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THE LCGC AWARDS

Fluorinated Stationary Phase Interaction Mechanisms

Problems with Reversed-Phase LC Columns at pH Extremes

2016 Lifetime Achievement Award Winner
Milton L. Lee

2016 Emerging Leader Award Winner
Debby Mangelings

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Data Integrity in Pharma QC Labs: What You Need to Know
Loren Smith, Agilent Technologies

What Every Pesticide Analyst Needs to Know: Expert Roundtable Discussion
Katerina Mastovska, Covance Laboratories; Hans Mol, RIKILT-Wageningen UR; Amadeo Fernandez-Alba, University of Almeria, Spain; and Walter Hammack, Florida Department of Agriculture's Division of Food Safety and Chemical Residue Laboratory

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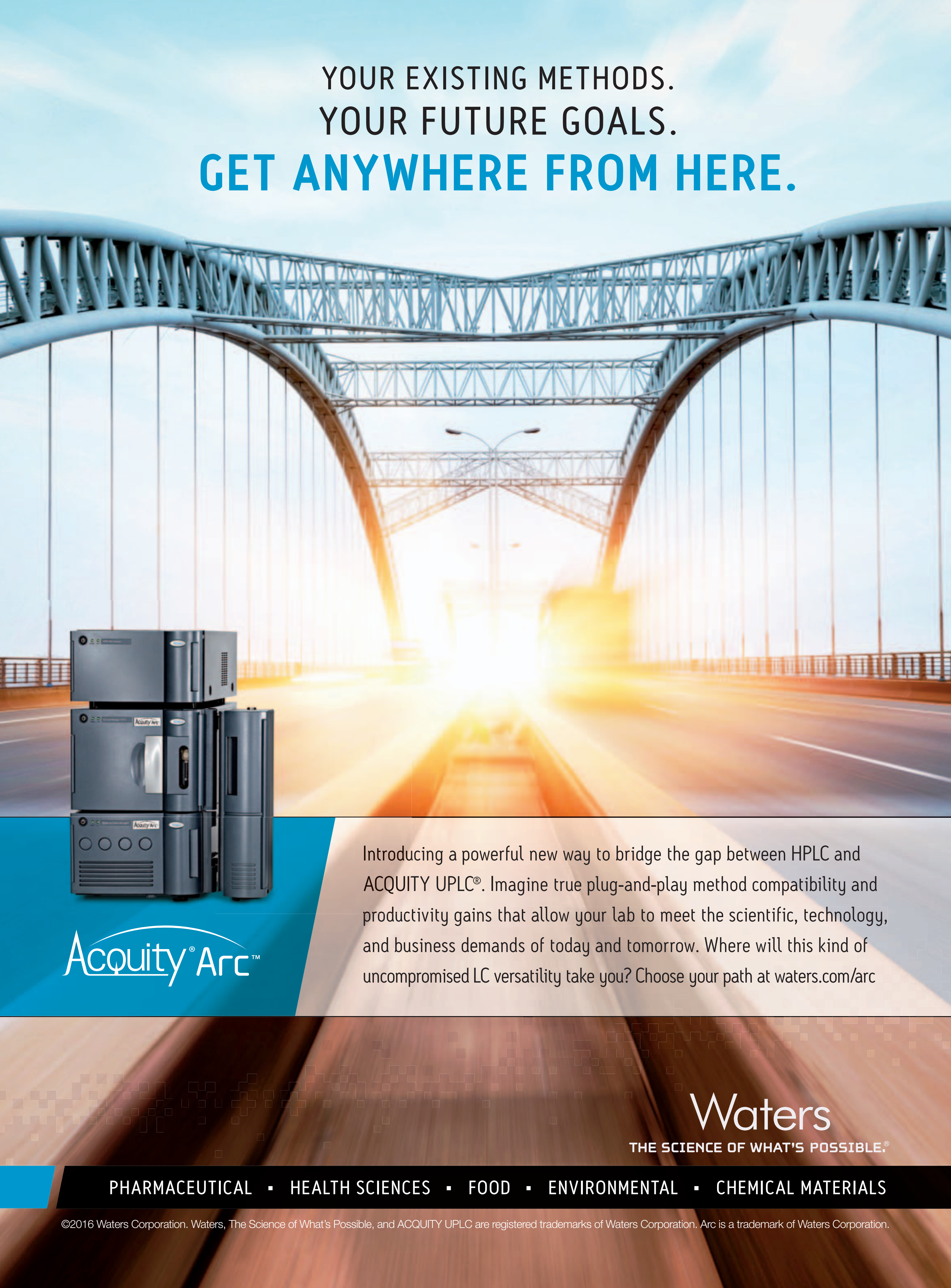
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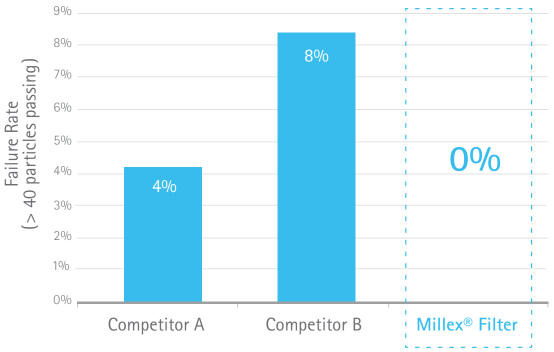

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PEAKS of Interest

The Effect of Microwave and Electron-Beam Treatments on Food and Packaging

A team of researchers based in France has examined the consequences of microwave and electron-beam treatments on food and its packaging using high performance liquid chromatography (HPLC) and gas chromatography (GC) (1).

The packaging of the food we eat poses a huge challenge to the food industry. Consumer demands place extra emphasis on the type of packaging that is produced. It should be compact, microwavable, and recyclable, not to mention biodegradable. It must also preserve the food contained within. As a result, manufacturers are turning to more-complex polymer materials to meet these demands. These materials need to be examined for their impact on human health and the environment; some polymers can release harmful substances when treated in certain ways.

Polypropylene (PP) films were prepared with a series of additives, and were then treated with electron-beam irradiation and microwaves. The PP extracts were separated into six fractions by normal-phase HPLC, followed by complete and automated on-line transfer of these fractions to GC (comprehensive two-dimensional analysis, HPLCxGC).

The potential toxicity of the extracts was assessed using three in vitro short-term bioassays, and migrations were performed using a standards-based approach. After the electron-beam treatment some additives decomposed, and there was a significant increase in the polyolefin oligomeric saturated hydrocarbons concentration. The electron-beam treatment had a stronger impact on the polymer than the microwave one. It produced degradation products from the additives as well as from the polymer. The additives reduced this degradation of the PP backbone significantly, but also formed degradation products themselves. The microwave treatments (800 or 1100 W) did not lead to structural changes in the PP film. However, GC coupled to flame ionization detection (FID)–mass spectrometry (MS) analysis indicated the presence of novel degradation products in the film (Irgafos 168 and Irganox 1076).

The team believes that this approach is a big step toward the assessment of the health risks of materials and objects in contact with food. It could also help to comply with the regulations concerning those materials that come into contact with food.

Reference

(1) A.M. Riquet et al., *Food Chem.* **199,** 59–69 (2016).

LGC Acquires Maine Standards Company

LGC (London, UK) has announced its acquisition of Maine Standards Company (Cumberland Foreside, Maine). A leading provider of calibration verification materials in the United States, Maine Standards Company bolsters LGC's portfolio of reference materials and proficiency testing schemes available for the clinical market.

Maine Standards Company will continue to operate from its recently opened facility near Portland, Maine. Tom Happe, Founder and President of Maine Standards Company, hoped the union of the two companies capabilities would ease the transition of Maine Standards Company into the global market and address growing interest in their services from overseas. ■

LCGC tv NEW VIDEOS FROM LCGC

JARED ANDERSON ON THE POTENTIAL FOR IONIC LIQUID-BASED COATINGS IN SPME

Jared Anderson of Iowa State University has designed new solid-phase microextraction (SPME) coatings based on polymeric ionic liquids. In a new interview, he discusses the potential for these coatings to improve SPME.

Other recent LCGC TV *interviews include:*

- Alan G. Marshall, on the impact of Fourier-transform ion cyclotron resonance mass spectrometry
- Apryll Stalcup on some interesting differences in the European versus American approaches to science education
- Giogia Greco on reversed-phase LC coupled to HILIC

Visit http://www.chromatographyonline.com to see these videos and more.

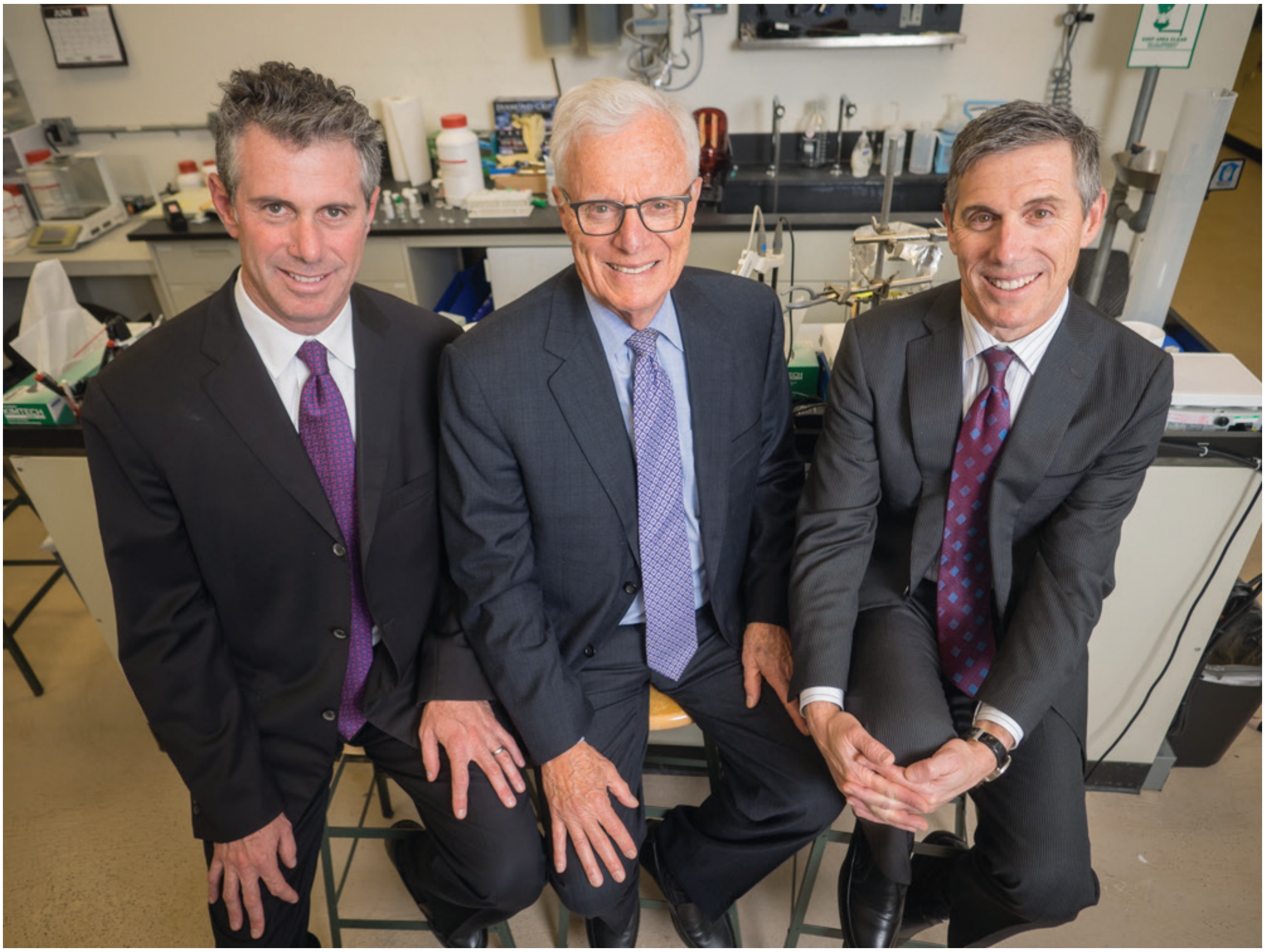
SPECIAL ISSUE HIGHLIGHTS

THE SECRETS TO SUCCESSFUL GRADIENT SEPARATIONS

Liquid chromatography separations using gradient elution have the potential to be faster, give better detection limits, and have more flexibility than separations using isocratic elution. But gradients can present challenges. In this e-book, John Dolan, the author of our "LC Troubleshooting" column, presents the secrets to achieving success with gradient separations. Topics covered include:

- **Intuition.** Gradient elution can seem counterintuitive. Yet with proper perspective, the things we intuitively understand about isocratic methods apply to gradients.
- **Equivalent Separations.** Is it possible to get the same separation under isocratic and gradient conditions?
- **Surprises.** Sometimes when changes are made to gradient conditions, the result isn't what was expected.
- **Dwell-Volume Problems.** Problems that arise when transferring a gradient method often can be traced to differences in dwell volume between the LC systems.
- **Baseline Drift Problems.** Can anything be done to correct for baseline drift in gradient separations?

Find this issue in the e-books section of our website, at www.chromatographyonline.com/lcgc-ebooks



FROM the EDITOR

Laura Bush
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An Insider's View

At the heart of liquid chromatography lies a column. Because of the importance of the column, we have dedicated a regular place to it in *LCGC* magazine: "Column Watch." And what has made "Column Watch" so informative over the years is that it has captured the insights of an insider—someone who really understood the workings of the column and its role in separations: Ron Majors.

Ron Majors served as our "Column Watch" editor for more than three decades, having started writing in the second issue of what was then called *LC Magazine* in 1983 (and then adding "Sample Prep Perspectives" to his duties in 1991). During that time, Ron—a senior scientist at Agilent for much of his career—always had his finger on the pulse of advances in chromatography, and he was also in the midst of several groundbreaking developments in HPLC. (For more details, see our November 2015 supplement, *The Best of Column Watch and Sample Prep Perspectives: A Salute to Ron Majors.*)

Yet despite his passion for chromatography, Ron Majors has decided, following his retirement from Agilent just over a year ago, that the time has finally come to retire from writing monthly columns in our pages. We are very sad to see him go! Fortunately, he is staying active in the field, and will stay on with us as a column editor emeritus and continue to contribute articles from time to time.

We are also very fortunate that "Column Watch" will be in excellent hands, because we have found a new HPLC column insider to take over the reins: Dave Bell.

Dave received his PhD in analytical chemistry from the Pennsylvania State University, and has spent the last 20 years working on the design, development, and application of chromatographic stationary phases to advance liquid chromatography and related hyphenated techniques. In his current role, as the manager of HPLC Surface Chemistry and Health Sciences Research at MilliporeSigma, Dave's main objectives are to create and promote novel LC technologies and to research molecular interactions that contribute to retention and selectivity in chromatographic processes. He presents his research results in journals and at numerous symposia around the globe.

It was Dave's reputation as a scientific speaker and author that led Ron to recommend him as his successor. "I had heard that Dave was a good lecturer, attended his presentation at HPLC 2014 in New Orleans, and read some of his scientific papers and was impressed," said Ron. "His presentations and writings showed a real in-depth understanding of the chemistry behind column technology." His subsequent guest contributions to "Column Watch" last year confirmed this view.

And of course, it is also Dave's position as an insider that convinced us he was the right person to take over this role. "Since Dave's position is an R&D leader in column technology at MilliporeSigma, I thought that this perspective would provide insight into the inner workings of column chemistry and help to point out future directions in the technology of HPLC and UHPLC columns," Ron continued.

What's more, Dave understands not only the theory of column chemistry, but what happens when you apply it to the real-world problems our readers face. He spent the first decade of his career in the pharmaceutical industry—performing analytical method development and validation using various forms of chromatography and electrophoresis—and his work at MilliporeSigma gives him plenty of exposure to other fields as well. "Dave is very knowledgeable about the applications of HPLC and UHPLC columns in a wide range of fields," Ron added.

So it is with great pride that I salute two column insiders for providing their insights to *LCGC*'s readers: I take my hat off to Ron Majors for all his great work in these pages over the past three decades, and I welcome in Dave Bell, recognizing the excellent work he began last year and will continue for years to come.

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A Sponsored
Q&A Discussion

UCT: A 30-Year Tradition of Excellence in Sample Preparation

Michael J. Telepchak, CEO, UCT Incorporated

Solid-phase extraction is a powerful sample preparation tool that is widely used in application areas as diverse as forensics, toxicology, pharmaceutical, environmental, and food safety. *LCGC Magazine* contributing editor Kari Hallenburg spoke with Michael Telepchak, CEO of UCT Incorporated, about the intriguing story of his discovery of the concept of solid-phase extraction and its enduring impact in the field of analytical chemistry. Michael holds several chromatography patents. He is the co-inventor of the technique of solid-phase extraction, and he is the co-author of *Clinical and Forensic Applications of Solid Phase Extractions*.

LCGC: How did the concept of solid-phase extraction come to you?

TELEPCHAK: It was a serendipitous accident. Tom Goode, Dr. Reginald Adams, and I were working for PerkinElmer at the time. Dr. Adams was developing instrumentation for steroid analysis, and Tom and I were responsible for making HPLC columns.

One afternoon in the laboratory, we accidentally spilled some HPLC column packing into one of Dr. Adams' test tubes with the steroid samples in it. He panicked and said that we had ruined his experiment. I suggested to him that if he just let the stuff settle to the bottom and pour it off in the morning, he would have his steroids back.

When I arrived in the office, there were three messages on my phone letting me know that, in fact, he didn't have his steroids back. It occurred to me that they were probably still stuck onto that HPLC column packing. I suggested that he throw some methanol in it and try it again. And, by jeepers, there they were! And that was the first accidental solid-phase extraction (SPE) that was performed.

LCGC: How did you manage to develop that idea into a widely used commercial technology?

TELEPCHAK: We presented the idea to PerkinElmer, but they informed us that they were an instrument company, not a chemistry company, and were not interested.

Tom and Dr. Adams then moved on to other endeavors, but they were instrumental in the discovery of solid-phase extraction. After a period of time and a lot of effort trying to figure out how to finanace a project like this, I decided to execute some market research to see if this type of technology would apply to forensic toxicology. At that time, you were still allowed to do manufacturing R&D in your garage, which is what I did, and I found a toxicology lab that gave me instrument time to test the ideas.

In 1986, the toxicology barrier was finally broken, and the mixed-mode product that I'd developed qualified as the basis for a high-quality, easy-to-operate device that would extract analytes out of biological fluids, clean enough to put on a GC–MS. And that was the actual birth of the forensic toxicology market.

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LCGC: Can you tell us what UCT's flagship product has been?

TELEPCHAK: Our flagship product is the same one that we developed 30 years ago —the DAU (Drugs of Abuse in Urine) line. As we developed this product, we found that it also applied to therapeutic drugs and additional applications in pharmaceutical, environmental, and food safety markets.

LCGC: Did you foresee the power this technology would provide in the sample preparation community?

TELEPCHAK: We never saw it coming. One afternoon, one of my clients said to me, "Where were you? The Society of Forensic Toxicology held a meeting in Montreal, and everybody there was talking about your products."

That was when we realized that we had one of those rare products that takes off and grows very quickly. Product sales increased from about $60,000 a year to in the millions in the matter of a year and a half. We knew then that this technology was something special and needed more exploration.

LCGC: How has UCT evolved over the past 30 years to remain in touch with developing technology?

TELEPCHAK: Tha basic product line is still intact. The DAU columns consistently provide high-quality and excellent results. To meet market demands for quicker extractions, we developed two additional lines: the Xcel line and the FASt line. FASt was developed to compete with dilute-and-shoot, an area we thought we had no chance in. Quickly thereafter, we realized there were other needs in the marketplace not being met other than SPE columns. UCT then began to supply additional sample preparation items such as buffers, enzymes for enzymatic hydrolysis, and HPLC columns. We established ourselves in developing products that people really need.

After spending a majority of our focus developing products geared towards the forensic science market, we decided, if we could execute it that well for that particular segment, we could do it for others. Soon after, we successfully ventured into the agricultural and food safety markets with a product line called QuEChERS, which is now one of our fastest-growing products throughout the world. We also quickly moved into the pharmaceutical and environmental fields.

LCGC: Can you tell us how the industry has changed over the past few years?

TELEPCHAK: Today, there are a number of SPE-type products on the market. There is a version- a -month that comes out with somebody trying to capitilze on the concept. As these products are introduced, the comment is, "This is going to put SPE out of business." Thirty years later, we're still going strong. We've seen the reintroduction of several notorious technologies just under a new name for example, solid–liquid extraction, but there are still difficulties getting all of these concepts to perform as consistenly as tradiitonal SPE.

In HPLC, we've seen core-shell technology and the introduction of expensive HPLC–MS systems. Many labs will budget for this exclusive instrumentation by cutting out consumable SPE product costs and not do any clean-up. Soon there after, there is often a quick transition back to some type of solid-phase extraction when analyts not only find how much instrument down-time is being spent cleaning out all of the unaccounted-for matrix, but also see the costs add up in replacing parts.

LCGC: Do you have any closing thoughts?

TELEPCHAK: This was an exciting year. Along with marking our 30th anniversary, we were delighted to learn that the American Academy of Forensic Science included us in their "Six Decades of Milestones in Forensic Science" for the development of solid-phase extraction.

From our establishment in Horsham, PA. in 1986, United Chemical Technologies, Inc. (UCT) has evolved into a major competitor in the field of silica based solid phase extraction technology. Following the acquisition of the specialty chemical branch of Huls America (formerly Petrarch) in October of 1993, UCT was then able to expand into silane manufacturing. This allowed for a stronger internal influence over the production of our high-quality bonded phases. Aside from the key institution of vertical integration, UCT also has always been second to none in technical support and customer service.

Over the past 30 years, UCT has continuously evolved to remain in touch with developing technology and market demands through the expansion of their product offerings. In addition to sample preparation columns and well plates, we offer QuEChERS tubes, Selectra® HPLC columns, vacuum and positive pressure manifolds (PPM), Select® pH buffer pouches, Selectrasil® reagents and hydrolysis enzymes. UCT's extensive product diversity along with our dedicated employees and loyal customers have significantly shaped the company into what it is today. As we mark the significant milestone of 30 years in business, we not only remember where we started, but more importantly, we celebrate the contributions and successes that got us here. Here's to 30 more years of excellence in sample preparation.

COLUMN WATCH

Mechanisms of Interaction Responsible for Alternative Selectivity of Fluorinated Stationary Phases

Fluorinated stationary phases, especially those including a pentafluorophenyl (PFP) moiety, have become popular alternatives to the more traditional alkyl (C8 and C18) phases. Many modern column lines have, in fact, been initially introduced with the standard C18 and a PFP phase because of their orthogonality. In this installment, the differences between alkyl phases and PFP phases are discussed in terms of fundamental interactions. The origin of the interactions is also interpreted to better understand how analysts can use and control them to develop effective and rugged analytical methods.

Carmen T. Santasania *is a guest coauthor.* ***David S. Bell*** *is the editor of Column Watch.*

Stationary phases based on alkyl-bonded silica particles are most often used in reversed-phase liquid chromatography (LC) (1). High performance liquid chromatography (HPLC) columns manufactured using alternative bonded phases have, however, become increasingly popular because of the differences in selectivity and retention that they provide. Fluorinated stationary phases, especially pentafluorophenyl (PFP) moieties, have gained acceptance as alternatives to common C18 and C8 phases because of their unique selectivity (2). PFP phases have been shown to provide enhanced dipole, π-π, charge transfer, and ion-exchange interactions when compared to traditional alkyl phases (2,3).

Fluorinated silica-based stationary phases such as the one depicted in Figure 1 have shown unique retention for small, polar analytes (4–6). In particular, PFP phases generally exhibit greater retention of basic analytes than their alkyl counterparts mainly because of the presence of strong ion-exchange interactions (2,6–8). The exact origins of the ion-exchange interactions, however, remain unclear. It is important to not only understand the interactions that stationary-phase chemistry provides, but also to have some knowledge of the source of those interactions so that the appropriate controls can be incorporated into method conditions. This work is intended to provide a discussion regarding the potential origins of ion-exchange character exhibited by PFP phases. In addition, differences in the ion-exchange character of seemingly similar PFP phases are discussed. It is expected that a greater understanding of the ion-exchange interactions will result in more robust and reliable methods as well as facilitate the choice of appropriate stationary phases during method development efforts.

Background Discussion

In a recent publication, West and colleagues (9) reported on a study of

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In 2015, David S. Bell was a guest author for several installments of "Column Watch," and Ronald E. Majors remained the column editor. Those installments provided readers with great insights into new column technology and developments, and thus Majors recommended that Bell become his successor. This installment marks the first column for Bell as *LCGC*'s new "Column Watch" editor and columnist. The editors of *LCGC* would like to thank Ron Majors for his many years of dedicated service to this column and welcome Dave Bell to the team!

