symposium on Chromatography (ISC 2016)
Cork, Ireland
www.isc2016.ie/

28 August–2 September 2016
36th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2016)
Florence, Italy
dioxin2016firenze.org/

12–15 September 2016
NANOSTRUC 2016: The 3rd International Conference on Structural Nano Composites
Aberdeen, Scotland
www.nanostruc.info/

12–15 September 2016
25th ICP-MS User Meeting & 12th Symposium Mass Spectrometric Methods of Trace Analysis
Siegen, Germany
icpms-anwendertreffen.de/

12–15 September 2016
Mass Spectrometry: Applications to the Clincal Lab (MSACL) 2016 EU, 3rd Annual Congress & Exhibition
Salzburg, Austria
www.msacl.org

13–15 September 2016
37th British Mass Spectrometry Society (BMSS) Annual Meeting 2016
Eastbourne, England
www.bmss.org.uk/bmss2016/bmss2016.shtml

18–22 September 2016
15th Human Proteome Organization World Congress (HUPO)
Taipei, Taiwan
www.hupo2016.org/index.html

18–23 September 2016
23rd International Symposium on Electro- and Liquid-Phase Separation Techniques
Minneapolis, MN
www.cegss.ptchem.pl/itp-2016-23rd-international-symposium-electro-and-liquid-phase-separation-techniques

18–23 September 2016
SciX 2016
Minneapolis, MN
www.scixconference.org/

18–23 September 2016
Forensic Isoptope Ratio Mass Spectrometry (FIRMS) Conference 2016
Auckland, New Zealand
www.forensic-isotopes.org/2016.html

AD INDEX

THE ESSENTIALS

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

How U Is Your UHPLC System?

Ultrahigh-pressure liquid chromatography (UHPLC) is a powerful tool for increasing high-performance liquid chromatography (HPLC) sample throughput, chromatographic efficiency, and sensitivity. However, how simple is it to transfer between HPLC and UHPLC applications? There are several parameters associated with both the chromatographic method and the system hardware that need to be considered when switching to UHPLC. This article gives you some practical tips to make sure your UHPLC system is as "U" as possible.

Tips for Reducing System Extracolumn Volume

Extracolumn volume is the total volume contributed by all system components and capillary tubing from sample injection to detection, which are not directly involved with the separation process. Extracolumn volume is the predominant factor in loss of efficiency when using narrow internal diameter columns because of the contribution of peak dispersion; therefore, it is very important to minimize extracolumn volume throughout the system. This can be done by using short lengths of tubing with a reasonable internal diameter.

Peak dispersion is related by the Aris-Taylor equation to flow rate, tubing length, and internal diameter, and the molecular diffusion coefficient of the analyte in the mobile phase, with tubing internal diameter having the greatest impact. Therefore, it would seem that to reduce peak dispersion we should be using the narrowest possible tubing. However, using very narrow tubing comes with a compromise. When tubing internal diameter is decreased, the pressure required to move mobile phase through the tubing increases—for example, plumbing a system with 0.002-in. tubing and running a flow rate of 3 mL/min requires a pressure input of 1500 bar (and this is only to move the mobile phase through the tubing), leaving no pressure capability in the system to be able to install a column and run a separation.

A good compromise between dispersion and pressure is to use 0.005-in. tubing, which at the same flow rate would require a pressure of only 200 bar. It is essential to use the correct column endfittings—ideally zero-dead-volume fittings should be used, with many manufacturers providing specialist UHPLC fittings. Incorrect fittings can lead to leaks or an increase in extracolumn volumes resulting from voids.

If you are using an in-line filter (possibly the most high value piece of the system as they prevent blockages), make sure it has a low dead volume.

Look carefully at the injection system—for example, for a flow-through system look at the apparently small contribution from the needle seat capillary. It is also worth considering the injection volume, which should be matched to the column geometry. A good rule of thumb is to maintain the injected volume at 1–5% of the column dead volume. Most UHPLC experiments are performed with a 50 mm × 2.1 mm column (V_0 = 120 µL), so the injected volume should be 1–5 µL to limit peak dispersion.

Tips for Minimizing Frictional Heating

Frictional heating is the viscous heating of the mobile phase as it passes through very small diameter particles, causing a rise in temperature over the column length. Temperature impacts efficiency, retention, and selectivity. Frictional heating effects can be minimized through the use of columns with smaller internal diameters (2 mm is the sweet spot), which are less susceptible to heating and enable more-effective heat dissipation. Column ovens will ensure that the column temperature is accurately controlled. Preheating mobile phases reduces differences in temperature between the inlet and outlet that can cause diffusion of the sample plug, which leads to peak dispersion. Conversely, post-column cooling can be applied to mitigate any peak dispersion. Superficially porous particles also have improved heat dissipation.

Tips for Detecting UHPLC Peaks

Smaller peak volumes and narrower peaks in UHPLC have an impact on both detector hardware and settings. Detector flow cells are often the main source of extracolumn volume; therefore, to minimize this, and in conjunction with the reduction in injection volumes, reduced flow-cell volumes are commonly used (≤2 µL). One compromise of reducing cell volume is the need for a reduction in pathlength, which can have an impact on sensitivity. If greater sensitivity is required, then selecting a larger cell volume and losing some efficiency may be necessary.

It should be noted that if injection volumes are not correctly scaled then overloading of the detector can be encountered. There is also the issue of how the detector acquires the data and reports to the data system. Detector sampling rates (measured in hertz) must be high enough that enough points are detected across the peak to allow proper quantitation. At low sampling rates, peak apices can be missed and numerical integration will be inaccurate. Low data sampling rates alone do not lead to peak broadening, rather a combination of sampling rate and filtering electronics do.

For example, in one system, if the detector is set to acquire data at 40 Hz many data points will be detected across the peak and the data will exhibit a fine structure. Reducing the sampling rate to 1.5 Hz results in fewer data points being detected, which results in a loss of some of the fine structure (for example, the peak apex). An alternative system may exhibit peak broadening when the detector sampling rate is reduced, which can probably be attributed to how the data are handled in the analogue or digital domain inside the instrument module or software.

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