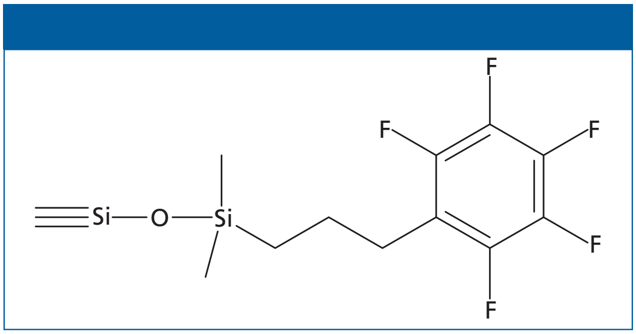


Figure 1: General structure of pentafluorophenyl (PFP) stationary phases. The structure is depicted here with a propyl spacer between the PFP ring and the silica substrate. Several variations in linker are commercially available.

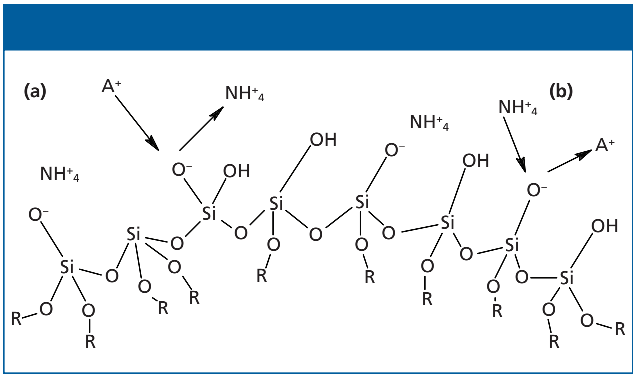


Figure 2: Depiction of an ion-exchange process on bare silica: (a) charged analyte, A$^+$, displaces mobile phase counterion, NH_4^+; (b) mobile-phase ion displaces adsorbed analyte. (Adapted with permission from reference 11.)

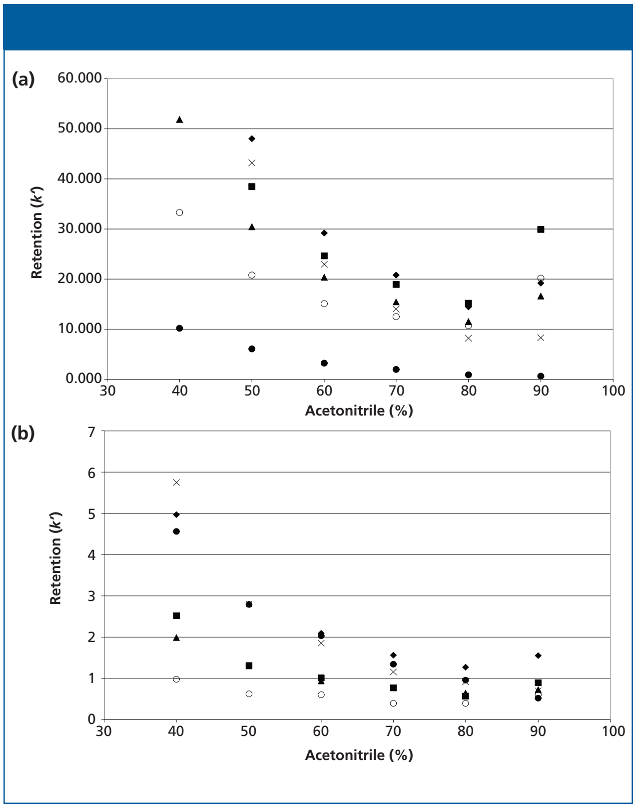
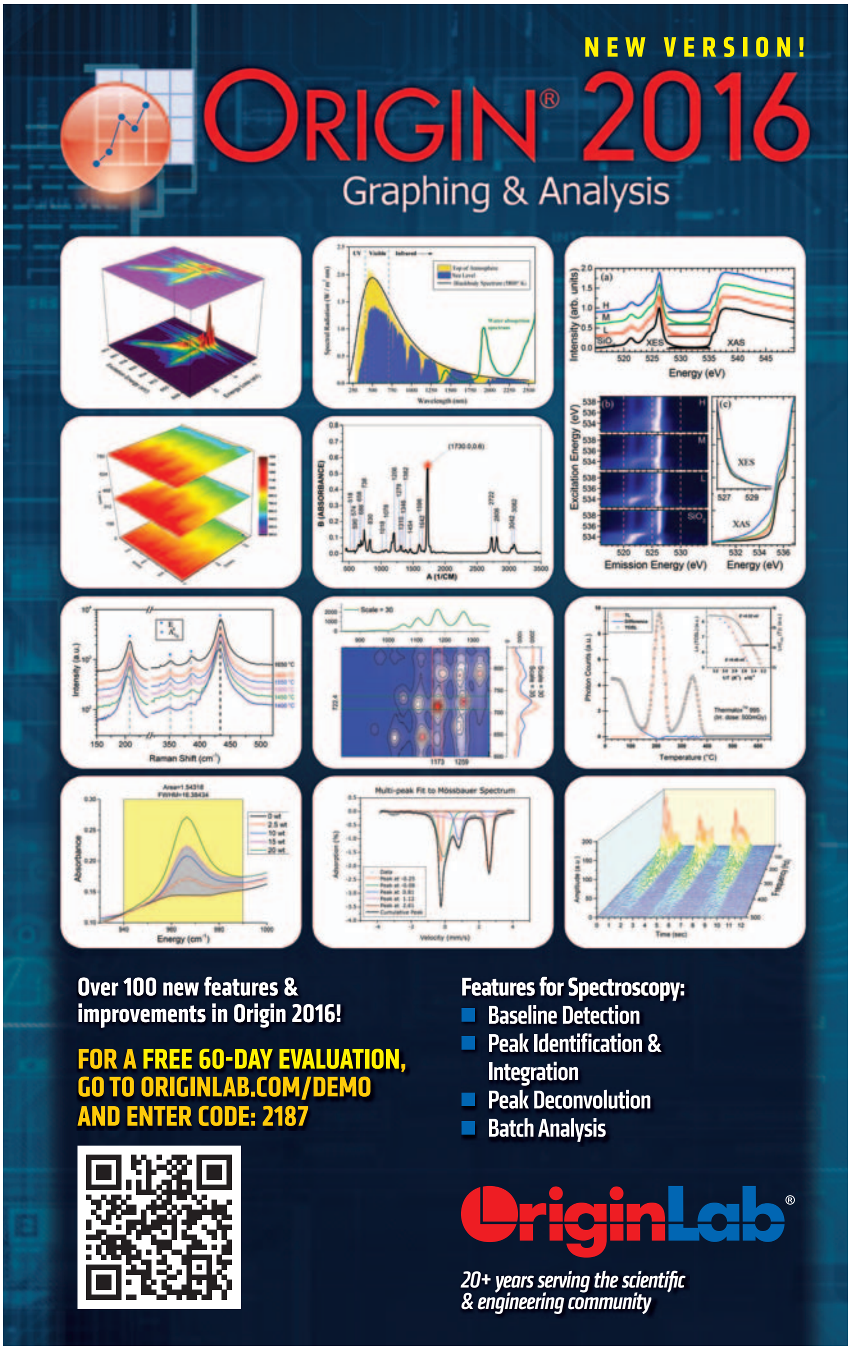


Figure 3: Retention (*k'*) of basic probes amitriptyline (♦), nortriptyline (■), diphenhydramine (▲), verapamil (x), alprenolol (●), and lidocaine (○) using (a) PFP and (b) C18 phases. Columns: (a) 5 cm × 4.6 mm, 5-µm d_p Discovery HS F5, (b) 5 cm × 4.6 mm, 5-µm d_p Discovery HS C18; mobile-phase A: 10 mM ammonium acetate, pH unadjusted (pH: 6.7); mobile-phase B: acetonitrile; gradient: 40–90% B; flow rate: 1 mL/min; temperature: 35 °C; detection: UV absorbance at 220 nm. (Adapted with permission from reference 7.)

ionic interactions exhibited by phenyl and PFP stationary phases in supercritical fluid chromatography (SFC). The authors noted that PFP phases exhibited greater cation-exchange properties than the phenyl phases they tested. This was attributed due to enhanced ion-exchange to the partial negative charge on the fluorine atoms in the PFP ring. The electronegativity of the fluorine atoms in the PFP ring imparts a partial negative charge on the outer edge of the ring system as well as a partial positive charge on the internal region of the ring. The ion-exchange properties may therefore stem from interactions with the ring system itself. The authors also note that the ion-exchange could possibly emanate from negatively charged silanol groups on the silica surface, but struggled to correlate the relative strength of the ion-exchange properties of various phases studied with reported carbon load data.

Retention in ion-exchange chromatography is governed by the exchange of the analyte ions with mobile-phase ions associated with the sorbent surface as depicted in Figure 2. The retention caused by exchange interactions

is based on the relative affinities of the analyte and mobile-phase ions toward the surface. Retention is primarily controlled through manipulation of the concentration and type of mobile-phase competing ion and the charge of the analyte or surface ion through adjustments in mobile-phase pH. Most ion-exchange materials are prepared by bonding a suitable ligand such as arylsulfonic acid to a silica surface; however, silanol groups on the surface of silica-based bonded phases may also act as ion-exchangers. Although HPLC column manufacturers have made attempts to reduce the effect of silanols in reversed-phase column chemistries, it is currently considered impossible to remove all silanol groups from interacting with cationic solutes (10). It is therefore important to understand and account for ion-exchange interactions that may be present even on modern "deactivated" stationary phases (11).



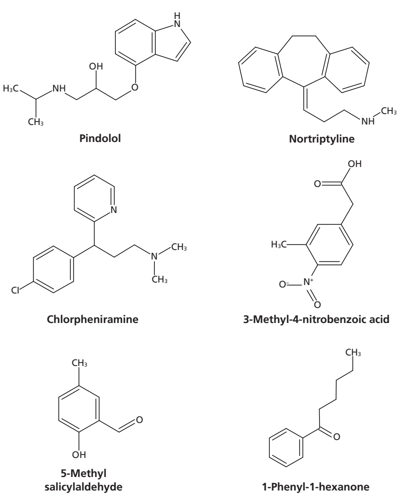


Figure 4: Acidic, basic, and neutral probe structures.

In an earlier study, Bell and Jones (7) investigated molecular interactions that contribute to "U-Shape" retention profiles on PFP phases. The authors reported that at low percentages of organic in the mobile phase, dispersive (hydrophobic) interactions dominated retention; however, as the organic percentage of the mobile phase increased, retention was dominated by ion-exchange interactions. A few key observations led to the conclusion that the ion-exchange properties were a result of surface silanol interactions rather than emanating from the PFP ligand. First, as shown in Figure 3, similar retention profile features were observed, though much attenuated, in the high organic region on a C18 phase manufactured using the same base silica as the studied PFP phase. It is in this region where the PFP exhibits dominant ion-exchange interactions. Since there is no strong dipole within the C18 ligand, the ion-exchange properties must be a result of the common silica support. Second, increasing the pH of the mobile phase from 4 to 6.7 resulted in an increase in retention for the strongly basic analytes used in the study. The pH change within this range would not be expected to alter the ionization state of a strong base (approaching 100% ionized at both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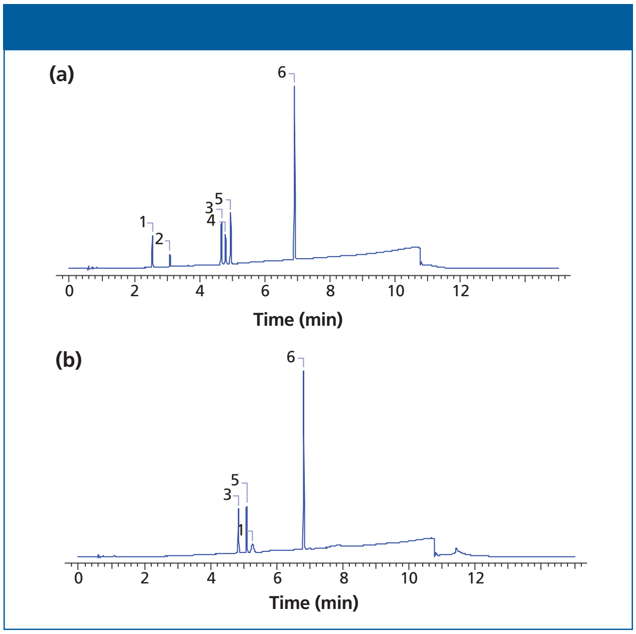
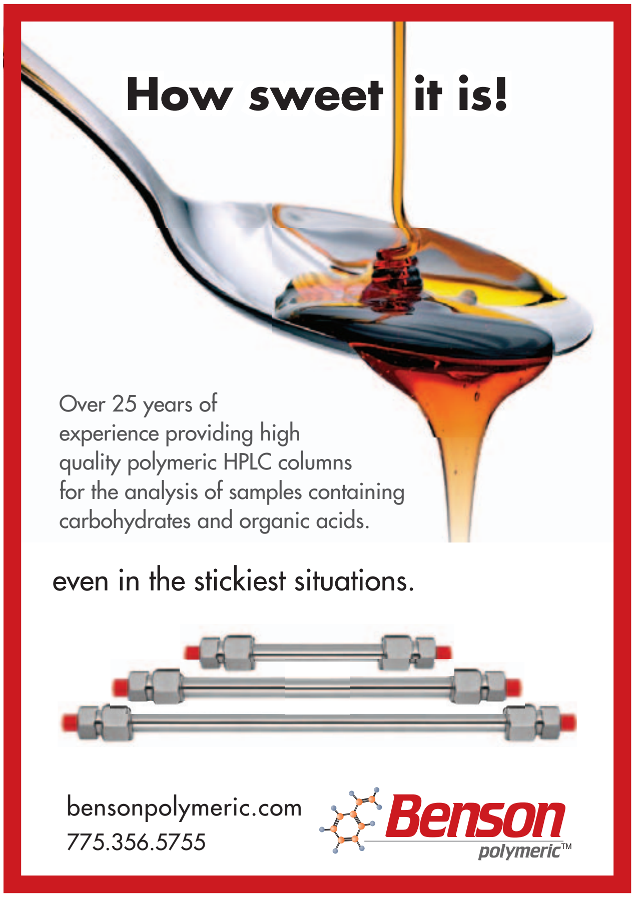


Figure 5: Comparison of PFP phases using formic acid in the mobile phase. Columns: (a) 10 cm × 3.0 mm, 2.7-µm d_p low ion-exchange PFP, (b) 10 cm × 3.0 mm, 2.0-µm d_p higher ion-exchange PFP; mobile-phase A: 0.1% formic acid in water; mobile-phase B: 0.1% formic acid in acetonitrile; gradient: 5–95% B in 10 min; flow rate: 0.8 mL/min; column temperature: ambient; detection: UV absorbance at 254 nm; injection volume: 1 µL; sample: 25 µg/mL each in 95:5 A–B. Peaks: 1 = pindolol, 2 = chlorpheniramine, 3 = 3-methyl-4-nitrobenzoic acid, 4 = nortriptyline, 5 = 5-methyl salicylaldehyde, 6 = 1-phenyl-1-hexanone.



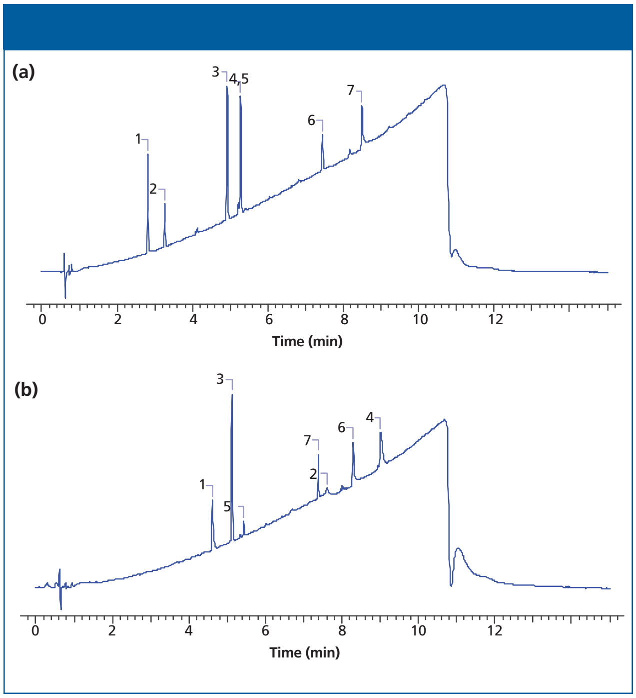


Figure 6: Comparison of PFP phases using ammonium formate in the mobile phase. Columns: (a) 10 cm × 3.0 mm, 2.7-µm d_p low ion-exchange PFP, (b) 10 cm × 3.0 mm, 2.0-µm d_p higher ion-exchange PFP; mobile-phase A: 10 mM ammonium formate adjusted to pH 2.0 with formic acid; mobile-phase B: 10:90 (v/v) 10 mM ammonium formate adjusted to pH 2.0 with formic acid : acetonitrile; gradient: 5–95% B in 10 min; flow rate: 0.8 mL/min; column temperature: ambient; detection: UV absorbance at 254 nm; injection volume: 1 µL; sample: 25 µg/mL each in 95:5 A–B. Peaks: 1 = pindolol, 2 = chlorpheniramine, 3 = 3-methyl-4-nitrobenzoic acid, 4 = nortriptyline, 5 = 5-methyl salicylaldehyde, 6 = background peak, 7 = 1-phenyl-1-hexanone.

pH levels), but would impact the degree of ionization of the silica surface which, for modern silica supports, exhibits an average pK_a value of around 6–7 (12,13). A pH change from 4 to 6.7 would clearly result in increased ionization of the surface silanols and explain the increase in basic analyte retention. Lastly, the base silica and both the PFP and C18 bonded phases were examined by monitoring the retention of a quaternary ammonium probe as a function of mobile-phase pH. The retention profile of the probe on bare silica and the PFP phase was shown to be nearly identical, indicating that the PFP structure allows the probe nearly unrestricted access to the surface silanol groups. In contrast, the C18 phase more effectively masks the probe from the surface silanols under the conditions studied resulting in little change in retention as a function of pH.

Recent Efforts

In classification studies focused on PFP phases, including the papers by West (9) and Euerby (2), significant differences in retention and selectivity have been noted between PFP offerings from various manufacturers, especially when ionic analytes were used. Since modern bonding technologies are, for the most part, similar,

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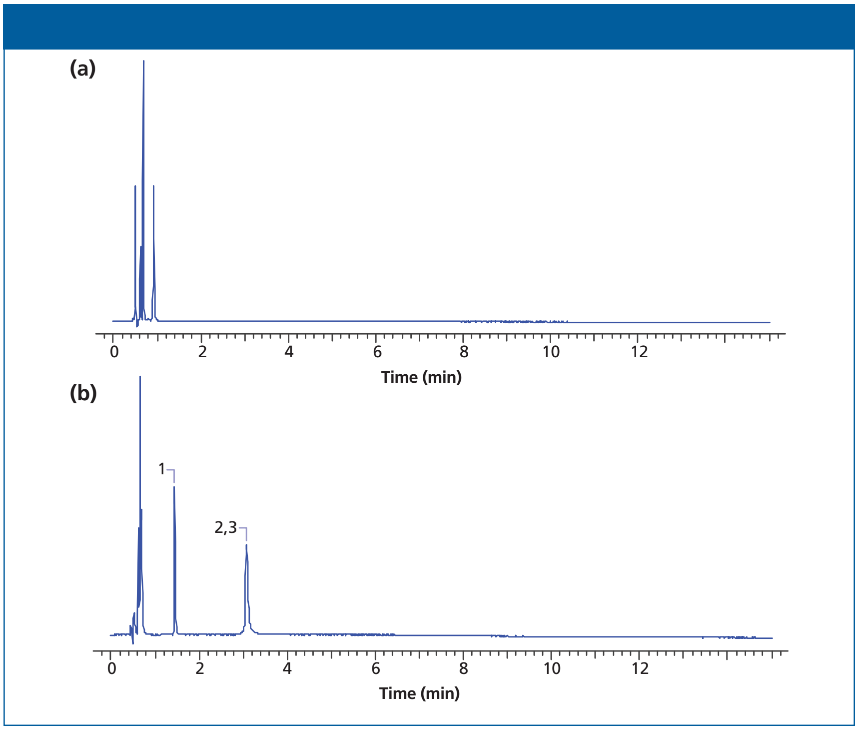

Figure 7: Comparison of PFP phases using HILIC conditions. Columns: (a) 10 cm × 3.0 mm, 2.7-μm d_p low ion-exchange PFP, (b) 10 cm × 3.0 mm, 2.0-μm d_p higher ion-exchange PFP; mobile phase: 10 mM ammonium acetate in 90:10 acetonitrile–water; flow rate: 0.8 mL/min; column temperature: ambient; detection: UV absorbance at 254 nm; injection volume: 1 μL; sample: 25 μg/mL each in 95:5 A–B. Peaks: 1 = pindolol, 2 = chlorpheniramine, 3 = nortriptyline.

these observations also point to differences in the silica surface as the main source of ionic interactions. A recent PFP product offering showed a comparison of retention for a set of acidic, basic, and neutral probes using several commercially available PFP columns. It was noted in this comparison that the basic analytes that were clearly eluted as sharp peaks using the new PFP phase were eluted as broad peaks or were fully retained using the comparison phases. These interesting results prompted further investigation within the author's laboratories.

The conditions noted in the marketing material were first replicated using the "new" PFP phase and several commercially-available PFP phases. Figure 4 provides the structures of the compounds used throughout the study. The compounds 5-methyl salicyladlehyde and 1-phenyl-1-hexanone represent neutral analytes whereas pindolol, nortriptyline, and clorpheniramine are basic. The compound 3-methyl-

4-nitrobenzoic acid provides an acidic component to the test mixture. The results of the initial experiment were in agreement with the findings noted in the brochure and are shown in Figure 5 (for brevity only one comparison column is presented). The data indicates that the new PFP phase exhibits less ion-exchange character than the comparison phases. The mobile-phase conditions used consisted of water and methanol acidified with formic acid (see the full conditions in the caption of Figure 5). Since ion-exchange interactions are dependent on the concentration of the competing ion in the mobile phase, a second set of experiments using 10 mM ammonium formate (pH adjusted to 2) in place of the formic acid were conducted. The chromatograms presented in Figure 6 show that the addition of the ammonium competing ion in the mobile phase for the column exhibiting greater ion-exchange character resulted in sharper and earlier eluted peaks for the basic analytes and a markedly different elution order. The elution order using the "new" PFP column remained similar. It is noteworthy to mention that low pH is often used to minimize surface silanol ionization and thus reduce undesired ionic interactions. A common misconception is that silanols are completely neutralized at low pH; however, silanols exhibit a wide range of pK_a values resulting in significant ionization even at low pH levels. The rationale for why many PFP phases seem to provide increased ion-exchange character of the surface silanols relative to other bonded phases is unclear and the subject of further investigations.

The experiments discussed above demonstrate the importance of understanding the interactions that are available from different phase chemistries and the source of the interactions. Although the new column name implies it is a PFP phase, it does not exhibit the same interactions and may potentially provide very different chromatographic results. Whether this is a good or bad attribute depends on the interactions

> ***The experiments discussed here demonstrate the importance of understanding the interactions that are available from different phase chemistries and the source of the interactions.***

required to generate the desired separation. The lack of ion-exchange may provide more reproducible results in some cases, but may also show a lack of retention or selectivity in another. For example, PFP phases have been used in hydrophilic-interaction chromatography (HILIC) where the dominant mechanism of interaction has been shown to be ion-exchange (14). Figure 7 shows a comparison of the new PFP and a competitor PFP for the same test mixture using HILIC conditions. The competitor phase, which exhibits ion-exchange character, provides greater retention and selectivity for the basic analytes in the test set and the subject PFP provides little retention because of the lack of ion-exchange interactions.

As the exact surface chemistry of the subject PFP phase is proprietary, it is speculative to state the exact reasons for the lack of ion-exchange character relative to other PFP phases. If one assumes, however, that the stationary phase is indeed dominated by PFP ligands, then this provides additional evidence that the ionic character does not emanate mainly from the ring system, but from the silica surface.

Choosing the PFP phase that exhibits the most appropriate interactions for a given separation task should greatly facilitate the development of robust and reliable methods.

Conclusions

Pentafluorophenyl stationary phases provide alternative retention and selectivity when compared to the commonly used alkyl phases. The differences in chromatographic results stems from the fact that the PFP phases may provide interactions such as π-π, dipole, hydrogen bonding, and ionic interactions not prevalent on standard alkyl modified surfaces. When positively charged analytes are present, ion-exchange interactions often dominate. It is important, therefore, to carefully control the conditions (pH, mobile-phase ion concentrations) that ion-exchange interactions are dependent upon. In this work, the origin of the ion-exchange interaction was discussed. Although some studies have implicated the partial charges emanating from the PFP ring system as the source of the surface ion-exchange, there is strong evidence to suggest that the ionized surface silanol groups are the dominant source. It has been demonstrated that

PFP phases such as the "new" column studied here provide relatively little ion-exchange interactions as compared to other PFP phases. This feature may provide more reproducible chromatographic results where ion-exchange is not desired or controlled well, but also may not provide resolution where the retention and selectivity are dependent on the presence of ion-exchange interactions. Although further study is necessary to completely understand the impact of bonding parameters such as ligand density and residual surface treatments, such as endcapping, it is clear that all PFP phases are not the same. Choosing the PFP phase that exhibits the most appropriate interactions for a given separation task should greatly facilitate the development of robust and reliable methods.

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LC TROUBLESHOOTING

Detective Work, Part IV: Chemical Problems with the Column—Chemical Attack

The silica-based packing in reversed-phase columns is not inert. Here we consider what happens when the mobile-phase pH is too high or too low.

John W. Dolan
LC Troubleshooting Editor

This is the fourth "LC Troubleshooting" column in a series related to problems that we associate with liquid chromatography (LC) columns. We started with a look at the major causes and symptoms of LC column failure (1). In the second installment (2), we concentrated on problems that are caused by physical problems with the column. Chemical problems (adsorbed sample and chemical attack) are associated primarily with changes in retention and selectivity (peak spacing). Last month (3), we started looking at trouble related to chemical problems with the column, specifically what happens when materials from the sample are strongly adsorbed on the column. This month's topic is related to what happens when the mobile-phase conditions are beyond the recommended limits and the column packing material is chemically attacked.

Reversed-Phase Column Packings

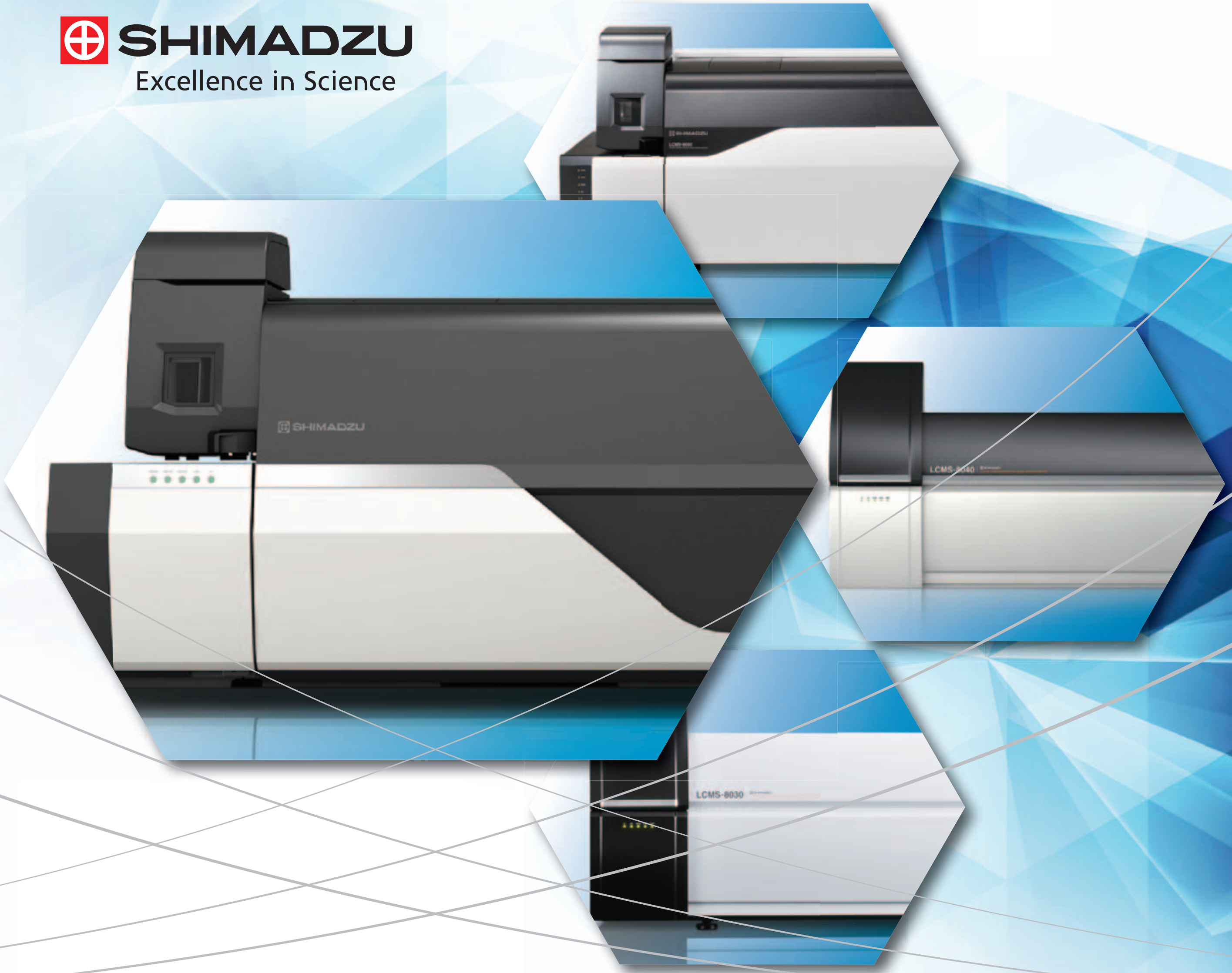
The eversed-phase mode is the most popular mode of liquid chromatography, with C8 and C18 columns accounting for somewhere around 80% of all columns sold. These columns typically comprise totally porous, spherical silica in the range of <2 µm to 5 µm in diameter. Superficially porous particles are becoming more popular, and particles beyond this size range also are used, but chemically they all behave in the same way in the context of the present discussion.

Silica is an amorphous polymer of silicon and oxygen, with a surface that terminates in silanol (Si-OH) groups. These silanols provide an attachment point for the bonded phase. For example, consider a silanol reagent $X\text{-}Si(CH_3)_2R$, where X is a reactive chloro or ethoxy group and R is $C_{18}H_{37}$; a C18 bonded phase could be created by forming a "silyl ether" as shown in Figure 1. Because the C_{18} group is quite bulky, only part of the free silanols on the silica surface are bonded in this reaction because of steric hindrance. Most C18 columns are further bonded through an endcapping process to increase column stability and reduce the population of unbonded silanols. This is the same reaction but uses a smaller trimethyl reagent: $X\text{-}Si(CH_3)_3$. Even with "complete" bonding and endcapping, approximately half of the silanol surface remains unbonded. As we saw last month (3), these residual silanols can be a source of strong chemical interaction with the analyte or other sample components.

Low-pH Problems

The bonded phase illustrated in Figure 1 is quite easy to form. Unfortunately, under the right conditions, it is also easy to destroy. When the mobile-phase pH is too low, the silyl ether on the right of Figure 1 can be hydrolyzed, gradually returning the surface to the condition at the left of Figure 1. This hydrolysis generally is a concern for mobile-phase pH < 2 for most reversed-phase columns, although there are exceptions as noted below.

The effect of continued exposure of the column to low pH is illustrated in Figure 2. Here the loss of bonded phase is followed by measuring retention of a hydrocarbon solute, such as phenylhexane, that is retained only by hydrophobic interaction with the bonded phase. The initial retention is normalized to 100%, and as retention drops over time,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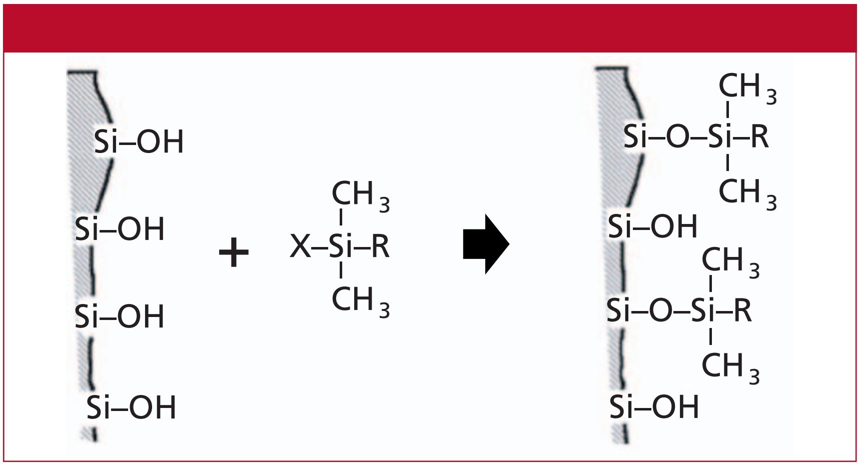
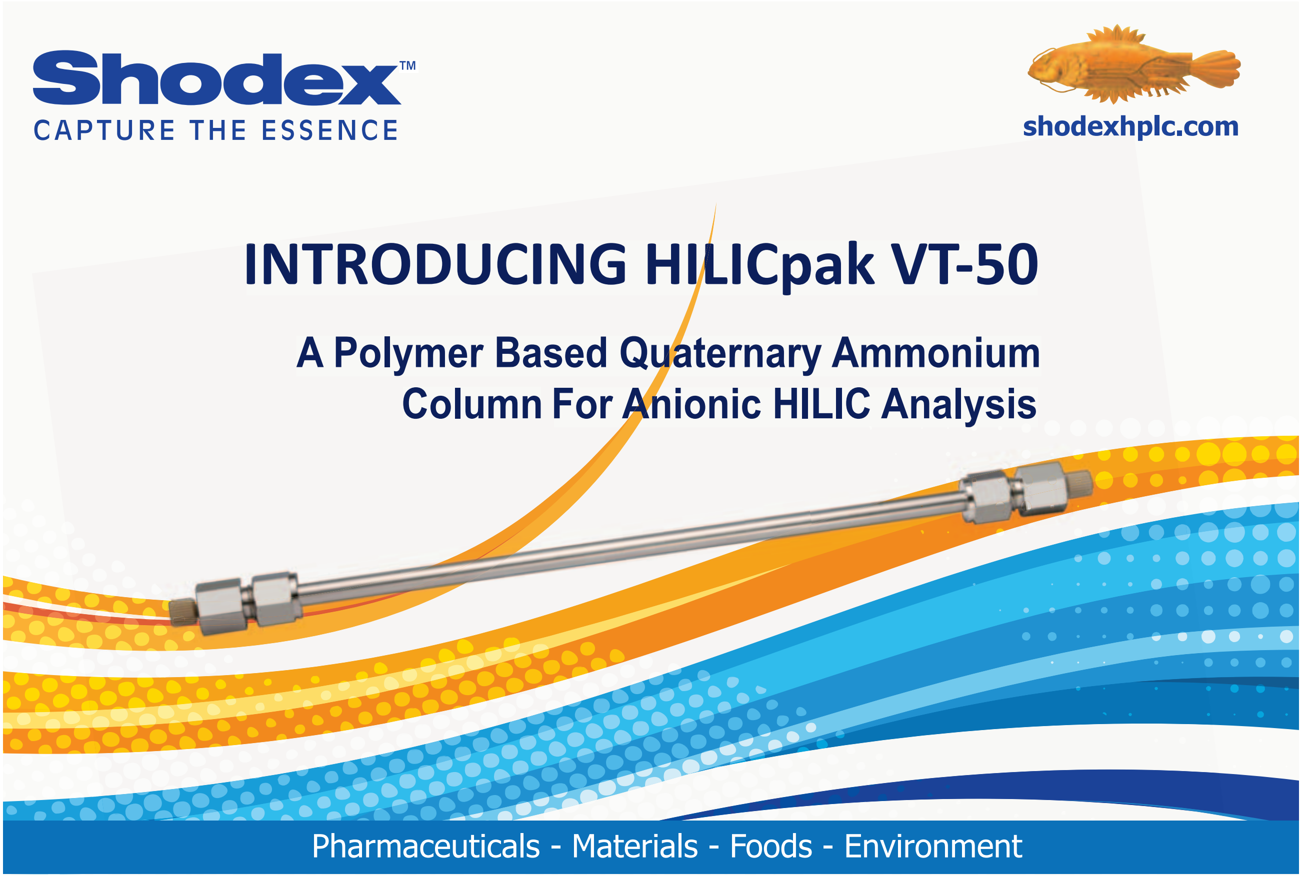


Figure 1: Illustration of adding the bonded phase to a silica surface. See text for details.

it is an indication of the loss of bonded phase. The conditions in Figure 2 are quite severe: 0.1% trifluoroacetic acid (pH ≈ 2) and 80 °C. The performance of four C18 LC columns is shown: a column using older, type-A, low-purity silica typical of most columns introduced before 1990; one of the early high-purity, type-B silicas introduced in the early 1990s; and examples of today's high-purity, type-B silica columns. You can see that the type-A column is not at all stable by today's standards, and while the early type-B column is an improvement, it also has a limited lifetime under these conditions. The current-day high-purity type-B columns are quite stable, but it should be recognized that any column will eventually deteriorate under sufficiently low pH if the exposure time is long enough. Before we leave Figure 2, let's consider what 200 h of exposure to these conditions means. The test columns had dimensions of 50 mm × 2.1 mm, with a column volume of ~100 µL; the flow rate was 0.2 mL/min, so the exposure rate was two column volumes per minute. The experiment went for 200 h, which is 12,000 min or 24,000 column volumes. For the traditional 150 mm × 4.6 mm column, this test would be the equivalent of running 36 L of hot acid through the column! If samples with a retention factor of 2 were run in a continuous sequence with a one-column-volume pause between injections, you could run 6000 samples during the 200 h test period. All this is to say that today's LC columns based on high-purity silica are amazingly stable.

What would you expect to see as the bonded phase is gradually hydrolyzed off the column? As is illustrated in Figure 2, retention times would generally drop as the hydrophobic stationary phase is lost. However, more of the bare silica surface would be exposed during this process, so the strong polar interactions between polar sample functional groups and the silanol groups would be expected to increase. This increase in polar interac-



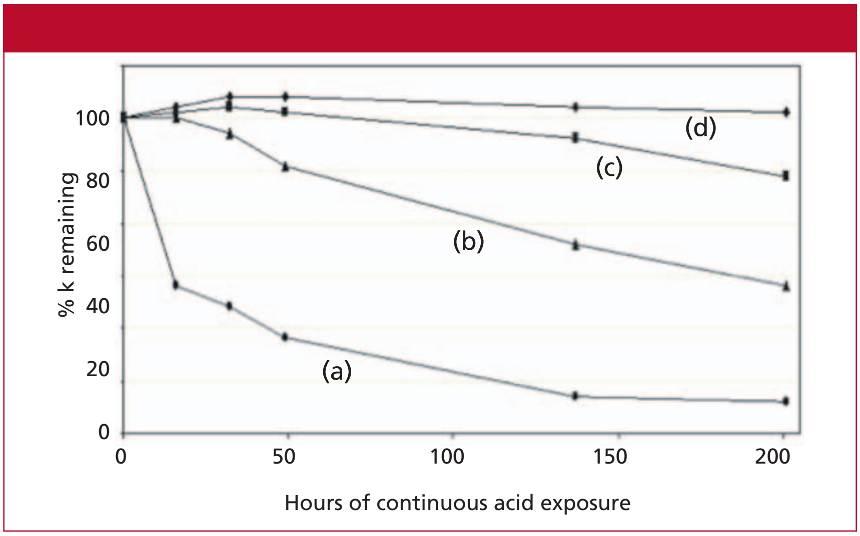


Figure 2: Stability of reversed-phase columns when exposed to pH 2 mobile phase at 80 °C, with 50 mm × 2.1 mm C18 test columns operated at 0.2 mL/min. Columns were packed with (a) older, low-purity, type-A silica; (b) early higher-purity, type-B silica; and (c,d) high-purity, type-B silica. See text for details. Adapted from data of Advanced Chromatography Technologies Ltd., Aberdeen, Scotland.

tions would likely change selectivity and increase peak tailing, as discussed in last month's "LC Troubleshooting" (3). Furthermore, as the bonded phase is hydrolyzed from the surface, it has to go somewhere. This gradual loss of bonded phase is called *column bleed*. Column bleed is a special problem with mass spectrometry (MS) detection—the hydrolyzed bonded phase is not volatile, so it will contaminate the interface at the point of solvent evaporation, and in some cases analyte adducts or complexes with bonded phase contaminants can confuse interpretation of the collected sample spectra. In any event, running with mobile-phase conditions under which the column is not stable would cause changes in retention, selectivity, and peak shape—all of which are undesirable for robust LC methods.

High-pH Problems

Hydrolysis of the bonded phase, which is unstable at sufficiently low pH, is not a concern when the pH is greater than 2–3 or so. However, at sufficiently high pH, the underlying silica becomes soluble and will dissolve out from under the bonded phase. Most silicas are stable below pH ≈ 8, so pH 8 is a somewhat arbitrary upper limit for mobile phases for generic reversed-phase columns. As noted below, there are many columns available today that can operate successfully with higher-pH mobile phases.

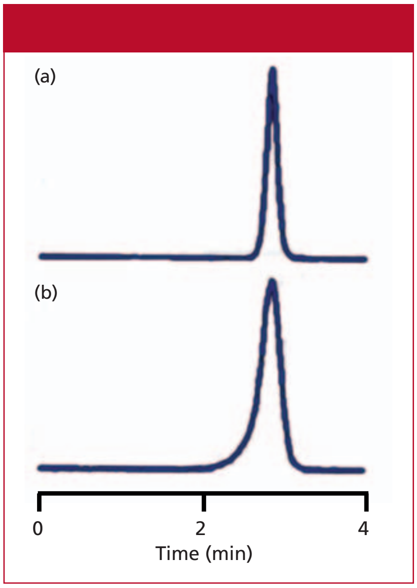
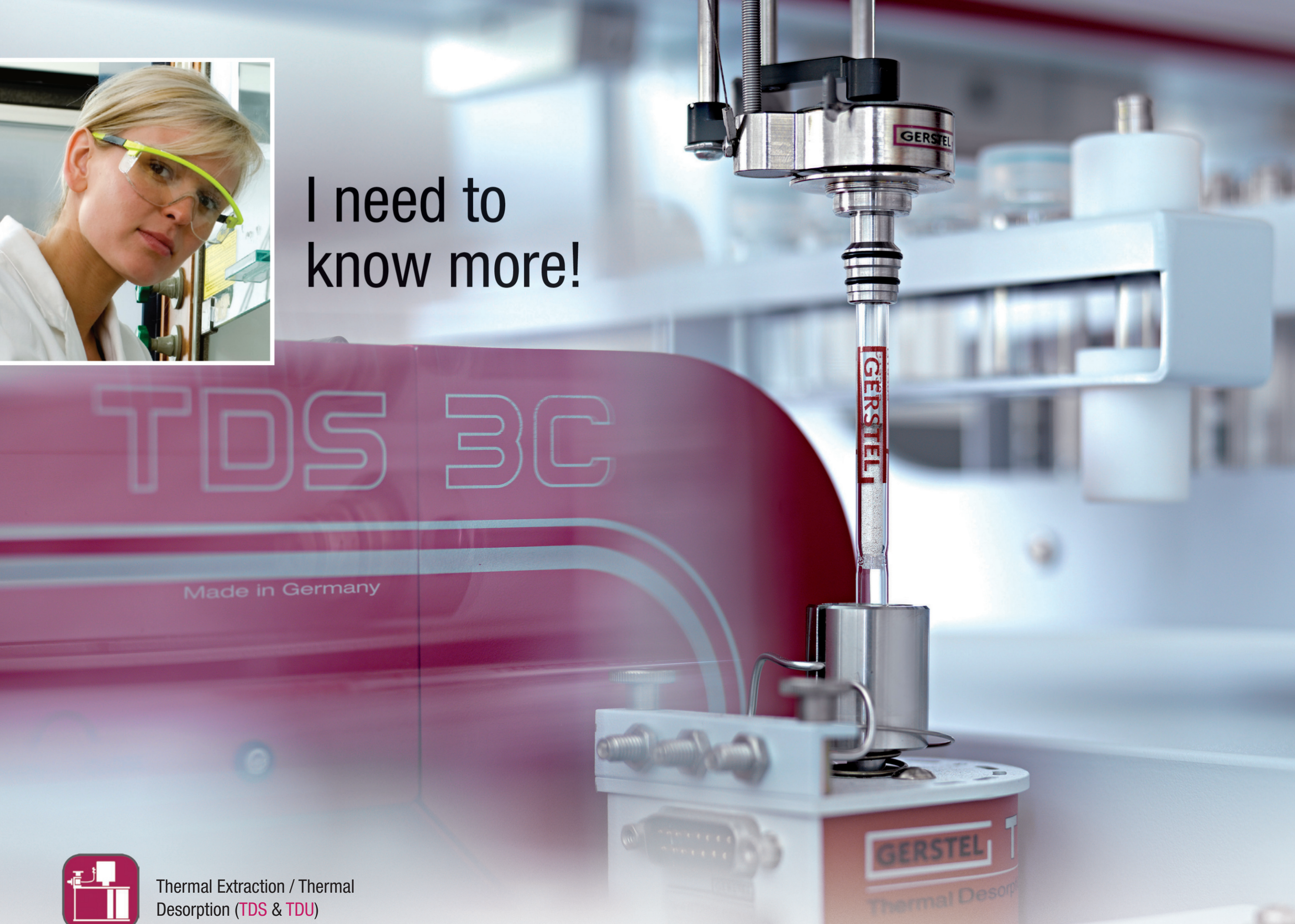


Figure 3: Chromatograms (a) before and (b) after structural collapse of the column packing bed. Conditions: pH 9.0 and 70 °C on a column with recommended upper limits of pH 7.0 and 45 °C. See text for discussion.

Short-term operation of silica-based columns at pH > 8 will probably not generate dramatic changes. There will be a gradual change in the surface of the column as more silanol groups are exposed and bleed of the bonded phase (perhaps with additional silica attached). These processes will give similar results to the loss of bonded phase discussed in the previous section: changes in retention, peak spacing, peak tailing, and possible additional problems if MS detection is used.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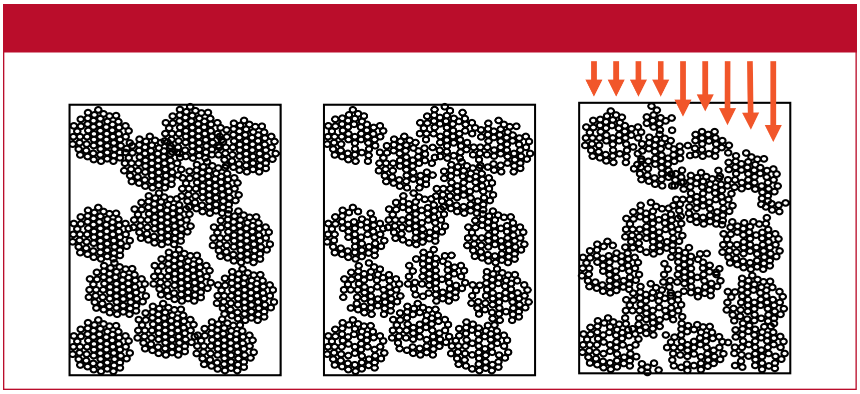

Figure 4: Conceptual drawings of dissolution of the silica packing within a column. Left, a well-packed column; center, column after partial silica dissolution within the particles; right, column after collapse. See text for details.

After sufficient exposure to high-pH mobile phase, the structural stability of the column will suffer, with physical collapse of the column packing bed at some point. A typical symptom of column collapse is shown in Figure 3. The upper chromatogram represents a normal chromatogram after approximately 500 injections; the lower one is the next injection. The lower peak can be seen to front strongly, and although it is not apparent from the chromatogram because of height normalization, the peak area is the same. Figure 3 is an example of operating a column for too long under too high a pH. The mobile phase is pH 9.0 and the column temperature is 70 °C. The column was designed for low-pH work, and the column care-and-use instructions clearly state the column limits (which were not followed . . .): the allowable pH range is 2–7, the temperature range is 20–45 °C, and a special caution regarding column stability is noted when both the pH and temperature limits are approached.

The cartoon of Figure 4 gives us a concept of what is likely occurring when symptoms such as those of Figure 3 are observed. The porous silica particles in the column are made up of much smaller nanoparticles much like the popcorn kernels in a popcorn ball. The spaces between these smaller particles are the pores in the packing. The left-hand column of Figure 4 represents a normal, tightly packed column. When the pH is too high, the particles dissolve, but the 5-μm packing particles do not dissolve sequentially from the outside inward as would happen when peeling layers off an onion. Rather, the mobile phase has full access to the pores in the particles, so the nanoparticles dissolve, gradually reducing the structural integrity of the larger particle. This is illustrated in the middle column of Figure 4, where the inner structure of some of the larger particles is missing. At some point, this particle structure becomes so fragile that any shock to the system, such as the pressure shock of cycling the sample injection valve, can crush a particle. As one particle collapses into another, it can result in a settling of the packing bed, as shown in the right column of Figure 4. If the collapse is even, there will be a void at the top of the column that can cause mixing and peak broadening. If it is uneven, as in Figure 4, it is possible for some sample molecules to flow further down the column before they reach the packing. This distorted profile is shown for the right column by the arrows at the inlet. This behavior would give some sample molecules a head start in the separation process, so they would lead the main sample band, resulting in a fronting peak as shown in Figure 3. At this point, there is nothing that can be done to salvage the column. Although you might try to fill in the void at the column inlet, it will have no effect on the damage done to the remaining particles in the column. The best advice to avoid this kind of problem is to read (and follow) the directions that come with

the column! (For further discussion on the example of Figure 3, see reference 4.)

The pH Sweet Spot

Silica makes a very convenient substrate for support of the bonded phase in reversed-phase LC, but it does have limitations, as discussed above. For most reversed-phase columns, it is safe to assume that they are stable in the $2 < \text{pH} < 8$ range. As was shown in Figure 2, the newer, higher-purity, type-B silica columns are more stable than the older, type-A columns. If you are using a method on an older type column, it may be good idea to be overly cautious and set the range to $2.5 < \text{pH} < 7.5$. It should be noted that column failure from inappropriate mobile-phase pH does not take place instantaneously, but is a gradual process over time; even the dramatic failure of the column of Figure 3 took ~500 injections to occur.

Today there are many columns that have been designed for extended pH ranges. Some of these will perform over $1.5 < \text{pH} < 12$ or other ranges advertised by the manufacturers. One way to extend the upper pH range is to use "hybrid" particles that are not pure silica, but are modified to be less soluble at high pH. Some manufacturers make particles with proprietary treatment of the silica surface or some kind of a coating on the silica that protects the silica from attack by the mobile phase. These extended-pH columns are widely advertised; consult the manufacturer's literature for descriptions of pH tolerance and (sometimes) a description of the physicochemical process used to protect the column.

There are hundreds of different reversed-phase columns on the market today, and sorting through the offering can be a daunting task. Whether you are using a column that is new on the market or one that has been available for decades, it is always a good idea to follow the age-old advice, "if all else fails, read the directions." Every column should have care-and-use instructions either packed in the column box or available on the column manufacturer's web site. As a final note, remember that no matter how the silica is manufactured, if you use it long enough at extreme pHs, it will die, so don't expect any column to be immortal.

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John W. Dolan

"LC Troubleshooting" Editor John Dolan has been writing "LC Troubleshooting" for LCGC for more than 30 years. One of the industry's most respected professionals, John is currently the Vice President of and a principal instructor for LC Resources in Lafayette, California. He is also a member of LCGC's editorial advisory board. Direct correspondence about this column via e-mail to John.Dolan@LCResources.com

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

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THE HISTORY OF CHROMATOGRAPHY

Leading Light: Pavel Jandera

Continuing our "History of Chromatography" series in which a younger scientist interviews an icon of chromatography, here Frantisek Foret interviews Pavel Jandera. Jandera spoke about building his own liquid chromatograph, the birth of "modern" high performance liquid chromatography (HPLC), the importance of exploring (and understanding) earlier research papers, current trends in contemporary chromatography, and his inspiring advice for aspiring chromatographers.

Frantisek Foret

Frantisek Foret: What brought you into the field of analytical chemistry and chromatography?

Pavel Jandera: During my master's studies at the Institute of Chemical Technology in Pardubice, Czech Republic, in the late 1960s, I was attracted by analytical and physical chemistry more than other branches of technology. The Department of Analytical Chemistry was founded and, by that time, still chaired by Professor Miroslav Jureček, the internationally recognized expert and author of excellent monographs in organic analysis.

Thanks to him, the profile of the department was focused on organic compounds more strongly than at other universities in Czechoslovakia and the students had the opportunity to study various aspects of chemical analysis in considerable detail including laboratory courses of elementary and functional analysis and microanalysis of organic compounds. The lecture course given by Associate Professor Jaroslav Churáček was focused on the applications of spectroscopic and separation techniques in organic analysis. It was there that I came into practical contact with gas chromatography (GC), paper chromatography, thin-layer chromatography (TLC), and electrophoresis. I was impressed by the possibilities of simultaneous analysis of several sample components in a single run that was offered by these techniques. With an Ing. diploma in my pocket, I asked Professor Churáček about the possibility of PhD study in his group of separation sciences and he agreed (by that time the equivalent degree to PhD, the Candidate in Sciences, CSc, was used in our country).

I started in 1967 and was sent for two months' experience to Jiří Štamberg's laboratory at the Macromolecular Chemistry Institute of the Academy of Sciences in Prague, where they developed plastic tubes with inner walls treated by grafting ion-exchange groups to them. I had to investigate the possibilities of using these products as analytical separation media. Necessarily, this attempt was a big failure because of the large inner diameter and the unsuitably thick and dense grafted layer. This type of open ion-exchange column did not provide any separation efficiency.

Interestingly enough, 40 years later, our group returned to the principal idea, preparing and using organic–polymer monolithic capillary LC columns, this time with more success!

By that time, our library was surprisingly relatively well supplied with journals from the field—including *Analytical Chemistry*, *Fresenius*, *Journal of Chromatography*, *Journal of Chromatographic Science*, and *Chromatographia*—from which I learned about the breakthrough theoretical work accompanying the introduction of modern high performance liquid chromatography (HPLC). What a promising field for my dissertation! However, there was no available instrumentation on the market by that time, so I had to build my own liquid chromatograph from the parts used for various purposes in analytical laboratories. This was hard and often frustrating work, but finally I had a liquid chromatograph (working up to 50 bar) on my desk. During this time I learned a lot about setting up and adjusting the instrument and packing glass columns with silica particles, on which I performed all the experiments for my dissertation.

Instrument Performance Standards: How to Test GC–MS Sensitivity and Performance Quickly and Efficiently for the Analysis of Dioxins and Furans

Dirk Krumwiede and Heinz Mehlmann, Thermo Fisher Scientific

A concept for testing GC–MS analytical instrument performance is described, which is based on a specifically designed set of Dioxin standards.

Dioxins and Furans need to be monitored at lowest levels in food, feed and other matrices. To achieve this, GC–MS instruments must be able to detect and quantify absolute amounts of Dioxins and Furans down to the low femtogram range in routine. Efficient tools to develop and optimize instrument methods which meet these requirements and fast tests on the instrument performance are needed.

Material and methods

All measurements were carried out on a Thermo Scientific™ DFS™ magnetic sector high resolution mass spectrometer coupled to two Thermo Scientific™ Trace™ 1310 GC supported by an extra-wide Thermo Scientific™ Triplus RSH™autosampler.

The concept for the instrument performance standards was developed and defined at the Thermo Scientific POPs application laboratory in Bremen (Germany).

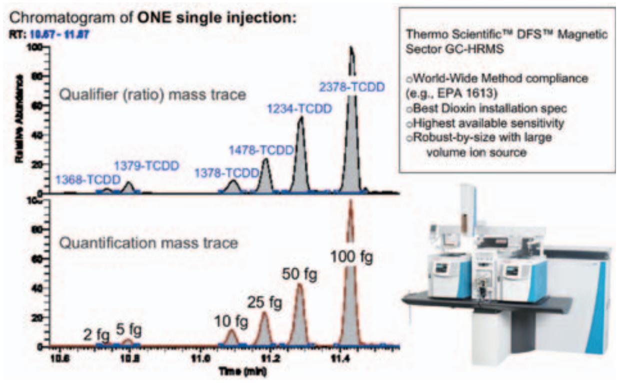

Figure 1: Sensitivity Standard on Thermo Scientific DFS GC-HRMS. Quantification and ratio mass trace of tetra Dioxins: TCDD congeners from left to right: 1368 - 2 fg/ul, 1379 - 5 fg /ul, 1378 - 10 fg/ul, 1478 - 25 fg/ul, 1234 - 50 fg/ul and 2378 -100 fg/ul (in nonane); 1 ul injected.

Results and Discussion

Typically the sensitivity of a GC–MS for Dioxin analysis is checked by measuring a low concentrated standard. Often the limits of detection and quantitation are then extrapolated from the concentration levels in these standards down to far lower values. Real measurements of these very low levels seldom take place and would require repeated injections of decreasing concentrations until the point of minimum signal-to-noise values are met. Most laboratories do not have time for this. This leaves significant uncertainty concerning the real instrument performance for very low levels. The special Instrument Performance standard 1 (1: "sensitivity/linearity standard") contains six different native tetra Dioxin congeners which can easily be separated via GC on 30 or 60 m 5-Phenyl type apolar columns. The concentrations of the different congeners rise from 2 fg/ul for the first to 100 fg/ul for the last eluting analyte. One internal standard is included at 5 pg/ul (2378 ^{13}C TCDD).

A single injection allows checks on the following parameters at six different concentration levels:

- Lowest level detected
- RRFs at different levels
- Signal-to-noise at different levels
- Correct ratios of peak areas (isotope ratios)
- Single injection calibration curve

Based on the results of such measurements it can be decided if further method optimization is needed to meet the sensitivity requirements in a given situation, how much sample should be processed to reach overall low level analysis goals of the complete method, if maintenance is needed to re-establish the former instrument performance, etc.

A second standard (2: repeatability/stability") is available. It contains the same congeners, but all at the same concentration. Here all congeners are equally concentrated (5 pg/ul).

Also the comparison of different analytical methods, instruments or technologies are possible.

The Thermo Scientific DFS Magnetic Sector GC-HRMS has been tested with these standards over years for its superior performance in Dioxins and POPs analysis.

Remark

The standards are commercially available from Wellington Laboratories Inc. (Ontario, Canada): TF-TCDD-MXB.

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Foret: Who was the most influential person for you?

Jandera: It would be difficult to select just a single person from those who influenced me in various ways. First, there were my teachers: Professor Jureček gave me a rare moral example of a most righteous and honest person, a scientist highly dedicated to his field of organic analysis; an excellent teacher, always willing to offer the best of his knowledge to a young generation of students and followers.

My dissertation advisor, boss, and good friend for years, Jaroslav Churáček, constantly encouraged me and offered good advice. I also owe a lot to another great person of Czech chromatography, the late Professor Karel Macek, who introduced me to the editorial board of my favorite journal, the *Journal of Chromatography*, where I have been serving for many years. Later, as a postdoctoral fellow, I had the opportunity to join for some time the research groups of the recently deceased guru of separation science, Professor Georges Guiochon, first at the École Polytechnique in France in 1980 and later at the University of Tennessee in Knoxville.

Foret: What do you think is your greatest scientific contribution and your biggest mistake?

Jandera: It became evident to me very early on that for fundamental reasons HPLC cannot compete in efficiency of separation with, by that time, the already well established technique of GC. HPLC has four orders of magnitude slower diffusion. However, HPLC outperforms GC in the separation of biologically and industrially important polar, ionic, and macromolecular compounds. To fully utilize the possibility of LC in this area, we can optimize the differences in the affinity of the sample components to the stationary and the mobile phases, that is, the selectivity of separation, by adjusting not only the chemistry of the stationary phase, but also by selecting and optimizing the composition of the mobile phase; this is not available in GC, where the mobile phase is a gas.


Pavel Jandera in the early 1970s.

Adjustment of the separation by selecting the appropriate composition of mixed mobile phases is the approach that has been used for years in planar (paper and TLC) chromatography, on the empirical base. In my opinion, the exact composition of most three-, four-, and even more-component mobile phases recommended in the published recipes for the separation of particular samples by planar techniques reflect the bottles of solvents standing on the authors' laboratory desk shelves, rather than a rationally justified selection. This can be OK with the cheap planar

Pavel Jandera studied at the Institute for Chemical Technology in Pardubice, where he graduated in analytical and physical chemistry in 1967. Later, he joined the staff of the Department of Analytical Chemistry of the Institute, which became the Faculty of Chemical Technology of the new University of Pardubice in 2004. In his PhD dissertation, he addressed instrumentation and retention mechanisms in HPLC. He developed a new comprehensive theory of gradient elution, which was the basis of the book *Gradient Elution in Column Liquid Chromatography,* published by Elsevier in 1985 (7).

In 1977, he visited Professor Halász's laboratory in Saarbruecken, Germany, where he learned how to prepare chemically bonded stationary phases and the packing of efficient HPLC columns. In 1980, he obtained a stipend from Hamilton's Science Exchange Foundation for a six-month visit to Professor Georges Guiochon's laboratory at Palaiseau in France. This started a long-term cooperation with Georges, and he stayed as a visiting scientist in Georges' laboratory at the University of Tennessee from 1990 to 1991. In 1989 he obtained the DSc degree and in 1992 he became full professor of analytical chemistry. At the University of Pardubice, he leads courses in organic analysis, separations, and spectroscopic methods. His research work in modern separation sciences has focused on the study of principles and the theory of separation, retention mechanisms, gradient and multidimensional LC techniques, evaluation and characterization of columns, and development, optimization, and application of HPLC methods in environmental, food, and industrial products analysis.

Pavel has been engaged in microcolumn LC techniques, particularly in the development of new efficient organic polymer monolithic capillary columns. He has published more than 250 papers in scientific journals. He has been cited more than 5200 times, coauthored four books, written 30 book chapters, and obtained seven patents. He has presented almost 300 lectures at foreign universities and at international symposia in the field of analytical separations, and has led a number of Czech and international research projects. He has served on the editorial boards of *Journal of Chromatography A, Journal of Separation Science,* and *Analytical Letters.* He has been awarded the Hanuš Medal of the Czech Chemical Society; the Waksmundzki award from the Polish Academy of Sciences; the Maria Curie-Sklodowska medal of the Polish Chemical Society; medals of the University of Torun (Poland) and of the University of Messina (Italy); the AJP Martin Medal awarded by the UK Chromatographic Society; the Janák medal of the Institute of Analytical Chemistry of Czech Academy of Sciences; and the silver medal of the Senate of Parliament of Czech Republic. Professor Jandera is married and has one daughter and two granddaughters.

chromatography techniques, but relatively expensive modern HPLC obviously needs a more systematic approach to the selection of mobile phases, which should be based on a rational retention model. I decided to orient my research activities in that direction.

Several early models can be found in the literature, but—in my opinion—the major part of the useful work should be attributed to L.R. Snyder and his group, particularly in normal-phase adsorption chromatography (1) and in reversed-phase chromatography introduced in the early 1970s, based on the work by J.J. Kirkland's, I. Halász's, Cs. Horváth's, and several other groups (2–5).

In 1977, I visited Professors I. Halasz and H. Engelhard in Saarbruecken, Germany, where I learned the practical basics of chemical modification of silica gel and the packing of efficient HPLC columns with 10-µm and 5-µm bonded phase particles (by that time standard)—techniques I later used at the Department of Analytical Chemistry in Pardubice, where I became a staff member. Reversed-phase separations on chemically bonded C18 and C8 alkyl silica columns in aqueous–organic mobile phases soon became so extremely successful that they—in the view of many users—became practically a synonym for the whole HPLC practice. Hence, the work on the theoretical retention models focused mainly on the reversed-phase chromatographic system; the plethora of other HPLC possibilities seemed to have fallen temporarily into oblivion. Snyder and coworkers have applied a simple linear correlation between the logarithm of distribution con-

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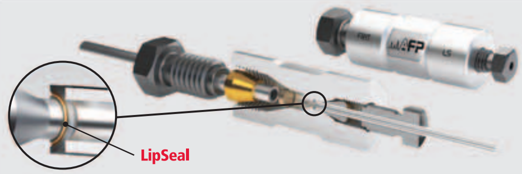

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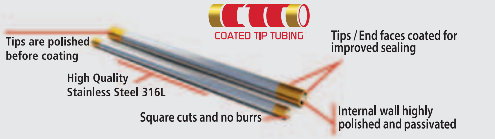

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233, Jalbert St. W., Thetford Mines, Qc, Canada, G6G 7W1 • 418-338-0004 • info@afproducts.ca • **www.afproducts.ca** stant and the volume fraction of organic solvent in binary aqueous–organic mobile phases in their linear solvent strength (LSS) model for prediction of retention in both isocratic and gradient HPLC, which was later incorporated into commercial software (6).

However, some systems are not very well described by the LSS model. We selected a more general approach to modeling, which was suitable both for LC systems where not only LSS but also other retention equations may apply. These can be combined with equations describing linear (and even nonlinear) mobile-phase programs, to predict the retention in various reversed-phase, adsorption-, normal-phase, and ion-exchange gradient elution modes. The basis of this approach, which I value the most from my achievements, was published in 1974 in the *Journal of Chromatography* and subsequently refined in a plethora of original papers, reviews, and a monograph published together with Jaroslav Churáček (7). A reduced Czech version appeared in the same year at the publishing house Academia in Prague (8).

My work in this period was largely based on modeling, which is an extremely useful tool for method development and optimization. However, a few words of warning: modeling should be used carefully, which is often not the case, as can be seen in some published papers. First, the retention model uses equations with parameters, as determined by the regression analysis of experimental data. Hence, the model can only be as reliable as the experimental data, which should be acquired in the mobile-phase range providing not too weak a retention is achieved, otherwise the model can be subject to gross errors. This is because the thermodynamic distribution constant of a solute between the stationary and the mobile phase is not directly proportional to the experimental retention time, t_R, but to the reduced retention time, t'_R, that is, to the difference between the measured retention time, t_R, and the elution time of a nonretained compound, t_0 (so-called dead or hold-up time of the column). Because the experimental errors in both t_R and t_0 combine, the uncertainty in t'_R significantly increases for weakly retained compounds eluting close to the column hold-up time. As almost all retention models acquire the parameters by the regression of the t'_R or their logarithms, the reliability of the model parameters becomes questionable if obtained employing too low experimental t'_R.

Furthermore, a good fit of the experimental data to the regression retention model equation alone does not prove the physical correctness of the model. From a mathematical point of view, the fit of experimental data to a model regression equation always improves with an increasing number of model parameters; however, this does not necessarily mean that the additional parameters have any physical meaning. The model should be tested with a variety of samples and separation conditions before it can be more or less generalized. This was very well characterized by Pat Sandra: "Models should be used, but not believed."

As far as the second part of the question is concerned, it is impossible to avoid mistakes when searching for new solutions to problems; I cannot count mine. However, the failures can be very useful and can often be turned to the advantage in the long term. I can give an example: Pardubice is a center of chemical industry, which has supplied us with numerous analytical problems. One such problem arose from the production of synthetic dyes, where a method for the control of purity of naphthalene sulfonic acid intermediates was badly needed. Ion-exchange and ion-pairing chromatography could separate them into groups containing 1–4 sulfonic functionalities (SO_3-), but failed to distinguish the isomers in the individual groups. The reason is that all acids are fully ionized and their acidity is comparable with strong mineral acids. Searching for another solution, we realized that the isomers show important differences in permanent dipole moments affecting the ion-exclusion from nonpolar stationary phases. The combined effects of hydrophobic and ion-exclusion interactions allowed us to achieve nice isomer separations on bonded C18 columns with suitable pore morphology in aqueous–organic mobile phases containing relatively high concentrations of salts such as sodium or ammonium sulfate (9).

Foret: What areas of separation science are you focusing on at the moment?

Jandera: There are two trends I can see in contemporary analytical science. The first is developing fast, simple, and reliable methods for detecting the presence and estimating the quantity of biologically, clinically, or environmentally important compounds: the solution is obviously finding suitable sensors for specific target compounds or their classes.

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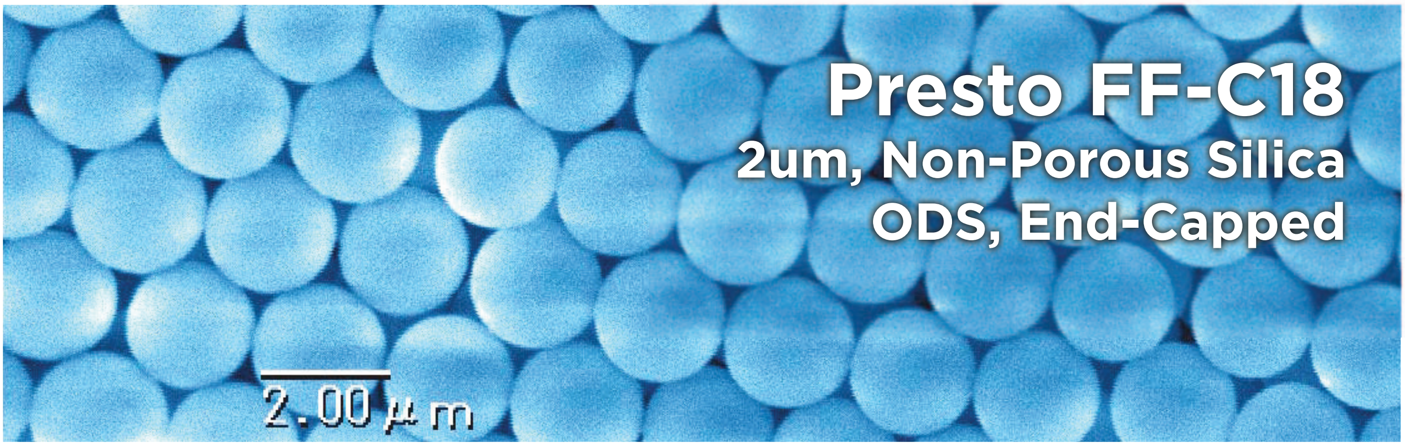

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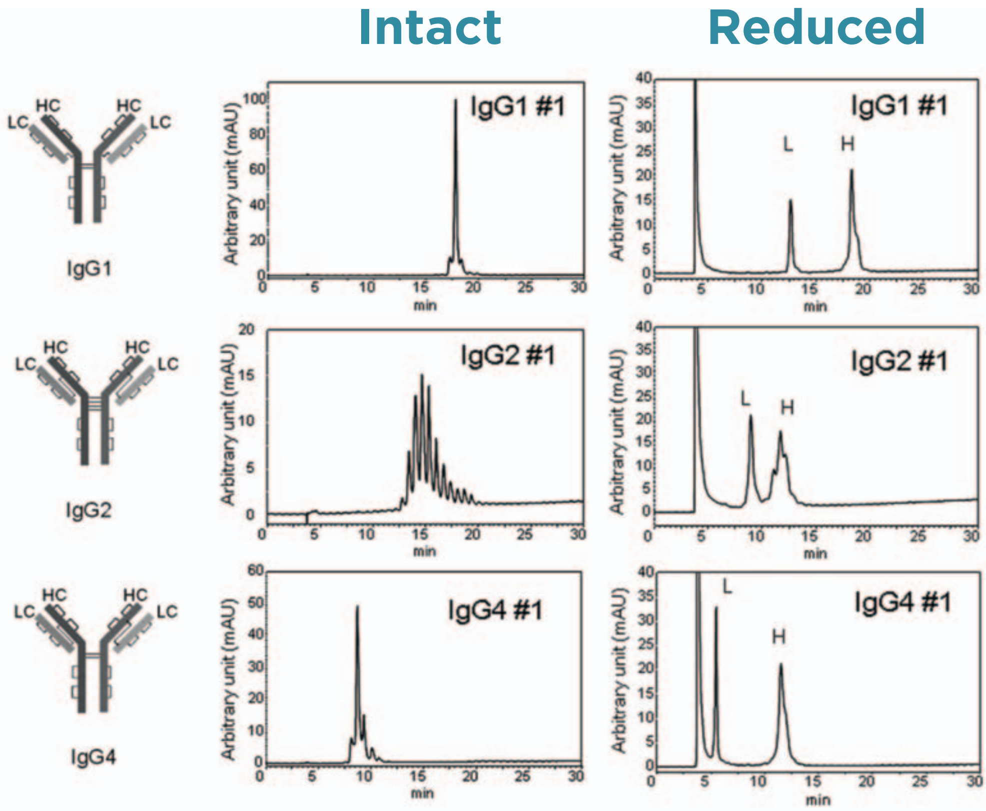
In this study by our friends at Japan's National Institute of Health, they are analyzing both the intact and reduced forms of some common antibodies. Try doing that on a standard wide-pore protein column. By being able to analyze both the intact AND reduced forms of these large proteins (~150 kDa) you can gather information about the cysteine-cysteine disulfide bridge structure which is lost when they are reduced to the heavy and light chains as common in current methods using wide-pore columns.



K. Sakai-Kato, K. Nanjo, T. Yamaguchi, H. Okuda, and T. Kawanishi. Anal. Methods, 2013, 5, 5899-5902

Jandera with Georges Guiochon at the 11th International Symposium on Separation Sciences (ISSS 2005) in Pardubice, which Jandera chaired.

The second trend is finding methods that allow simultaneous determination of as many components as possible in complex samples, which is my present area of interest. Two-dimensional liquid chromatography (LC×LC) allows a significant increase in the number of separated sample components, which cannot be achieved in a comparable time on a single column. My main focus is on setting-up two-dimensional LC systems with large differences in selectivity between the first- and the second-dimension systems, such as combining reversed-phase and hydrophilic interaction liquid chromatography (HILIC).

At the ISSS in Ljubljana, Slovenia, with Professor František Švec and Jandera's co-worker Tomáš Hájek.

Almost all stationary phases used in contemporary HPLC are bonded on silica (less often on zirconia or other metal oxides) because of the excellent separation efficiency. However, organic polymer stationary phases still remain largely unexplored (a few commercial products are rare exceptions), in spite of excellent temperature stability, and a large variety of chemistries that can be prepared by polymerization, either in particulate or in monolithic format.

Organic polymer monolithic capillary columns and microcolumns are the second area of my present research interest. So far, we have succeeded in preparation of monolithic stationary phases showing different properties when changing the mobile phase. When used in combination with an efficient short second-dimension column, the same two-dimensional setup can be used in different phase combinations (reversed-phase × reversed-phase and HILIC × reversed-phase) in successive runs with alternating gradients of increasing and decreasing concentration of acetonitrile in water (10).

Foret: What major challenges remain in separation sciences? What areas of separation science do you see as most exciting?

Jandera: The main problem is that we are often faced with the analysis of samples containing millions of compounds in concentrations spanning over several orders of magnitude. On the basis of very limited knowledge, we guess which of them are the most important. This

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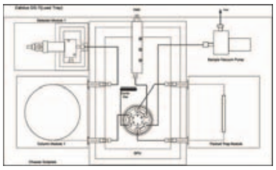

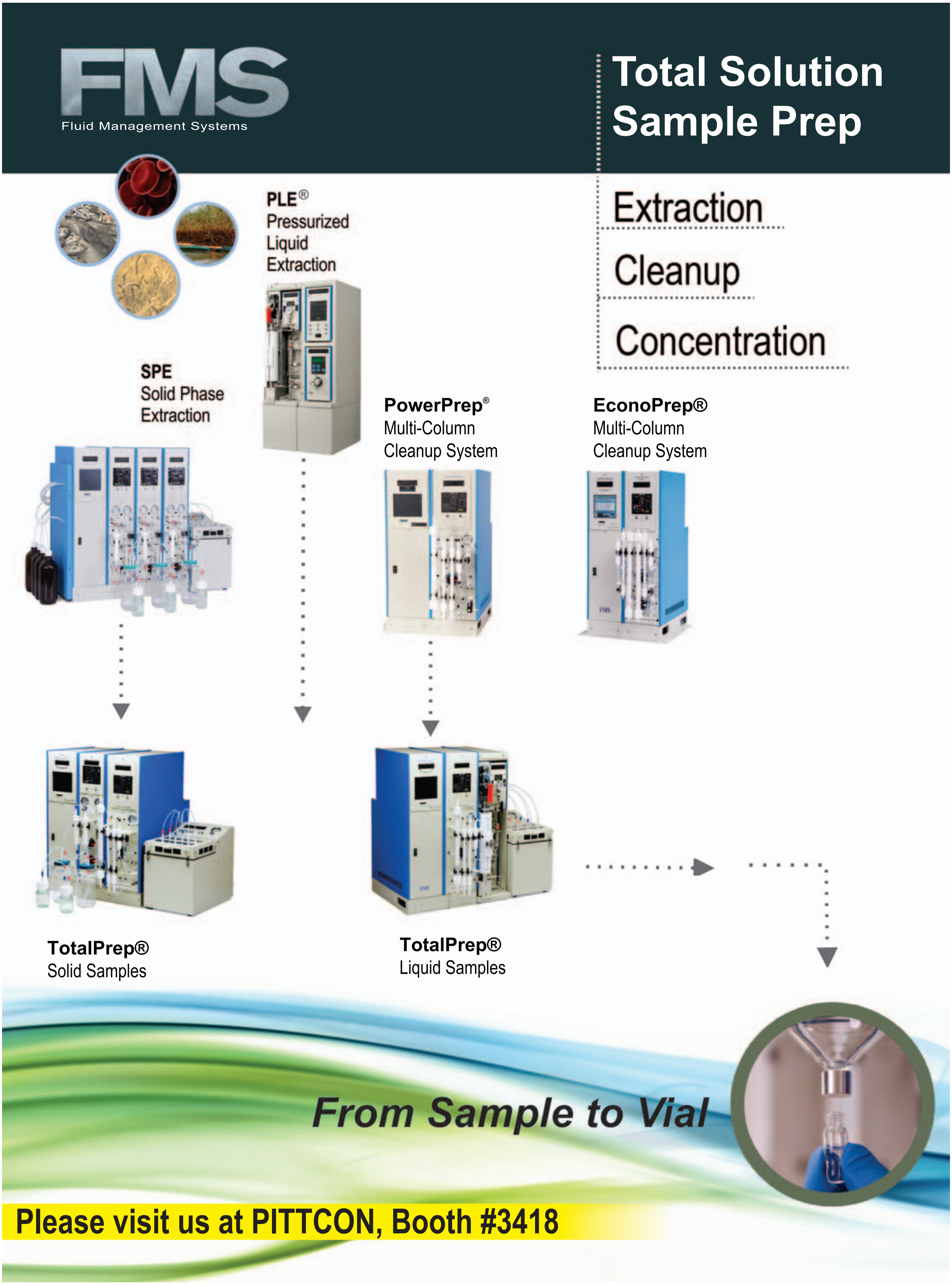

guess may or may not be right. We can only approach the information on such samples by detecting (not to say identifying) a few thousand, or even a few tens of thousands of them using multidimensional chromatography coupled to mass spectrometry and—possibly—to other spectroscopic techniques. It is frustrating that the more chromatographic dimensions we couple on-line, the more we dilute the sample, and the more we lose in terms of sensitivity. Any approach towards a solution to this problem would be very welcome. A possible step on this way may be using selective off-line multidimensional systems, using new stationary-phase chemistries.

Foret: Among all the awards you have received, which one was the most remarkable award to you?
Jandera: The highly prestigious AJP Martin Medal awarded by the Chromatographic Society. I also greatly appreciate the Jaroslav Janák medal, which I recently obtained from the Institute of Analytical Chemistry of the Czech Academy of Science. I hold Jaroslav in high esteem as the coinventor of GC, a giant of Czech chromatography, an example to all scientists, and also a friend of mine.


A recent photo of the Pardubice University group "Separations in liquid media." From left: Magda Stanková, Petr Cesla, Jirka Urban, Verca Škeríková, Jandera, Tom Hájek, Katka Vynuchalová, and Honza Soukup.

Foret: Do you think scientific evaluation is becoming too bureaucratic?
Jandera: This is a very good question. Obviously, the original intention was to control the funding of science from public sources and to distinguish between the "good" and the "bad" (or "less good") research to allocate the funds most efficiently. However, I am afraid that the output of this effort has been counterproductive so far. It is based on the idea of providing an objective tool that can be simply evaluated and used by anyone. The frequent scientometric criteria used in the common databases such as the number of citations and h-index have often been criticized because they can give an approximate idea on the relative merits of the research results achieved by experienced researchers, but are not very suitable for administrative workers to evaluate the quality of scientific work. So another system was developed that gave point values for results published in less important local journals and for presentations at some selected indexed conferences, often not the highest level ones. The point system is usually meticulously controlled and used as the basis for evaluating the research institutions and the individual scientists, for the purpose of distribution of public research funds.

In addition, the criteria are often strange, essentially counting the num-

bers of the publications, and do not distinguish between, sometimes very significant, differences in the quality of articles published in the same journal. The quality of the results can be best judged by the experienced scientists working in the field; however, because there are usually only a few knowledgeable people working in a specific research area, they are a-priori suspected to not provide an objective evaluation because of a possible conflict of interest. Hence, scientists are motivated by acquiring the highest point values rather than doing high quality research, which often does not bring immediate output in the points. I am afraid that this system of hunting for points often results in average or even sub-average research, probably contrary to the original intention.

Foret: If you had a second chance would you select science for your career?

Jandera: Scientific research has changed a lot since I started my academic career, turning more and more towards business, and oriented on fast output rather than on thorough fundamental understanding of the problems. Of course, on the one hand, the advances in the instrumentation available for research has been formidable. On the other hand, some researchers do not find time to read up on the earlier scientific literature related to their research and instead rely on the automated search software, but by doing this they may miss something very important. Some do not even read the literature they present in the list of references, which is often irrelevant or even incorrect. Quite frequently, "new" reports appear on the research essentially similar to that described in publications 10, 20, or even 40 years ago. Even though the editors and reviewers should take care to avoid this, the problem is becoming more serious with an ever-increasing abundance of new open access journals desperately looking for articles. Finally, scientists have less and less time to spend in their laboratories because they have to struggle for the research funds by preparing new projects and writing reports on the ongoing ones.

To answer your question: In spite of these problems, I think I would select science as a career, preferably again at a university. I like solving problems and sharing my knowledge with students. I appreciate being among young people. One does not have time to realize how he or she is growing old!

Foret: Is there any advice you would give to young scientists embarking on a career in analytical chemistry?

Jandera: Practicing analysts are likely to find good jobs. Young people who are intent on pursuing a research career in analytical chemistry may have a harder life. For them, it is essential to find a good team with long-term research program objectives.

Almost 400 years ago, the famous French writer and satirist La Rochefoucauld said that "Old men usually give good advice when they cannot give bad examples anymore." Hence, I am not sure if the scientists starting out now would really appreciate any of my

advice and take it seriously. If I am to send a message to them anyway there are two general, not original, but sometimes neglected items: Before starting the experiment, it is useful to check in the literature if anybody has performed the same or similar research earlier; believe me, this is not a waste of time, you may avoid re-discovering America. Furthermore, the more preliminary information you get on the problem, the better your chance for success. When you get the experimental results, check the quality of the data and be careful in its interpretation; try to avoid “wishful thinking”—often there may be more possible explanations of your observations than the first one that comes to mind.

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Frantisek Foret *obtained his PhD in 1991 from the Czechoslovak Academy of Sciences and in the same year joined the Barnett Institute in Boston as a postdoctoral research fellow in the group of Professor Barry L. Karger. He stayed at the Barnett Institute for nine years as a research group leader and in 2001 resumed his position in Brno as the deputy director and head of the Department of Bioanalytical Instrumentation at the Institute of Analytical Chemistry, CAS. Since 2011 he has been the group leader at CEITEC—Central European Institute of Technology. His main research interests include capillary separation techniques, laser-induced fluorescence detection, miniaturization, and mass spectrometry coupling. He has authored and coauthored over 150 publications including a monograph on capillary electrophoresis, 12 book chapters, and 15 patents. He currently serves as the senior deputy editor of* Electrophoresis *and on the editorial boards of* Journal of Separation Science, Biomacromolecular Mass Spectrometry, *and* Current Analytical Chemistry. *He is also an Associate Director of CASSS—an International Separation Science Society (since 2009) and member of the Learned Society of the Czech Republic (since 2011).*

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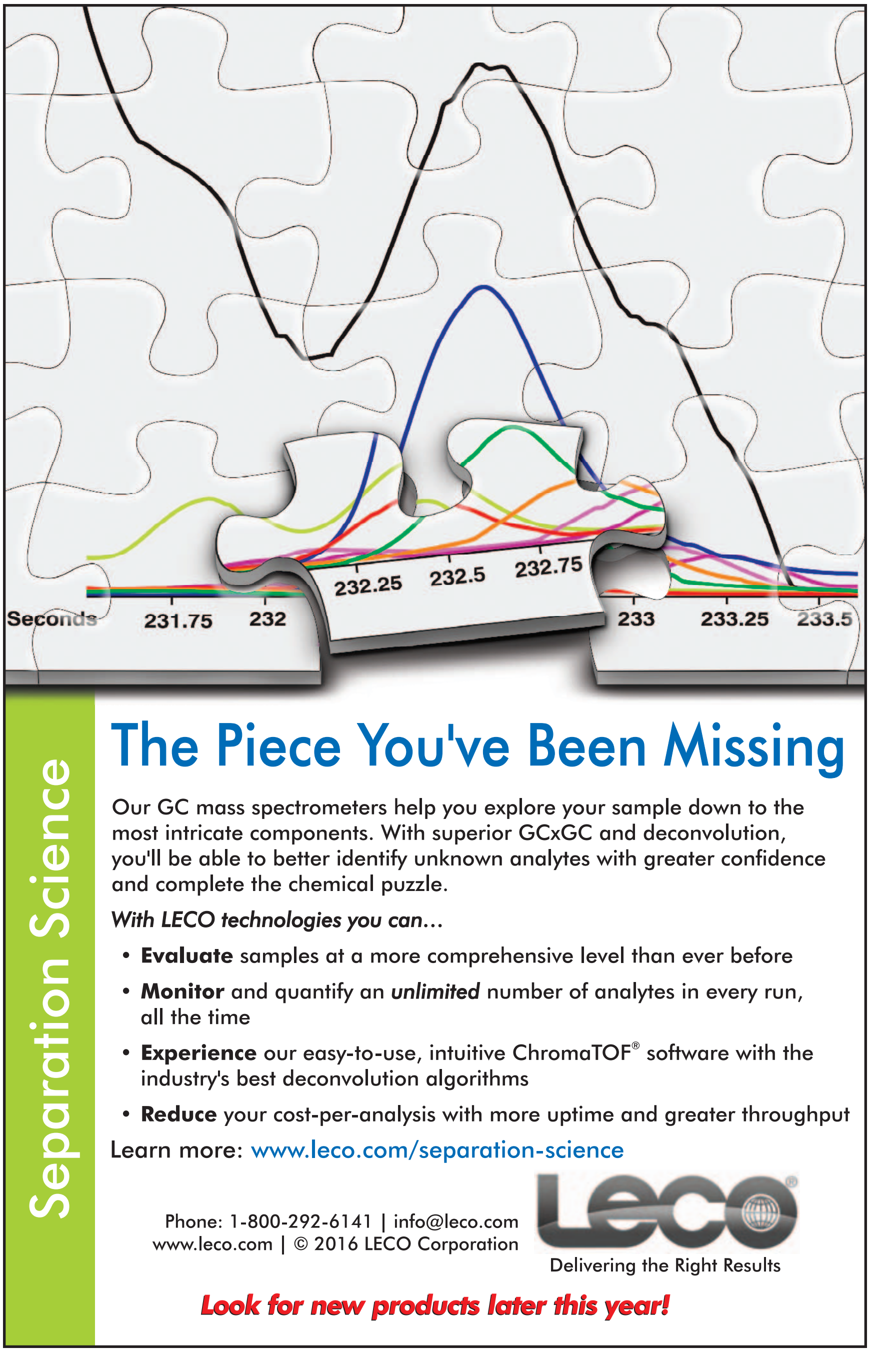


The 2016 *LCGC* Awards

The winner's of *LCGC*'s ninth annual awards are highlighted.

Milton L. Lee

Debby Mangelings

Meg L'Heureux

The *LCGC* Awards have made their mark in chromatography history during the last eight years by honoring the work of iconic figures in the field as well as up-and-coming scientists (Table I). In 2015, we had the opportunity to go a step further by honoring the award winners at an oral symposium as part of the Pittcon 2015 technical program, featuring talks by both of the award winners and Mark R. Schure, Lloyd R. Snyder, and Terry A. Berger. The winners of the ninth annual *LCGC* awards also will be honored at Pittcon 2016.

This year, we are pleased to honor two outstanding separation scientists: Milton L. Lee, the winner of the 2016 Lifetime Achievement in Chromatography Award, and Debby Mangelings, the winner of the 2016 Emerging Leader in Chromatography Award. Lee and Mangelings will receive their awards on Monday, March 7, during an oral symposium at Pittcon 2016 in Atlanta, Georgia.

Milton L. Lee

Milton L. Lee graduated from the University of Utah in 1971 with a B.A. in chemistry. From there, he went on to receive his PhD in analytical chemistry in 1975 from Indiana University under the guidance of Milos V. Novotny.

"I have been very fortunate to have many great graduate and postdoctoral students during my career," said Novotny, now a Distinguished Professor Emeritus at Indiana University. "Milton was one of the best." He was also Novotny's first. Novotny explained that the two began their work together on several aspects of capillary chromatography and that Lee was "enormously helpful" in starting his research laboratory. Lee was also able to get his PhD in four years with a high number of publications, Novotny noted. "This was because he rapidly learned the experimental skills and knowledge of chromatography and became incredibly motivated and organized in his research," said Novotny.

After receiving his PhD, Lee briefly worked as a chemical engineering research associate at the Massachusetts Institute of Technology under Ronald A. Hites. In 1976, he took an assistant professor position in the Department of Chemistry and Biochemistry at Brigham Young University (BYU) in Provo, Utah, a position he held until 1981. Lee was an associate professor at BYU from 1981 to 1984, and then in 1984 he became a full professor. In 1985, Lee was named the H. Tracy Hall Professor of Chemistry at BYU, which is his current position.

Lee: A Scientist in Motion

Lee is known for a wide range of achievements. In addition to his research and teaching at BYU and his work advancing capillary chromatography, electrophoresis, and other techniques, Lee obtained 20 patents and successfully commercialized three instruments. He also founded three analytical instrument companies (Lee Scientific, Sensar, and Torion) as well as one journal (*Journal of Microcolumn Separations*). In addition, he has published more than 570 journal articles and given more than 500 presentations.

We interviewed several of Lee's colleagues, friends, and former students about his achievements, impact, and relationships. One common theme was

Table I: Winners of the *LCGC* awards

Year	Lifetime Achievement	Emerging Leader
2008	Walt Jennings	Gert Desmet
2009	Harold McNair	Kevin Schug
2010	Georges Guiochon	Jared Anderson
2011	James W. Jorgenson	Dwight Stoll
2012	Lloyd Snyder	Emily Hilder
2013	Peter W. Carr	Davy Guillarme
2014	Fred E. Regnier	André De Villiers
2015	Joseph Jack Kirkland	Caroline West
2016	Milton L. Lee	Debby Mangelings

Evaluating some complex chromatograms obtained through capillary GC in 1973 at Indiana University (IU). From left to right: Doctoral students, Mike McConnell and Milton Lee, Dr. Milos Novotny, and an IU sophomore Raleigh Farlow. The photo secured through the courtesy of Department of Chemistry, Indiana University.

that Lee's abilities and work have been wide-ranging, making it difficult to name a single greatest contribution to the field.

"When you look back on Lee's body of work in separations, he has continued to transform himself and his expertise," said Mary Ellen P. McNally, a Technical Fellow at Dupont Crop Protection. She explained that Lee's work focused on a wide range of techniques, including supercritical fluid chromatography (SFC), electric field gradient focusing of proteins, high-speed thermal gradient gas chromatography (GC), polymer monolithic column technology for liquid chromatography (LC), sampling and concentration of target organic compounds in air, thermochemolysis–methylation of microorganisms for the generation of characteristic biomarkers, toroidal ion trap mass spectrometry (MS), and fluidic sieving of nanoparticles.

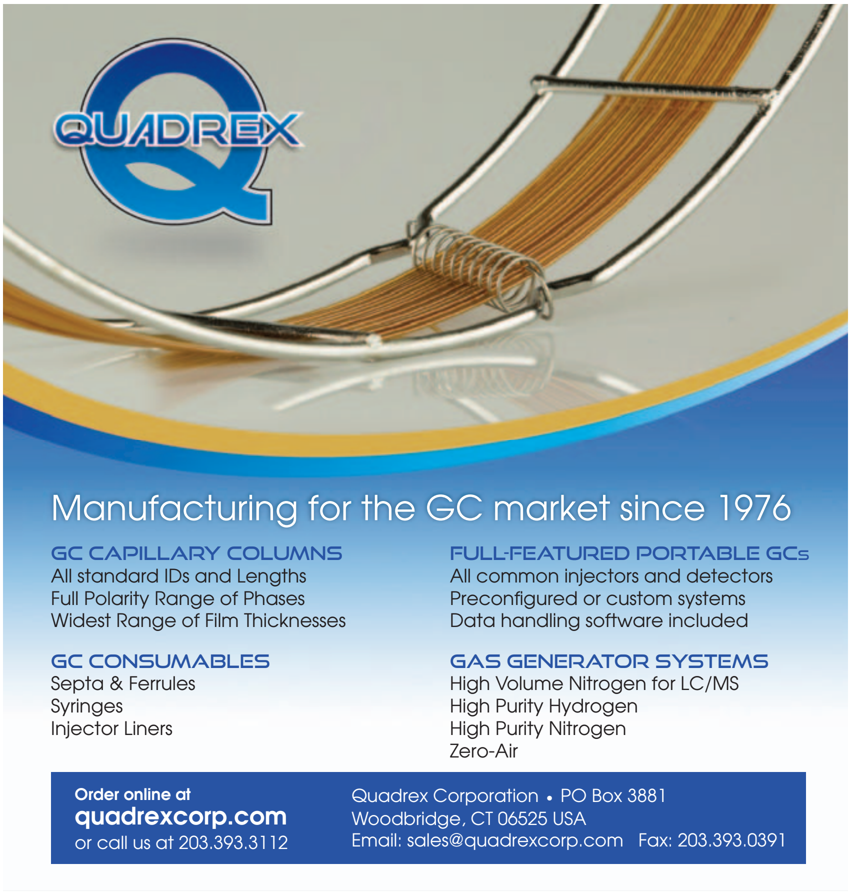
Former graduate school labmate and long-time friend James Jorgenson, the Kenan Professor of Chemistry at the University of North Carolina at Chapel Hill, agreed. "Lee has worked on so many divergent things, from analyzing tobacco smoke, SFC, and the design of mass spectrometers to monolithic LC columns, and the list goes on and on," he said.

"He is a giant in separation science with an endless list of accomplishments," echoed Jim Luong, Associate Technology Director with the The Dow Chemical Company. "What is unique about Milton is that he is a scientist in motion—he continues to invent and to drive science forward!"

Pat Sandra, an emeritus professor at the Research Institute for Chromatography, agreed that Lee has significantly contributed to many fields of separation science. "His portable analyzers are definitely some of the highlights of his achievements," he added.

Theory and Practice

But it is not just Lee's breadth that is remarkable. It is his ability to connect the diverse areas of his work. Lee's research philosophy is unique in the way that fundamental chemistry, physics, biology, and mathematics are intertwined with the corresponding applied sciences and technology, noted one of Lee's postdoctoral researchers, Karin Markides, now the senior advisor to the president and CEO of Chalmers University of Technology. "In this way, theory and practice meet in a most stimulating way to enhance the ability to sample, separate, and detect valuable chemical information," she said. "His ability to spin out chemical instrumentation of high complexity to the market



Milton Lee working in the Novotny Laboratory at Indiana University in 1975.

and at the same time continue as a leading researcher and teacher is an amazing achievement. The impact of his career is too large to measure."

Former student and colleague Bruce E. Richter, an R&D manager at Agilent Technologies, agreed that Lee is impressive in his ability to connect theory and practice. "While Lee understands the theories, his approach has always been to develop practical solutions for real problems," he said. "Whether the technique was GC, SFC, LC, or capillary electrophoresis (CE), Lee helped to improve the ability of these techniques to solve real problems by providing new tools and insights that demonstrated higher levels of practicality and usability."

As an example, Richter cited Lee's work to develop a better understanding of surface deactivation of fused-silica capillaries and then new and innovative stationary phases all for GC. "Lee has always pushed the envelope of separation science by looking at what problems existed and trying to develop ways of overcoming those problems," he concluded.

As a result, noted Edward Yeung, a Distinguished Professor Emeritus at Iowa State University, Lee has had a significant impact on separation science. "Lee's work has not only conceptually changed separation science, but also has had a major influence on the users of separation science," he said. "This is evident from the many patents that are derived from his work and the different companies that Lee founded to adapt his inventions to practical applications."

Lee's influence on instrumentation goes beyond his specific developments, said Markides. "The significance that Lee always gave to the separation part of the analysis and [his insistence] that the column is the most important component of separations instrumentation has strongly influenced the way that instrument companies care for sample separation and transfer to detection," she said. "This emphasis on separation has been crucial for the successful development of the whole area of microfluidic separations."

While Lee's impact in the separations science community has been felt on the industry side, many consider his academic contributions to be his biggest legacy. "Lee spread his knowledge by training around 100 graduate students in his career," said Pankaj Aggarwal, a senior scientist at Pfizer and a former graduate student of Lee's.

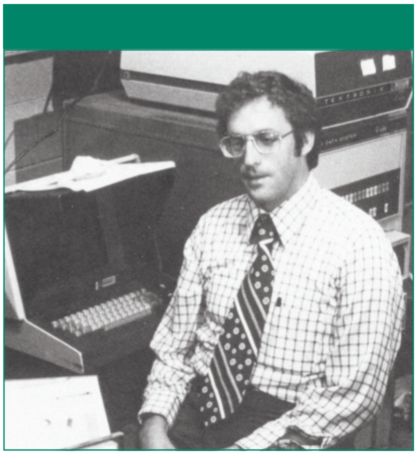
Another former student of Lee's, Sheldon Sumpter, who is an operations and program manager at DuPont Crop Protection, agreed with Aggarwal. "Lee's greatest contributions to the field of separation science are his

graduate students and post docs," he said.

Luong also mentioned Lee's contributions in academia. "Lee is an incredible educator and a lifelong learner himself," he said.

Jorgenson agreed. "I suppose in the end, as with many people working in universities, Lee's greatest contribution will be all of the students he has introduced to the field of separations," he said.


Lee and Novotny during a lecture tour in South Africa in 1988.


Lee in a BYU department graduate student recruiting brochure from 1982.

In the Broader Community

Lee has also been very active with analytical chemistry journals. In addition to founding and editing the *Journal of Microcolumn Separations*, Lee has been on the editorial advisory boards of 12 different publications at various times during his career. Some of those include *Chromatographia* (1981–2006), *LCGC* (1985–1987), *Journal of Supercritical Fluids* (1988–1999), *Polycyclic Aromatic Compounds* (1989–2002), *Analytical and Bioanalytical Chemistry* (1999–2003), and *Analytical Methods* (2012–present).

In addition, Lee has been involved with several scientific organizing committees over the years. From 1983 to present, Lee has been on the scientific organizing committee for the International Symposia on Capillary Chromatography. Lee was also on the scientific organizing committee for the International Symposia on Microcolumn Separations from 1987 to 1990, and the chairman of the organizing committee for the International Symposia on Supercritical Fluid Chromatography from 1987 to 1991. Finally, Lee was the chairman of the organizing committee for the International Symposia on Capillary Chromatography and Electrophoresis from 1995 to 2005. Lee is currently on the International Advisory Board of Mediterranean Separations Science Foundation Research and Training Center, which he joined in 2005.

Numerous Awards

Over the course of his illustrious career, Lee has been the recipient of 35 awards. Some of those awards include the First Annual Governor's Medal for Science and Technology, State of Utah (1987); the American Chemical Society (ACS)

Award in Chromatography (1988); the Russian Tswett Chromatography Medal (1992); the Martin Gold Medal (1996); the Karl D. Maeser Distinguished Faculty Lecturer Award, BYU (1998); the Dal Nogare Award (1999); The California Separation Science Society (CASSS) Award for Distinguished Contributions to Separation Science (2005); the Pittsburgh Analytical Chemistry Award (2008); and the *LCGC Europe* Lifetime Achievement Award (2014).

A Teacher's Legacy

As indicated earlier, Lee has played a significant role in the lives of a large number of students who have gone on to achieve great success in analytical chemistry and related fields. To date, Lee has mentored 62 PhD students, 26 post-doctoral researchers, and eight MS students.

Markides said that Lee inspired his students, partly through his own passion to advance separation science. "Lee's ability to continuously foster the forefront of basic and applied research mixed with a unique ability and interest to make students understand and strive for more knowledge is a true gift," she said.

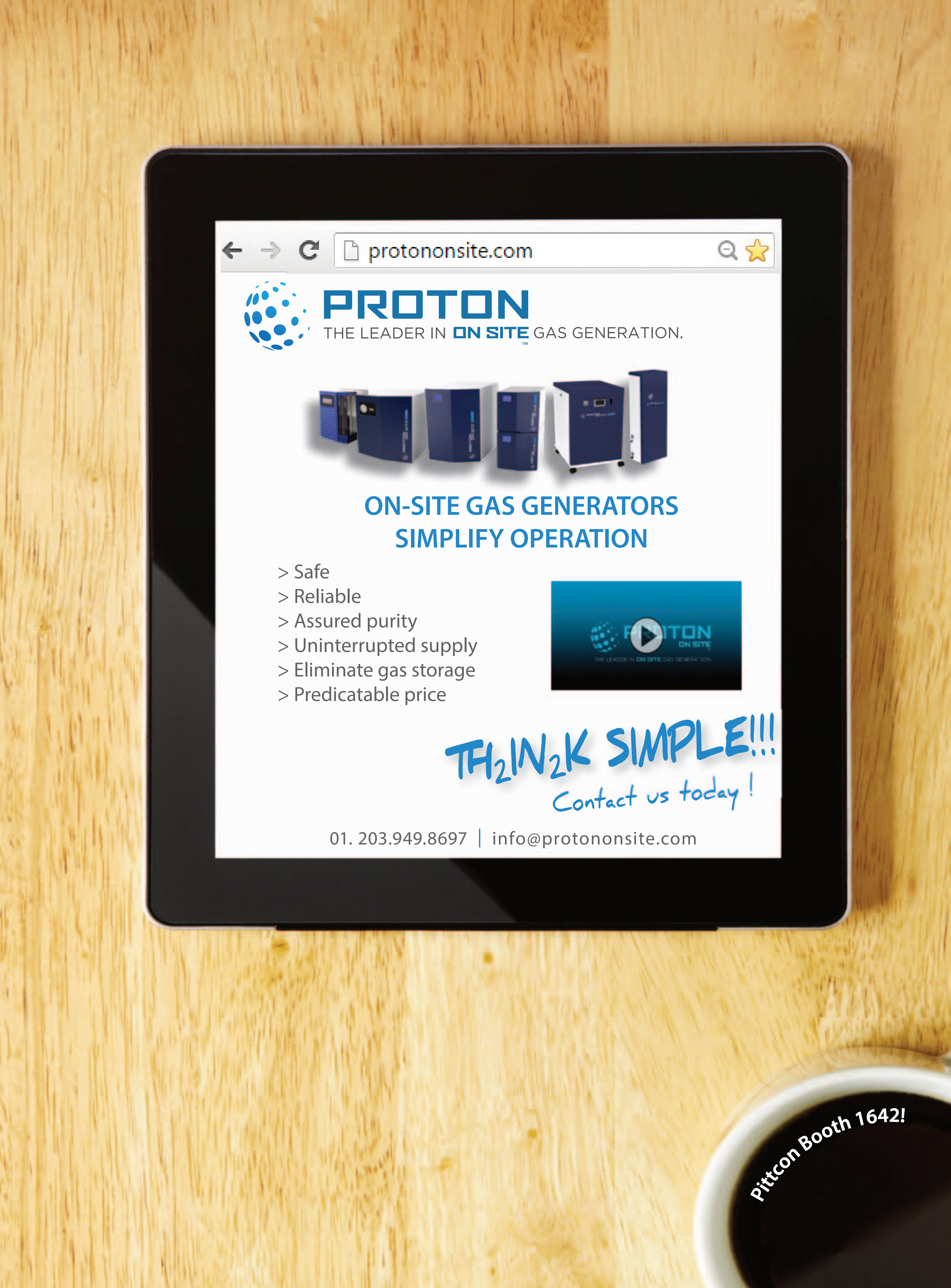
Kate Jackson, 2008 EAS President, presenting Milton Lee with the 2008 Eastern Analytical Award for Contributions to the Fields of Analytical Chemistry. The Fields Award is the top award given at the EAS conference. (Photo courtesy of EAS.)

Former PhD student Iuliana M. Lazar, an associate professor at Virginia Tech, echoed Markides's sentiments. Lazar said that Lee had a relentless commitment to science and was extremely creative in providing solutions to difficult problems. "His patience, wisdom, and vision, were—and will continue to be—a source of inspiration throughout my entire career."

Aggarwal agreed. "The one statement from him that always kept me going was: The feeling you get upon discovering something new cannot be described in words," he said. "He was absolutely correct about that and I was able to feel the same way while working in his laboratory."

That excitement of discovery, Lee also taught his students, requires hard work; Lee is described as being meticulous in his pursuit of excellence and asking the same from his students. Richter recalls some late nights working in Lee's lab with fellow students when they would get a call from Lee to check in. "He wanted to know that we were putting in the time to be successful in our endeavors," Richter explained. "He taught us that nature does not give up its secrets easily and for those who did not put in the extra work, it would be harder to find the answers."

Ray West, who was one of Lee's early graduate students and is now the Director of Business Development at Moxtek, Inc., says he also learned to be a dedicated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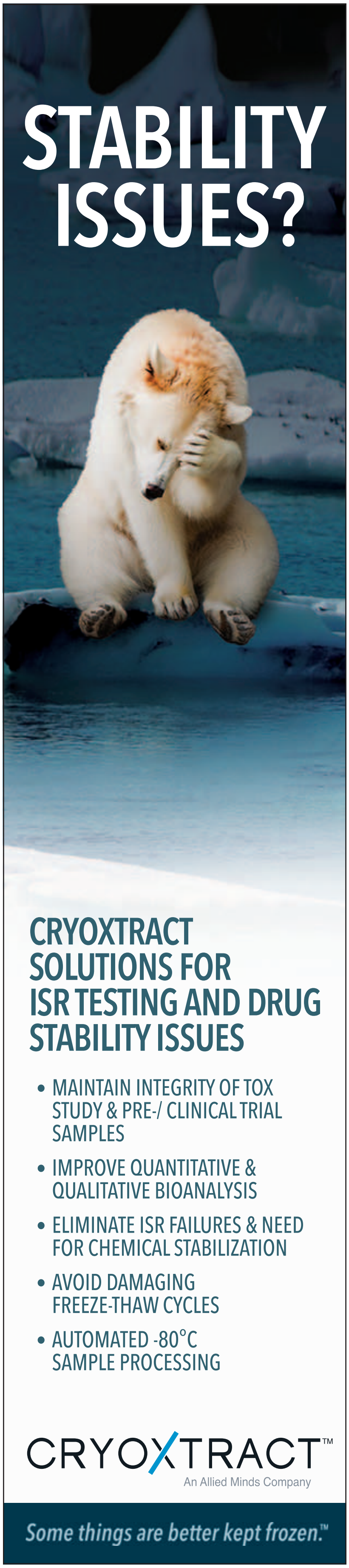


scientist from Lee "He taught us to work hard and had a cot in the lab so we could be there late in the night taking care of critical experiments," he recalls. "I don't think I could have had any better training in graduate school."

Doug Raynie, an associate research professor at South Dakota State University and another former graduate student of Lee's, agreed that Lee's training prepares students to shine once they graduate. When a graduate school labmate went on to work at a leading chromatography supply company, he reported back to his friends that it was the easiest job he ever had. "'You develop such a strong work ethic in Lee's lab that when you go to work you think you're slacking off, but everyone else is impressed by how hard you're working,'" Raynie told us.

But Lee was not just tough; he was supportive, too. "While Professor Lee set the bar high for his students, he was a very generous and caring supervisor," said former PhD student Naijun Wu, the Director of Analytical Research and Development at Celgene Corporation.

Wu also mentioned Lee's dedication to supporting his students at conferences. Wu recalled the 1999 International Symposium on Capillary Chromatography and Electrophoresis, where he was giving a talk as a student in the last session on the last day of the conference. "Prior to the session, Professor Lee asked his other five students attending the conference to show up to my talk," Wu said. "Without his thoughtful support, I would have been talking to an audience of one," Wu laughed.

A Generous Friend and Colleague

Lee's mentorship abilities extend beyond his students. "I am grateful and indebted to Lee for his mentorship and support throughout my career as a scientist," said Luong, who first met Lee at the International Symposium of Capillary Chromatography in 1995. Lee offered Luong his first opportunity to give a plenary lecture, at the International Symposium of Capillary Chromatography in 1997. "I became a better person and a more successful separation scientist because of mentors like Lee," he said.

The panel from the 2008 EAS Award for Outstanding Achievements in the Fields of Analytical Chemistry session. From left to right: Gene Berry (section chair), Abdul Malik, Naijun Wu, Milton Lee, Milos Novotny, Adam Woolley, and Doug Later. (Photo courtesy of EAS.)

Lee is also a very generous colleague, added Adam Woolley, a professor and the Associate Chair in the Department of Chemistry and Biochemistry at BYU. "Lee has been both generous and instrumental in my career development in terms of helping me get connected with funding sources, securing invitations to present at conferences, and successfully nominating me for various awards," he said.

Woolley recounted a specific example of Lee's collegiality. Lee became aware of a call for papers from the National Institutes of Health (NIH) to develop methods to test for bacterial infections. "This wasn't an area where Lee was actively working, but he knew of several of us here at BYU who would be able to put together a strong proposal, so he got us together," said Woolley. After the team was assembled, Lee told them his expertise was not needed and suggested they go ahead without him. "Lee was instrumental in helping us secure a five-year, multimillion dollar grant, even though he is not an investigator on it," Woolley concluded.

Family First

Many friends, colleagues, and past students described Lee as being very family-oriented. "More than once he was offered positions at higher-ranking universities with substantial financial incentives, but he turned them down because moving would mean suddenly putting his family in an unfamiliar place that would require a lengthy adjustment period," said Yeung.

Markides also felt the effect of Lee's devotion to family when she was working for him. She explained that one morning before work her oldest son

announced that he had to build a wooden car for a Scout competition the next day. Markides and her husband did not know how to get it done in one day, but she went to work because they were very busy in the lab at the time. When she got there, Lee could tell that something was bothering her so she told him about the car her son needed to build. “Lee did the most fantastic thing: he said that we needed to prioritize my son’s car and he called in some professors and graduate students to make a winning construction,” Markides said. “I have referred to this story many times in my life when talking about setting priorities.”

Lee’s family orientation also extended to his students. “As his students, we felt like a big family—Lee would often host birthday parties for students and invite us to his home for Thanksgiving or Christmas,” said Wu. “He also organized group hikes for us in the beautiful areas around Provo, Utah.”

Raynie recounted a funny story he heard related to Lee’s family. “Upon hearing his students call him ‘Dr. Lee,’ one of his children didn’t believe he was a doctor, so Lee brought them into his lab so they could see him ‘give a shot’ (injection) to a chromatograph,” said Raynie.

In a Few Words: Who Is Milton L. Lee?

The friends, colleagues, and students we interviewed for this article all provided glowing praise for the type of person Lee is and what it is like to work with or for him. This year we also asked everyone to describe Lee’s personal character or work ethic in three words. A few examples of the responses we received include *brilliant, innovative, honorable, loyal, curious, dedicated, tireless*, and *cooperative*. The three words that came up most often were: *integrity, reliable*, and *creative*.

Novotny expanded on the three words he chose. “Lee has high scientific standards and great organizational skills,” he said “He is a valuable and reliable scientific colleague.”

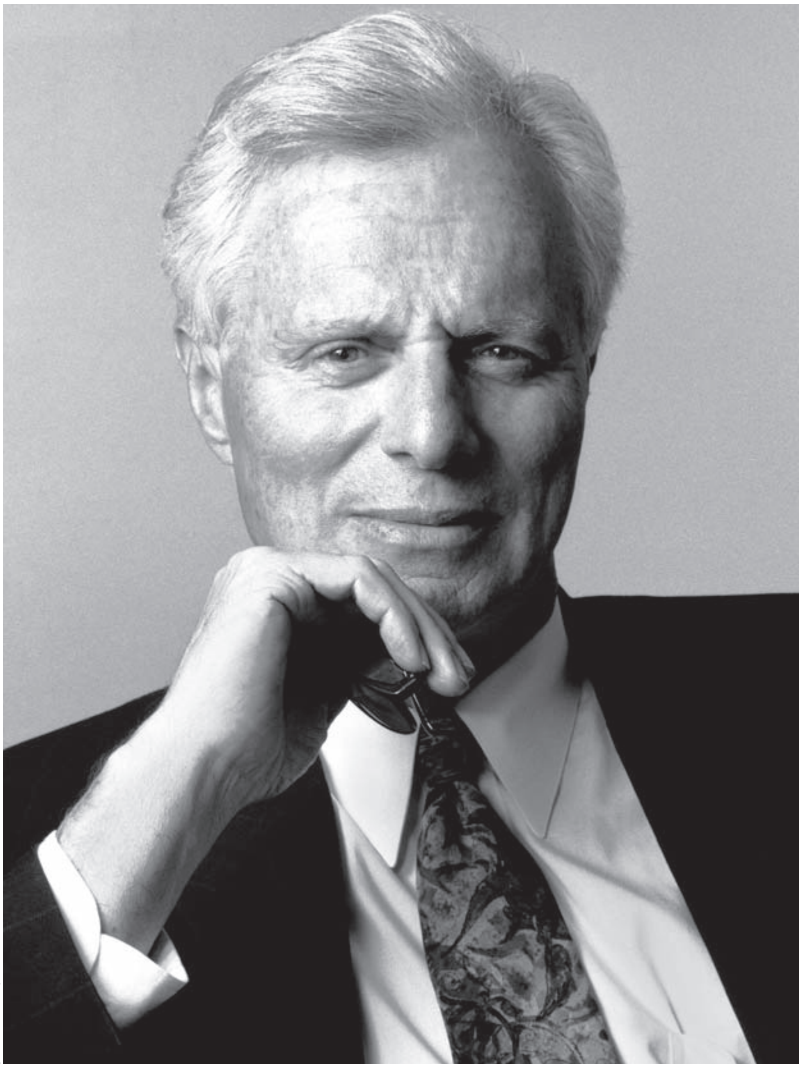
Richter also said a bit more than just three words about Lee’s personal character and work ethic. “He seems to have unlimited energy and ideas that need developing. He is a man of integrity and loyalty,” said Richter. “He expects a lot from those that work for him, but he also provides an example of someone who is hard-working and dedicated to his field of endeavor.”

Indeed, Lee certainly seems to exemplify a highly creative man with great integrity.

Debby Mangelings

LCGC’s 2016 Emerging Leader in Chromatography, Debby Mangelings, obtained her pharmacist diploma in 2001 from the Vrije Universiteit Brussel and a PhD in pharmaceutical sciences in March 2006 from the same university.

Mangelings’s PhD supervisor was Yvan Vander Heyden, a professor in the Department of Analytical Chemistry and Pharmaceutical Technology at Vrije Universiteit Brussel, who took notice of her work during her early graduate studies. “During her master’s thesis work, which was also performed in our department, we noticed that she was a logically thinking and hard working student, so we asked her to start a PhD,” said Vander Heyden. Mangelings agreed.



Mangelings presenting her invited lecture at the 26th International Symposium on Pharmaceutical and Biomedical Analysis which took place in Tbilisi, Republic of Georgia in July 2015.

After completing her PhD in 2006, with a thesis entitled “Evaluation of Capillary Electrochromatography as Chiral Separation Technique—Definition of a Generic Separation Strategy,” Mangelings became a postdoctoral fellow with the Research Foundation Flanders (FWO) within the Department of Analytical Chemistry and Pharmaceutical Technology at Vrije Universiteit Brussel. She became a part-time professor at Vrije Universiteit Brussel in 2009 and was promoted to associate professor in 2010, which is the position she holds today. In this role, Mangelings has supervised five successful doctoral students and is currently supervising five additional students who are working on their degrees.

A Focus on Chiral Separations

Mangelings’s work has focused primarily on chiral separations and the use of miniaturized separation techniques. She has focused on the definition and updating of chiral separation strategies for various modes of high performance liquid chromatography (HPLC)—including normal-phase, reversed-phase, and polar organic solvent chromatography—as well as for CE and capillary electrochromatography (CEC). Her current research efforts are focused on developing supercritical fluid chromatography (SFC) methods for chiral analyses and nonchiral analysis for drug impurity profiling, the robustness of chiral CE methods, the use of new chiral stationary phases in CEC, and the development of fingerprint chromatograms of herbal extracts using ultrahigh-pressure liquid chromatography (UHPLC) with mass spectrometry (MS) detection. 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 such as the boron cluster species.

Mangelings has received two awards for her research. At the 17th International Symposium on Microscale Separations and Analysis in 2004 (HPCE 2004) held in Salzburg, Austria, Mangelings received the Applied Physical Chemistry Award of the Istvan Halasz Foundation for outstanding achievements in the field of chromatography. In 2007, she was the Laureate of the National Prize of the Belgian Society of Pharmaceutical Sciences.

Vander Heyden said that Mangelings is clearly one of the most promising experts in chiral separations.

Martin Schmid, an associate professor at the University of Graz, Austria, said that Mangelings’s work to make chiral method development more strategic and systematic is important because chiral separation behavior is difficult to predict and often remains a trial-and-error attempt—despite great advances in method development for enantioseparation in the last 20–30 years. “Mangelings’s group has made a lot of progress introducing chiral separation strategies, such as for CEC,” he said “With their help it will be easier to get positive results for enantioresolution of a broad spectrum of analytes.”

Bezhan Chankvetadze, a Professor and Director of the Institute of Physical and Analytical Chemistry at Tbilisi State University, agreed. “Based on our current understanding of chiral separations, we cannot predict separation results based on molecular descriptors of a chiral selector and screening remains the major way for achieving and optimizing enantioseparations,” said Chankvetadze. “There-

Members of the Department of Analytical Chemistry and Pharmaceutical Technology at the Vrije Universiteit Brussel (VUB). Back row, from left to right: Johan Viaene, Bart De Cock, Sven Declerck, Debby Mangelings, Yvan Vander Heyden, Peggy Verbuyst, and Andres Ceuterick. Front row, from left to right: Solange Peeters, Katrien Decq, Charlene Galea, Dima Albals, and Mahmoud Hefny Gad.

fore, optimizing the existing screening strategies is very important for both academia and industry, in particular the pharmaceutical industry."

Caroline West, an associate professor at the University of Orléans, echoed the comments of Schmid and Chankvetadze. "Mangelings's work is definitely practical, with an intention to rationalize and simplify the strategies to achieve chiral separations with a minimum of time, effort, and costs," West said. "She is devoted to producing something useful and easy for end-users."

Another scientific peer, Serge Rudaz, an associate professor at the University of Geneva, made similar remarks. "Mangelings is strong in both the theoretical and practical aspects of separation science and has a keen eye for topics with a broad interest," he said. He noted her important contributions in chiral CEC specifically, which he monitored closely during a period when his group was involved with the countercurrent approach in capillary zone electrophoresis. "It was the pioneering period for coupling electro-driven techniques with mass spectrometry," he said. "It was difficult for all groups, therefore we were really following all contributions that could help us to have success with the hyphenation of CE and MS."

Mangelings has also shared her work and expertise with the scientific community at large. Her publication record includes 8 book chapters and 64 manuscripts in peer-reviewed journals, and she has given 45 oral and 71 poster presentations at national and international congresses and symposia. Since 2012 she has been a member of the editorial board of *Chromatographia* and *Acta Chromatographica*.

West noted that Mangelings's work is cited often, with a Scopus count of 770 external citations and an h-index of 15. "Among her papers are some of the most influential works on the development of generic screening strategies for chiral separations," said West.

Teresa Kowalska, a professor in the Institute of Chemistry at the University of Silesia, is also impressed by the impact Mangelings's expertise has had so far, despite her young age. "Mangelings's outstanding expertise, particularly in the fields of capillary electrophoresis and capillary electrochromatography, makes her an internationally recognized leading figure, an invited speaker at many separation science conferences, and a highly demanded reviewer for multiple separation science journals," she concluded.

Indeed, Chankvetadze stated that he enjoys Mangelings's presentations at international conferences because they always contain solid scientific results and are well organized and clear. As a result, Chankvetadze has invited Mangelings to be a speaker at all of the international conferences he has organized in recent years. He also commended Mangelings's work as an author and reviewer. "Mangelings greatly serves the separation science community not only as a good author, but also as a very competent and fair reviewer of submissions to international journals," said Chankvetadze. "Knowing her ability to be a principled, fair, and punctual judge, I frequently ask her to review manuscripts for the *Journal of Pharmaceutical and Biomedical Analysis*, which I edit."

Kowalska had a similar assessment of Mangelings's work as a reviewer. "Whenever I invite her to review a manuscript submitted to a chromatography journal that I edit, *Acta Chromatographica*, she always accepts my invitation," she said. "Manuscript reviews provided by Mangelings are exemplary masterpieces of competence and thoroughness, voluminous and not just critical, but appreciably educative for the manuscript authors."

Chankvetadze said that although Mangelings is young and can still be considered an emerging scientist, she is already a well-established leader worldwide in the field of enantioseparations. "The proof of this is not only her impressive list of publications in international journals and the presentations she makes at international conferences, but also the network of PhD and master's degree students around her at Vrije Universiteit Brussels, as well as the international collaborations she is involved in," he said.

Chankvetadze also enjoys his own direct collaborations with Mangelings, who is a very pleasant colleague who always considers the contribution of all parties in a joint project. "She has a good ability to generate new projects and is a very constructive team player," he said.

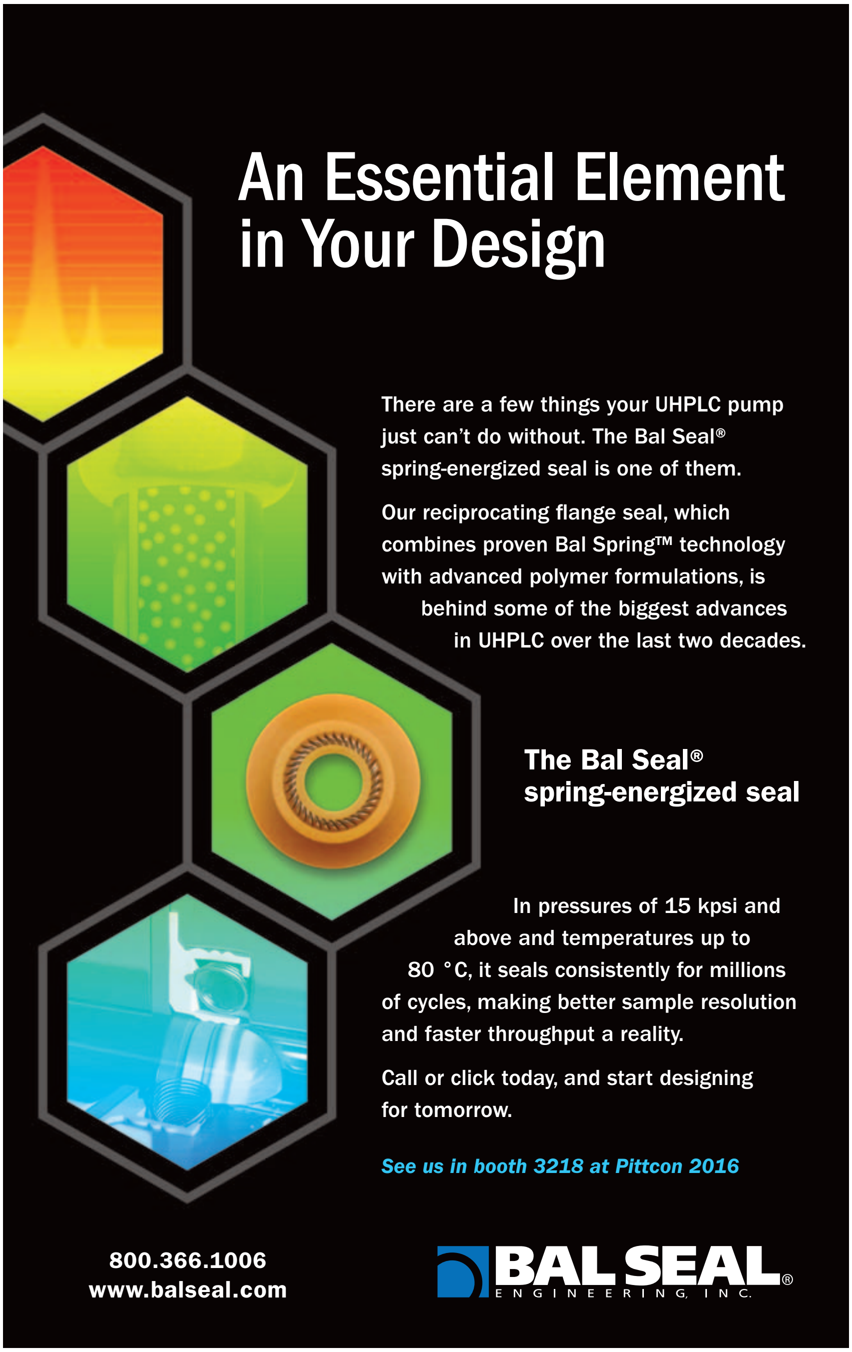
Kowalska anticipates that Mangelings's career will continue in a challenging and rewarding way. "In my view, Mangelings will continue moving toward challenging and pioneering applications of selected instrumental separation techniques to solve demanding scientific problems in pharmaceutical and other life sciences," she said.

Rudaz foresees a similarly bright future for Mangelings. "Mangelings is well qualified to be a future leader in separation science and has the intellect and skills to contribute massively to the study of chromatography and other separation techniques," he concluded.

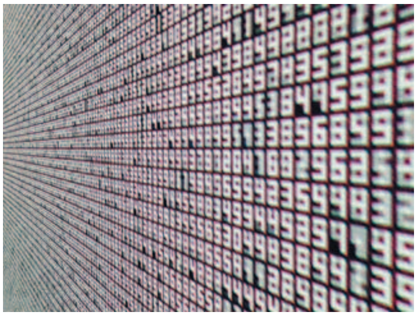
More About the Winners

In-depth interviews with Milton L. Lee and Debby Mangelings, focused on their research, challenges, and accomplishments, will be published in upcoming editions of our newsletter, *E-Separation Solutions*.

Meg L'Heureux *is the managing editor of* LCGC North America *and* Spectroscopy *magazines in Iselin, New Jersey. Please direct correspondence about this article to mlheureux@advanstar.com* ■



The Ideal Chromatography Data System for a Regulated Laboratory, Part IV: Ensuring Regulatory Compliance



JODIE GRIGGS/GETTY IMAGES

The first three articles in this series discussed where and how a chromatography data system (CDS) fits into a regulated laboratory, the overall requirements for the architecture of a future system, and additional items to enable effective electronic ways of working. The final part of this series looks at regulatory compliance of a future system and provides a summary of the 15 recommendations made in this series.

Chris Burgess and R.D. McDowall

In the first article in this series (1) we looked at the role of the laboratory and discussed the concept of the analytical factory together with the controllable and uncontrolled factors influencing the analytical process. In addition, we looked at the requirements for ensuring data integrity throughout the analytical process. We began the second installment (2) by defining the overall system architecture for a compliant chromatography data system (CDS) in a regulated laboratory in more detail. In the third part (3), we described the new functions required to create fully electronic processes and workflows that should be incorporated into a future CDS to improve efficiency and effectiveness. In this, the last part of the series, we look at regulatory compliance features that must be present in any CDS for trustworthy and reliable electronic records and electronic signatures, thereby ensuring data integrity. To complete this series, we summarize all 15 recommendations made, to describe what the future CDS should look like.

Where Are We Now?

Although chromatography data systems operating in regulated laboratories have basic controls for regulatory compliance there is still a lot that is driven by paper, such as system configuration and instrument qualification. The latter is particularly the case, as suppliers use their service personnel to deliver qualification services, but provide reams of paper for them to fill in for their customers to review. Mistakes, especially by the service personnel, abound as the authors have found when reviewing such documents when advising clients. Moving to an electronic process will eliminate many of these problems and allow fast, electronic review by the laboratory staff. Other areas for compliance improvement include increased data integrity features, improving audit trail content and review, as well as handling unattended working.

Where Do We Want to Be?

From the regulatory perspective a CDS operating in a GXP (good manufacturing, laboratory, or clinical practice) regulated environment should be capable of the following functions:

- documenting the software and instrument configurations of the system,
- automated instrument qualification,
- securing metadata and ensuring data integrity,
- enhanced audit trail functionality to meet current regulatory requirements, and
- compliance control for unattended working.

Each of these areas is discussed in turn in the sections that follow.

Requirement 1: Documenting Configuration Settings

A CDS consists of configurable software that is good automated manufacturing practice (GAMP) Software Category 4 (4), and when used in a regulated laboratory, the system must be validated. One area that needs to be documented is the configuration of the system. This consists of two parts: the first is the software and the second is the overall instrument configuration. Typically, the software settings that need to be configured to meet the business and regulatory needs of a laboratory or organization are definition of user types and the corresponding access privileges, password length and complexity, use of electronic signatures, and electronic records protection. Currently few, if any, chromatography data systems allow a user to document these settings without resorting to a paper-based process. Because the data are contained within the system, would it not make sense to have a function that performed this automatically? Incorporating a search function could allow the system to document the changes over time.

Similarly, the configuration of data servers and chromatographs attached to the CDS should also be available to be documented via the software rather than requiring documentation outside of the system as paper records.

Requirement 2: Automated Instrument Qualification

As noted above, execution of operational qualification protocols is traditionally performed manually with the attendant issues of incomplete signing and dating of all appropriate sections. In addition, the documentation review by the laboratory staff may take time and the engineer may be off-site before errors are found. What we envisage is that the operational qualification protocol for each instrument together with the predefined or user-defined acceptance criteria will be available in the CDS and each protocol will be preapproved by electronic signature before execution.

A service engineer or third-party agent will have limited access to the data system to execute the operational qualification (OQ), gather results electronically, where necessary entering data manually, and document and resolve any discrepancies. The CDS must identify the individual carrying out the work via the log-on credentials. Unless the OQ is reviewed and approved by laboratory staff the instrument cannot be used for regulated work; thus there is a driver to ensure timely review and approval of the data and results versus acceptance criteria.

Based on a user-defined period, the time for the next OQ will be set in the CDS and reminders will be sent before expiry to the instrument owner or the person responsible for instrument qualification. If required, a user-defined grace period can be specified in the system after which the instrument would become unavailable for use if an OQ had not been performed.

The automated instrument qualification procedure is defined by the vendor, but the scientific soundness is attested to by the user. Therefore the procedures and qualification standards employed must be defendable both in terms of good science and traceability to a national or international standard. Currently some vendor practices do not meet these requirements in the second respect. Hence, it would be

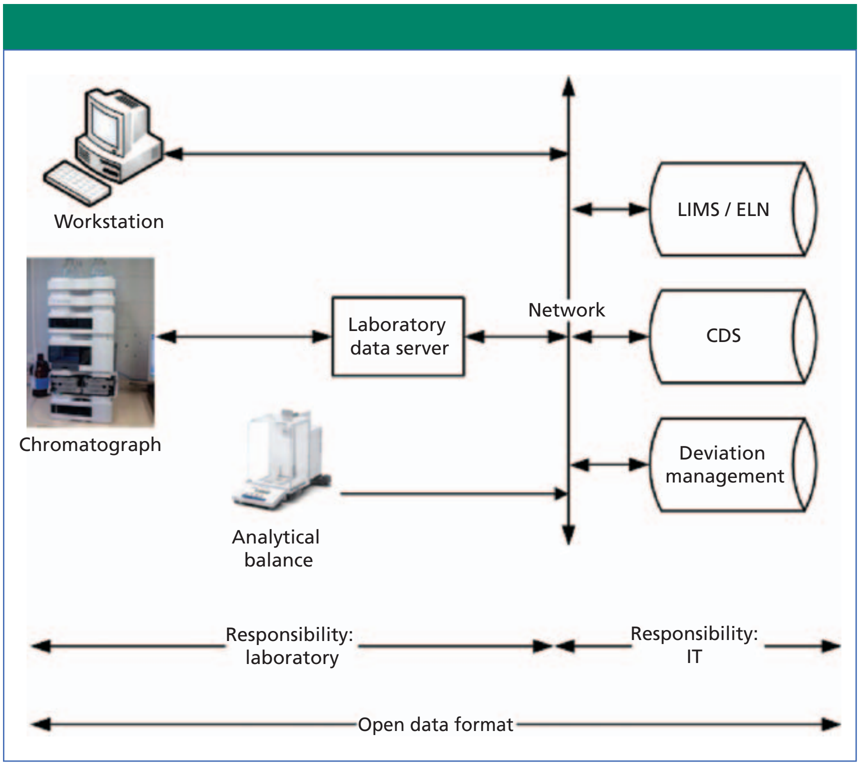


Figure 1: Overall CDS system architecture, informatics connectivity, and responsibilities.

ideal if the vendor provided the automated tools, but allowed the user to configure the reference materials used to determine criteria such as wavelength accuracy, response linearity, and resolution. However, any change in the acceptance criteria would have to be scientifically sound and justified within the system.

Requirement 3: Securing Metadata for Ensuring Data Integrity

One of the reasons for writing this series is the issue of data integrity in falsification cases found during European Union (EU) and Food and Drug Administration (FDA) inspections (5). The data files generated by any CDS are checksummed to detect and prevent tampering with them. However, examination of data falsification warning letters shows that the main thrust of falsification attempts are manual changes of factors, purities, sample weights, and integration parameters. Therefore, of necessity, data integrity and the associated audit trail entries must cover any changes made to the contextual metadata generated during any chromatographic analysis. This is vitally important as a value of 7.5 is useless without the context of the measurement with respect to units, composition, analysts, instrument, column, lot number, analytical method, and so forth. These contextual metadata are also essential for long-term retention and archiving.

Therefore, in the new-generation CDS it is essential to ensure that only changes to sequence, instrument control, data acquisition, and processing files can only be made by authorized users. This is particularly important for integration parameters. The overall requirements in the data integrity life cycle can be seen in Figure 5, which we presented and discussed in part I of this article series (1).

Requirement 4: Improved Audit Trail Review

Although all CDS applications used in regulated laboratories have audit trails, they are not adequate to meet today's regulations in an effective way. The key requirement is for audit trail entries to be reviewed by a second person (6–9). According to *Annex 11* (6), data entries that have been modified or deleted need to be tracked. This applies to both the chromatography data files, for example, manual intervention in the integration of peaks as well as monitoring changes to the associated metadata used by the run such as sequence file and instrument, acquisition and processing methods, and so forth. The design of the audit trails needs to be smarter as well—it is not the sole purpose in a reviewer's life to trawl through hundreds of audit trail entries as a chromatographic version of Indiana Jones. CDS suppliers need to define an audit trail dashboard that covers all data and metadata in a run and present this as a traffic light. Traffic lights would work on the principles that green shows where no operator changes or deletions have been made to data, yellow shows where there have been modifications, and red show any data deletions (if allowed by access privileges). This would allow a second person to review by exception only those entries in yellow or red. An alternative approach could be a function that automatically identified modifications or deletions then notified a supervisor or administrator at the start of the second-person review.

The new function also needs to record that the audit trail has been reviewed by a second individual and no action was needed (all green entries) or modifications have been reviewed and that they are acceptable and within the laboratory's procedures. Also, the ability to set review frequencies on each audit trail (policies, if you prefer) would be a good feature as the function could generate a reminder when a review interval is reached.

For the future CDS, we also need a function that tracks the export of data to other systems via audit trail entries. Many stand-alone systems permit a person to run an assay several times, then pick their favorite run and forward to a laboratory information management system (LIMS). These systems do not track the forwarded runs, so there is no way to quickly identify raw data that is still not included in some test record (at least justified in the CDS as to the rationale for the selection of the data forwarded). Agreeing on injection naming conventions—linked to CDS functionality would help here, along with a simple secure injection sequence log, where appropriate justification is provided as to why each injection in the sequence is performed. Although this may seem draconian, it could make instances of incomplete data, or where the wrong naming convention has been applied, visible in a second-person review.

After all these audit trail functions have been validated, a laboratory can ensure that many second-person reviews can be made speedier and much more efficient.

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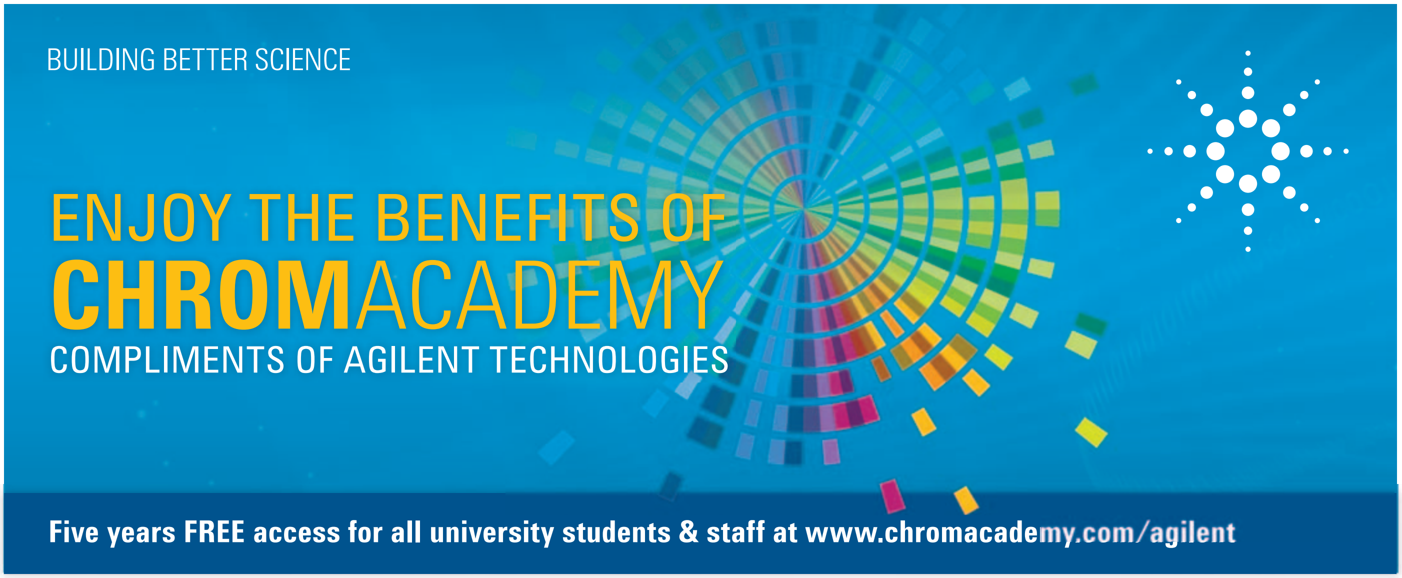

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Agilent Technologies

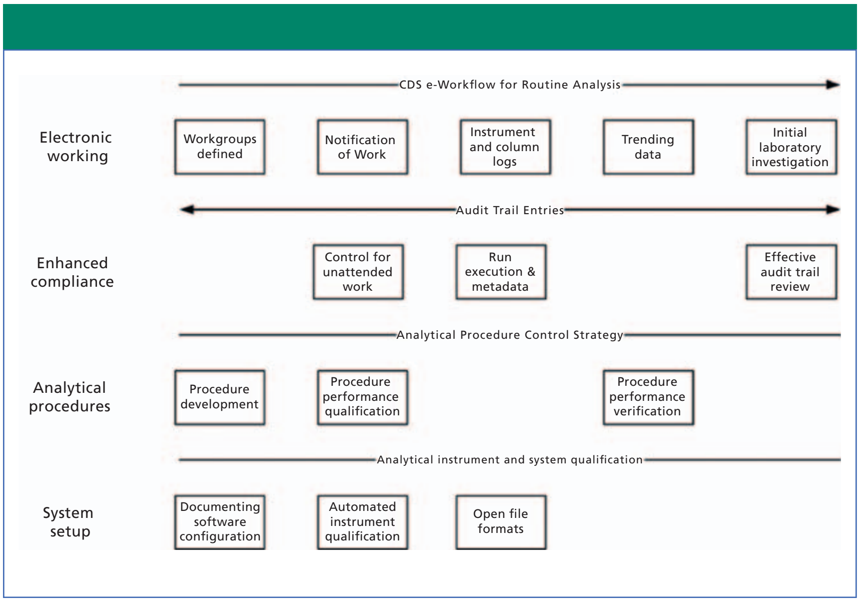

Figure 2: Additional functions and features for a future CDS in a regulated environment.

Requirement 5: Compliance Control in Unattended Analysis

One of the issues with current networked chromatography data systems is that if a run is started and a user goes home how can any changes be made to the run by an authorized user? The assumption made by most, if not all, chromatography data systems is that the user logged in at the start of the run is the same one that makes any subsequent changes, which may not be the case. There needs to be a function, linked to the audit trail, that if an authorized user needs to access a run when the initiating user is not available they can log on and make changes that are attributed to the new user's identity rather than the originating user.

Regulatory Enhancement Summary

In this article, we have looked at five areas that we believe will bring better regulatory compliance when using a CDS in a GXP laboratory. The ability to document configuration settings quickly and effectively will be useful in initial validation of a CDS, audits, and inspections as well as periodic reviews. Automated electronic qualification of instruments should be the norm rather than slow and error prone execution of paper protocols. Securing metadata in combination with effective audit trails are key compliance features. These additions, along with a documented review of key audit trail entries by exception during the second-person review, are essential productivity and compliance enhancements of any CDS while maintaining compliance with the regulations. Finally, the ability to secure a system during a long analytical run but also have another user access the system and make authorized adjustments under their own name is a key compliance requirement that is not addressed currently.

Bringing It All Together

In this series of articles, we presented 15 areas for improving a CDS for operating in a regulated environment and in this final section we collate and present them in two diagrams.

Figure 1 presents the high-level view of a future networked CDS system where data are stored in a database. The system is interfaced in the laboratory to the chromatographs, but also to an analytical balance to avoid manual transcription of sample weights. Acquired data and metadata are stored in open file formats to allow long-term record retention. The CDS is also interfaced with other informatics applications such as a LIMS or electronic laboratory notebook (ELN) and a deviation management application. Responsibilities for the system are also outlined in Figure 1, with the laboratory staff who are responsible for analytical aspects of the application and IT staff who are responsible for the configuration of the application, user account management, and backup. Data must be acquired, processed, and stored using open file formats for long term retention and interoperability.

The working of a future CDS in a regulated environment is shown in Figure 2. This figure is based on the overall process flow used in Figures 3 and 5 from part I of this series (1). Under this we have placed four threads: system setup, enhanced compliance, analytical procedures, and electronic working.

- System setup covering documentation of system configuration, electronic qualification protocols and their execution by the CDS, and open file formats for the data files and the contextual metadata
- Enhanced compliance features for a new system include compliance control for unattended operation of instruments, means of securing the contextual metadata of an analysis, and effective audit trails to enhance data integrity and second-person data review by exception.
- Analytical procedures covering the spectrum from procedure development, qualification (validation), and verification upon transfer to another laboratory.
- Electronic working including the set-up of workgroups with notification of work to be performed (either analysis or review of data), electronic instrument and column logs that are completed by the CDS rather than manually, trending of data within and between runs, and a user-defined module for performing the initial stages of a laboratory investigation

Although we show these features and functions as stand-alone items this would not be the case in practice. Take, for example, the development of a procedure and its associated procedure performance qualification, data generated during these stages would input into the trending module for the procedure. The analytical control strategy would define the extent of any change that would be allowed without the need to requalify the method, see the process flow in Figure 2 from part I (1). There are further linkages and interactions between other suggested enhancements shown above.

Summary

In this four-part series we have positioned a CDS or similar informatics solution in terms of a regulated environment. The business process that a CDS automates is envisioned as an analytical factory with controlled and uncontrolled factors. The enhancements suggested in this series are intended to ensure that a future CDS can work electronically in an efficient and effective way to generate data with its integrity ensured. Furthermore, the data and metadata are generated in a format that ensures that they can be retained throughout the record retention period.

The 15 proposed areas for enhancement are the major ones envisaged for a future CDS in the short to medium timeframe. They are not intended to be exhaustive or complete. However, these functions will not appear magically in the next release of your CDS system. To be fair to the suppliers of these applications, users need to demand them as these companies are market-led. If you think that these features will be of use in the future, what are you going to do about it?

Acknowledgments

The authors would like to thank Lorrie Scheussler, Heather Longden, Mark Newton, and Paul Smith for comments and suggestions made during the writing of this series of papers.

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PITTCON PRODUCT SHOWCASE

Phenyl columns

Waters' solid-core Cortecs C8 and Cortecs Phenyl columns are designed for small-molecule HPLC, UHPLC, and UPLC separations. According to the company, each column is available in 1.6- and 2.7-µm particle sizes. **Waters Corporation,** Milford, MA. www.waters.com/cortecs

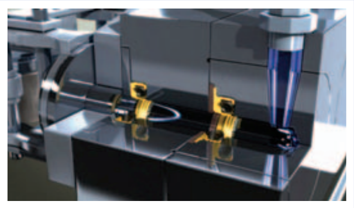
SEC columns

AdvanceBio size-exclusion chromatography columns from Agilent are designed to help scientists achieve reproducible results in the development of biopharmaceuticals. According to the company, the columns provide high resolution for quantitation and the sensitivity required to quantitate aggregates. **Agilent Technologies,** Santa Clara, CA. www.agilent.com

TLC–MS system

Advion's Plate Express sample analysis system is designed to analyze samples directly from thin-layer chromatography plates and a range of other planar surfaces by mass spectrometry. According to the company, the system is suitable for synthetic organic, natural product, and peptide chemistry, and can identify analytes in complex mixtures without additional sample preparation. **Advion, Inc.,** Ithaca, NY. www.expressioncms.com

Reciprocating flange seals



Bal Seal's reciprocating flange seals for HPLC and UHPLC equipment are designed to fit around the pump piston and prevent seepage of eluent. According to the company, the seals are effective in a range of temperatures, pressures, and media types, and provide resistance to abrasion and increased service life in mobile phases when buffer salts are present. **Bal Seal Engineering, Inc.,** Foothill Ranch, CA. www.balseal.com

Microwave sample preparation system

CEM's MARS6 sample preparation system reportedly includes non-contact sensors that provide in situ temperature measurement of every vessel. According to the company, the iWave temperature control feature eliminates the need for the control vessel and allows operators to remotely monitor and control the system using a mobile device. **CEM Corporation,** Matthews, NC. www.cem.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

Neonicotinoid standards

Neonicotinoid standards are available from Chem Service. According to the company, the certified analytical reference standards range from acetamiprid to thiamethoxam and are available neat, in a solution, or in a mixture. **Chem Service, Inc.,** West Chester, PA. www.chemservice.com

Syringe filters

EMD Millipore's Millex syringe filters are designed to prepare samples for analyses such as HPLC, UHPLC, or mass spectrometry. According to the company, the filters are manufactured to pharmaceutical standards with automated equipment and 100% visual and physical inspection, and in-process testing ensures that each filter is made correctly and that the membrane is fully sealed in the device. **EMD Millipore,** Billerica, MA. www.emdmillipore.com

HPLC columns

Hamilton's silica-based and polymeric HPLC columns are designed to provide a range of retention characteristics and performance benefits. According to the company, 17 polymeric HPLC columns are available for reversed-phase, anion-exchange, cation-exchange, and ion-exclusion separations, and two silica-based columns are available for reversed-phase separations.

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

Viscometer

The ViscoStar viscometer from Wyatt Technology is designed as an on-line chromatography detector for determining specific and intrinsic viscosities. According to the company, the viscometer has a transducer protection system that prevents accidental damage to transducer membranes.

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

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

HPLC brochure

Macherey-Nagel's Nucleosil brochure is designed to help HPLC users find information about the manufacturer-packed columns. According to the company, the spherical silica phases are available with various modifications (including C_{18}, C_8, base-deactived C_{18} HD or C_{18} AB, polar CN, NH_2, and SiOH), were originally developed in Germany, and are exclusively available from Macherey-Nagel and authorized distributors.

Macherey-Nagel, Inc.,
Bethlehem, PA.
www.mn-net.com

Ion chromatography system

Metrohm's ion chromatography system is designed to combine with Waters' Empower chromatography data software to determine anions, cation, and polar substances in concentrations ranging from percent to ultratrace. According to the company, the integration controls all aspects of the system from sample preparation to detection schemes.

Metrohm USA,
Riverview, FL.
www.metrohmusa.com

UHPLC connectors

A-Line connectors produced by Agilent and distributed by Neta Scientific are designed to resolve the problems of broad or tailing peaks and loss of resolution. According to the company, the connectors are stable at pressures as high as 1300 bar for UHPLC connections.

Neta Scientific,
a distributor for Agilent Technologies,
Hainsport, NJ.
www.netascientific.com

Headspace vials

MicroLiter's headspace vials reportedly are cleaned and packaged in a Class 10,000 clean room, eliminating particulate contamination. According to the company, closures with a variety of seals also are available.

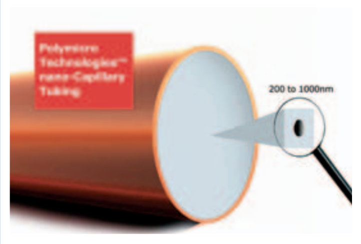
MicroLiter,
a WHEATON brand,
Millville, NJ.
www.wheaton.com

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.optimize.com

Capillary tubing



Polymicro's nano-Capillary tubing is designed with internal diameters ranging from 200 to 1000 nm for scientific, medical, and industrial applications. According to the company, the tubing has a polyimide coating and can interface with existing 375-µm connectors.

Molex – Polymicro Technologies,
Lisle, IL.
www.molex.com

Inert coating

SilcoTek's SilcoNert inert coating is designed to eliminate reactivity between active samples and the stainless steel or glass in an instrument's flow path. According to the company, instruments coated with SilcoNert allow resolution down to parts-per-trillion levels without tailed, split, or broad peaks.

SilcoTek Corporation,
Bellefonte, PA.
www.silcotek.com

Micro synthesis set

The SiliCycle MiniBlock micro synthesis set is designed to provide a platform for 6–48 parallel reactions (40–4 mL) with its own heating, cooling, and inerting capabilities. According to the company, the set is suitable for route scouting peptide synthesis and scavenging studies.

SiliCycle, Inc.,
Quebec City, Canada.
www.silicycle.com

GC columns for FAME analysis

Supelco's model SP-2560, and SLB-IL111 200-m capillary gas chromatography columns,are designed and tested for the analysis of cis–trans FAME isomers. According to the company, the columns are suitable for food composition, nutrition, and food safety applications.

Supelco/Sigma-Aldrich,
Bellefonte, PA.
www.sigma-aldrich.com/gc-food

GC–MS system

Thermo Fisher's DFS magnetic sector high-resolution gas chromatography–mass spectrometry system is designed for the analysis of dioxins and persistent organic pollutants. According to the company, the system provides compliance with any official dioxin, PCB, or PBDE method.

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

QuEChERS sorbent

UCT's Chlorofiltr sorbent is designed for the removal of chlorophyll from QuEChERS extracts without sacrificing the recovery of planar pesticides. According to the company, the sorbent is used as an alternative to graphitized carbon black within the dispersive extraction cleanup process, requiring no further method modifications.

UCT, LLC,
Bristol, PA.
www.unitedchem.com

Glycan profiling kit

Waters' GlycoWorks RapiFluor-MS N-glycan kit is designed to allow laboratories to go from native glycoprotein to ready-to-analyze sample in 30 min. According to the company, the kit is available in 24- and 96-sample formats.

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

GC–MS system

Agilent's model 5977B High Efficiency Source (HES) GC/MSD tandem gas chromatograph and mass spectrometer is designed to provide low limits of detection. According to the company, the system 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, Inc., Santa Clara, CA. www.agilent.com

Polymeric LC columns

The BP800 series LC columns from Benson Polymeric are designed for the analysis of carbohydrates and organic acids. According to the company, the columns are available in calcium, hydrogen, and lead-based forms and provide alternative choices for analyzing common samples found in the food and beverage industry.

Benson Polymeric, Inc.,
Reno, NV.
www.bensonpolymeric.com

Sample preparation system

The Total Rapid Prep system from FMS, Inc., is designed to automate the difficult, error prone, labor-intensive manual processes involved in sample preparation for trace analysis. According to the company, the system automatically performs extraction, sample cleanup, and concentration in hours for the trace analysis of persistent organic pollutants.

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

Robotic autosampler

Gerstel's MPS roboticPRO multipurpose sampler is designed to provide increased GC–MS throughput. According to the company, the autosampler automatically changes between liquid injection, headspace, and solid-phase microextraction. Thermal desorption and extraction and automated liner exchange can be added.

Gerstel GmbH & Co., KG,
Linthicum, MD.
www.gerstel.com

Analytical SFC system

JASCO's SF-4000 analytical supercritical fluid chromatography system is designed with a range of column oven arrangements that can house as few as two columns for simpler configurations, and ovens that can accommodate up to 10 columns for more-complex configurations. According to the company, each oven has a separate temperature control for combining chiral and achiral SFC in a single system.

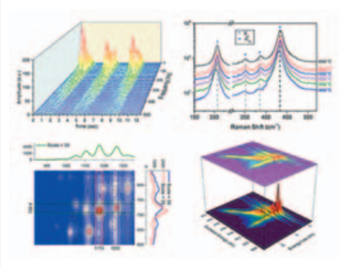
JASCO, Easton, MD.
www.jascoinc.com

GC–MS system brochure

A full-color brochure that provides information about the company's Pegasus GC-HRT 4D gas chromatography–mass spectrometry system is available from LECO. According to the company, the brochure details the system's various industry advantages and technologies, such as its use of folded flight path high-resolution deconvolution and ChromaTOF-HRT brand software.

LECO Corporation,
St. Joseph, MI.
www.leco.com

Data analysis and graphing software



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

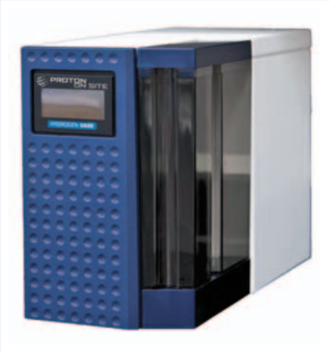
OriginLab,
Northampton, MA.
www.originlab.com

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

Gas generator



Proton OnSite's G600–HP high-purity hydrogen gas generator is designed with proton exchange membrane technology. According to the company, the generator can produce hydrogen at 99.99999% purity.

Proton Onsite,
Wallingford, CT.
www.protononsite.com

GC capillary columns

Quadrex's 007-5MS GC capillary columns are designed for GC–MS analysis of trace-level compounds in petrochemical, pharmaceutical, environmental, food and beverage, and other common GC applications. According to the company, the columns are based on a nonpolar, low-bleed, high-temperature silphenylene siloxane phase and are available in all standard lengths and film thicknesses.

Quadrex Corporation,
Woodbridge, CT.
www.quadrexcorp.com

LC columns

Restek's FluoroPhenyl Phase Raptor columns are designed to run in reversed-phase or hydrophilic interaction mode for analyzing a variety of compounds. According to the company, because of their efficiency with acidic mobile phases, the columns also are suitable for LC–MS.

Restek Corporation,
Bellefonte, PA.
www.restek.com

Mass spectrometer

The LCMS-8060 triple-quadrupole mass spectrometer from Shimadzu is designed to provide a scan speed of 30,000 u/s while maintaining mass accuracy and multiple reaction monitoring speeds of 555 ch/s. According to the company, the instrument has a polarity switching speed of 5 ms.

Shimadzu Scientific Instruments,
Columbia, MD.
www.shimadzu.com

HPLC columns

Shodex's HILICpak VG-50 HPLC columns are designed to support the analysis of saccharides and reducing sugars such as fructose, mannose, glucose, and sucrose. According to the company, applications are available from the company's database.

Showa Denko America, Inc.,
New York, NY.
www.shodex.net

HPLC and UHPLC columns

TOSOH BIOSCIENCE
TOSOH

Tosoh's TSKgel UP-SW3000, 2-μm size-exclusion chromatography columns are designed for the analysis of monoclonal antibodies and other biopharma products and can be used on both HPLC and UHPLC systems. According to the company, the columns are packed with 2-μm silica-based beads shielded with a hydrophilic diol-type bonded phase that prevents the silica surface from interacting with protein samples.

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

SEC–MALS detector for UHPLC

The μDAWN multiangle light-scattering detector from Wyatt Technology is designed to be coupled to any UHPLC system to determine absolute molecular weights and sizes of polymers, peptides, and proteins or other biopolymers directly, without column calibration or reference standards. The detector reportedly connects to the company's Optilab UT-rEX differential refractive index detector.

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

LC columns

YMC America's Triart ExRS HPLC and UHPLC columns for hydrophobic substances and isomers are designed with an organic and inorganic hybrid silica particle that uses polymeric bonding. According to the company, the columns are chemically stable at pH extremes (pH 1–12) and are mechanically stable at elevated pressures and temperatures.

YMC America, Inc.,
Allentown, PA.
www.ymcamerica.com

Frozen sample aliquotter

CryoXtract's CXT 353 semi-automated benchtop aliquotter is designed to extract multiple frozen aliquots from sample types including tissue, feces, serum, plasma, whole blood, urine, and other biofluids. According to the company, the parent sample and aliquots are maintained between -80 °C and -150 °C, eliminating freeze–thaw cycling and preserving sample quality.

CryoXtract Instruments, LLC,
Boston, MA.
www.cryoxtract.com/products/CXT353

CALENDAR

6–10 March 2016
The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon)
Atlanta, GA
pittcon.org/

16–18 March 2016
Chemistry Conference for Young Scientists (ChemCYS 2016)
Blankenberge, Belgium
www.chemcys.be/

3–7 April 2016
32nd International Symposium on Microscale Separations and Bioanalysis
Niagara-on-the-Lake, Ontario, Canada
www.msb2016.org/registration/registration/registration.html

9–12 April 2016
26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)
Amsterdam, The Netherlands
www.eccmid.org/

24–28 April 2016
61st ISA Analysis Division Symposium
Galveston, TX
www.adsymposium.org/

2–4 May 2016
Minnesota Chromatography Forum (MCF) Spring Symposium: The Upper Midwest's Largest Chromatographic Instrumentation and Supplies Exhibition
Minneapolis, MN
www.minnchrom.com/welcome-to-the-mcf/spring-symposium

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 3rd Annual Microbiology Week
Philadelphia, PA
www.cbinet.com/conference/pi16056#.Vl4Ogt-rREI

16 June 2016
5th International 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
Washington, DC
www.ivtnetwork.com/conference/pc16120

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

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
Cork, Ireland
www.isc2016.ie/

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

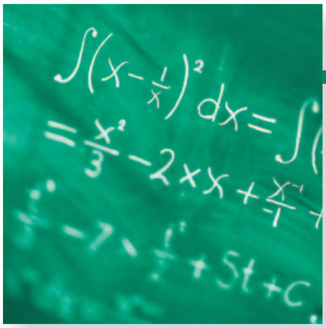
18–23 September 2016
23rd International Symposium On Electro- and Liquid-Phase Separation Techniques
Minneapolis, MN
www.cegss.ptchem.pl/itp-2016-23rd-international-symposium-electro-and-liquid-phase-separation-techniques

5–6 October 2016
SFC 2016 — 10th International Conference on Packed Column SFC
Vienna, Austria
www.greenchemistrygroup.org

7–9 November 2016
5th International Conference on Forensic Research & Technology
San Francisco, CA
forensicresearch.conferenceseries.com/

14–16 November 2016
2016 Eastern Analytical Symposium and Exposition
Somerset, NJ
eas.org

26–29 November 2016
14th Asia–Pacific Federation for Clinical Biochemistry and Laboratory Medicine Congress
Taipei, Taiwan
www.apfcbcongress2016.org/



SHORT COURSES

GC

5 March 2016
Getting the Most Out of Capillary Gas Chromatography
Atlanta, GA
www.pittcon.org

5–6 March 2016
Practical Gas Chromatography
Atlanta, GA
www.pittcon.org

7 March 2016
Introduction to Gas Chromatography/Infrared Spectroscopy
Atlanta, GA
www.pittcon.org

7 March 2016
Analytical Ionic Liquids in GC and Mass Spectrometry
Atlanta, GA
www.pittcon.org

7 March 2016
Method Translation in Gas Chromatography to Get the Same Chromatogram
Atlanta, GA
www.pittcon.org

7 March 2016
Instrumentation and Methods of Petroleum and Gas Analysis by Gas Chromatography
Atlanta, GA
www.pittcon.org

8 March 2016
Analytical Organic Mass Spectrometry
Atlanta, GA
www.pittcon.org

HPLC

5 March 2016
Introduction to Chromatography of Proteins, Peptides, and Related Molecules
Atlanta, GA
www.pittcon.org

5 March 2016
Introduction to Two-Dimensional Liquid Chromatography
Atlanta, GA
www.pittcon.org

5 March 2016
Chemistry Laboratory Techniques for the Chemist and Technician – A Comprehensive Review
Atlanta, GA
www.pittcon.org

6 March 2016
Two-Dimensional Liquid Chromatography for Pharmaceutical Analysis
Atlanta, GA
www.pittcon.org

7 March 2016
Basic HPLC – Fundamentals, Applications, and Troubleshooting
Atlanta, GA
www.pittcon.org

8 March 2016
Basic HPLC Method Development
Atlanta, GA
www.pittcon.org

8 March 2016
LC–MS for the Beginners
Atlanta, GA
www.pittcon.org

8 March 2016
Basic Preparative HPLC – Fundamentals and Applications
Atlanta, GA
www.pittcon.org

8 March 2016
Highly Successful Strategies for LC–MS Quantitation: Current Applications and Emerging Technologies
Atlanta, GA
www.pittcon.org

9 March 2016
Separations: Fundamentals of Advanced Gel Permeation and Size Exclusion Chromatography Detection
Atlanta, GA
www.pittcon.org

9 March 2016
HPLC and TLC Analysis of Herbal Medicines/Supplements for Purity and Content
Atlanta, GA
www.pittcon.org

9 March 2016
Light Scattering Techniques for Protein, Polymer, and Nanoparticle Characterization
Atlanta, GA
www.pittcon.org

10 March 2016
Hydrophilic Interaction Chromatography (HILIC) - Companion to Reversed Phase HPLC
Atlanta, GA
www.pittcon.org

10 March 2016
Practical Ion Chromatography – Steps to Successful Method Development
Atlanta, GA
www.pittcon.org

AD INDEX

Pesticide Residue Analysis Webinar
Utilizing the Power of GC-Orbitrap MS technology for the Multi-residue Analysis of Pesticides

LIVE WEBCAST: Wed., March 2 , 2016 at 8 am PST | 11 am EST | 4 pm GMT | 5 pm CET
Register free at: www.chromatographyonline.com/lcgc/utilizing

BONUS CONTENT: Attend to receive a FREE executive summary of the webcast

The introduction of high resolution, accurate mass (HRAM) Thermo Scientific™ Orbitrap™ Technology coupled to Gas Chromatography (GC) brings a new level of performance and flexibility in GC-MS full scan acquisition and more, and perfectly complements recent developments in new MS/MS acquisition modes using LC-Orbitrap. Together these technologies provide truly comprehensive workflow solutions for pesticides residues analysis with the capability of performing simultaneously quantitative target residues whilst screening for pesticides not expected to be present in the samples; all in a single analysis.

This webinar will provide pesticides residue analysts with new information from an independent thorough evaluation of the capabilities for and potential application of GC–Orbitrap technology to the analysis of pesticide residues in QuEChERS extracts of food. Hans Mol will share his experience with the development of a GC-Orbitrap workflow solution including optimization of the instrumental parameters and method validation, for qualitative and quantitative multi-residue analysis. Information on precision accuracy and reproducibility, identification capabilities and the ease of use and effectiveness of the software will also be presented.

Who Should Attend

- Researchers and analysts in pesticides analysis
- Food scientists interested in learning the latest technologies for targeted and non-targeted analysis of pesticides in food
- Anyone struggling with method development challenges for pesticide residue analysis in food, especially for complex matrices

Presenter

Dr. Hans Mol,
Senior Scientist,
RIKILT-Wageningen UR

Moderator: **Laura Bush,**
Editorial Director, LCGC

Key Learning Objectives

- An opportunity to understand the unique capabilities of QExactive™ GC Orbitrap™ GC-MS/MS
- Critical aspects for optimization of the GC-HRAM system for pesticides analysis
- Validation of the methods in compliance with international criteria

Sponsored by Thermo SCIENTIFIC A Thermo Fisher Scientific Brand

Presented by LCGC

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

THE ESSENTIALS

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

Troubleshooting Sample Filtration

Some of the most difficult problems to identify and solve originate from the sample preparation aspects of our work. This short article, taken from a recent Chromacademy webcast, delivers some key tips and tricks associated with troubleshooting sample filtration, an often overlooked source of potential problems.

Sample filtration, designed to remove particulate material from the sample before injection, is ubiquitous in sample preparation workflows, yet few of us pay enough attention to our filters. There are several materials of construction for both the filter body and the filter material, and the application and solvent system to be used will dictate the choice. A small range of typical filter materials is shown in Figure 1 alongside applications, solvent, and pH compatibility to indicate the importance of choosing the correct filter material.

These is only a small subset of the materials available; consult your supplier for further information on the chemical compatibility of the filter materials and the suitability for various application types.

One must be sure when using organic solvents or more extreme pH levels that there will be no disintegration of the filter membrane or, as is more often the case, leaching of components into the filtrate. Any leachates may act as interferents within the chromatogram and affect both the qualitative and quantitative aspects of the analysis, especially when using mass spectrometric detectors. If there is any doubt regarding the possibility of leachates, one should rinse the filter with an aliquot (1 mL is typically enough for most syringe filter devices) to preclean the filter. This approach can dramatically clean up the resulting chromatogram in a host of applications!

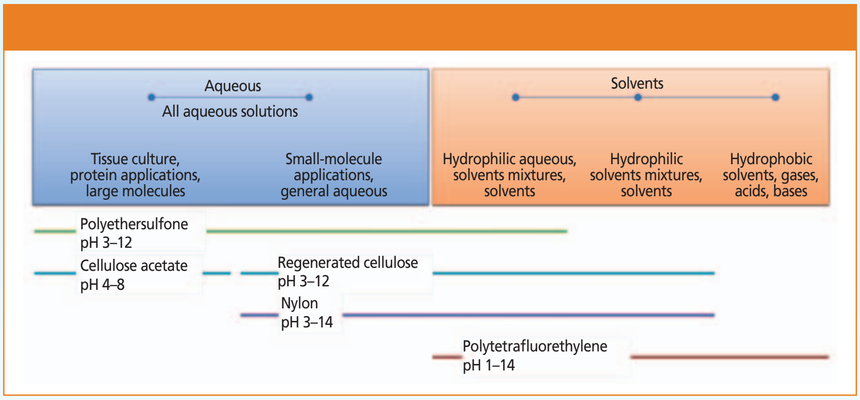


Figure 1: Typical filter materials, suitable applications, and chemical compatibility.

More Online:
Get the full tutorial at
www.CHROMacademy.com/Essentials
(free until March 20).

Analyte adsorption (analyte binding) is a problem sometimes encountered and can severely impact the quantitative performance of the method as the degree of adsorption will vary between filters and will be affected by changes in the sample matrix. In terms of specific applications, nylon and glass fiber materials show very high bonding for proteins and peptides, and polyvinylidene fluoride (PVDF) or polyethersulfone (PES) filters are generally much more suitable. Hydrophilic membranes such as PVDF and polytetrafluoroethylene (PTFE) tend to give the lowest nonspecific binding for lower molecular weight analytes. One should always carry out a filter binding investigation during method development by assessing the instrument response for each analyte with a filtered and unfiltered sample (usually possible with all but the most heavily particulate laden samples).

For analytes that are very heavy in particulates, successful, facile processing requires a prefilter to avoid blockage of the lower porosity membrane. These are often called *multilayer syringes* and will typically allow five times more sample to pass through. Be aware that most prefilters are glass fiber, which is incompatible with protein filtration and one should identify a filter with a PVDF or PES prefilter material.

Choosing the correct filter size is a balance. Large-diameter filters allow the sample to pass quickly at lower pressure and clog less; however they risk higher levels of extractables, higher nonspecific analyte binding, and have larger hold-up volumes, which trap more sample. For samples less than 1 mL use a 4-mm filter, less than 10 mL use a 13-mm filter, less than 100 mL use a 25-mm filter, and for samples greater than 100 mL use a 30–50 mm filter. For reference, 4-mm filters have a hold-up volume of around 10 mL, whereas 30-mm filters have a hold-up volume of 60–80 mL.

It is also important to choose the correct filter porosity and for ultrahigh-pressure liquid chromatography (UHPLC) analysis, one should ensure that the pore size of the filter material is less than 2 mm.

Troubleshooting tips on a wide range of sample preparation techniques can be found in the original webcast at www.chromacademy.com/sample-preparation-troubleshooting.html.


Where Innovations in Chromatography Go to Play.
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SHIMADZU
Excellence in Science

Rugged, High-Sensitivity GCMS Provides Unparalleled Performance

Shimadzu's New **GCMS-QP2020** Offers Excellent Qualitative and Quantitative Performance, Outstanding Reliability, and Smarter Operation

Adopting a proprietary multi-function ion source and a new large-capacity turbomolecular pump with heightened exhaust efficiency for all carrier gases, including nitrogen, the **GCMS-QP2020** elevates GCMS performance to a new level. New Quick-CI and Smart SIM functionality reduces analysis times and running costs while front access to the ion source enables easier routine maintenance. See for yourself why the GCMS-QP2020 is the smart, reliable solution for your application.

Learn more about Shimadzu's GCMS-QP2020.
Call (800) 477-1227 or visit us online at
www.ssi.shimadzu.com/QP2020

Order consumables and accessories on-line at http://store.shimadzu.com
Shimadzu Scientific Instruments Inc., 7102 Riverwood Dr., Columbia, MD 21046, USA

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
- Quick-CI function – allows users to introduce reagent gas while using the EI source to look for the molecular ion
- 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.