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Taking the Red Pill: Alleviating Matrix Effects for Small Molecule Quantitation in Food and Feed

Lewis Botcherby

Rudolf Kraska and Michael Sulyok from the University of Natural Resources and Life Science in Vienna, Austria, discuss their recent work developing a multi-analyte approach using liquid chromatography tandem mass spectrometry (LC–MS/MS), and the issues surrounding agrocontaminants in animal feeds.

Multivariate Optimization Procedure for Dynamic Headspace Extractions Coupled to GC(×GC)

Ryan P. Dias, Seo Lin Nam, Sheri A. Schmidt, A. Paulina de la Mata, and James J. Harynuk

A generalized approach using DoE is presented for the optimization of DHS extraction parameters.

Essentials of LC Troubleshooting, Part 2: Misbehaving Retention Times

Dwight R. Stoll

There are some problems with LC separations that will probably never go away, such as shifting retention times.

Is the Solution Dilution? Hidden Uncertainty in Gas Chromatography Methods

Nicholas H. Snow

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May Update
Welcome to the May issue of LCGC Europe! In our cover story this month, Rudolf Krška and Michael Sulyok from the University of Natural Resources and Life Science in Vienna, Austria, discuss how matrix effects continue to be an issue in small molecule quantitation in food analysis, and present their work developing a multi-analyte approach using liquid chromatography tandem mass spectrometry (LC–MS/MS).

This month’s peer review article discusses how headspace (HS) and dynamic headspace (DHS) methods for specific sample types are available, but a more generalized procedure is lacking. The authors seek to remedy this by presenting an approach using design of experiments (DoE) for the optimization of DHS extraction parameters in two different food samples.

As always, we also provide practical tips to help in the laboratory. In LC Troubleshooting, Dwight Stoll helps you address shifting retention times in reversed-phase LC.

GC Connections looks at experimental uncertainties that may be present in gas chromatography (GC) methods. It is important to understand that experimental error and uncertainty are inherent in all analytical techniques and plan accordingly.

This month we continue with our focus on the latest cutting-edge developments in separation science, this time in the field of LC and sample prep. Both Column Watch and Sample Preparation Perspectives present the very latest products and accessories that have been introduced in the past 12 months.

LCGC is a multimedia platform that helps chromatographers keep up to date with the latest trends and developments in separation science, and supports them to perform more effectively in the workplace. Keep updated with our multimedia content by visiting the global website (www.chromatographyonline.com), subscribing to our newsletters, and attending our wide range of educational virtual symposiums and webinars.

Mike Hennessy Jr,
President and CEO, MJH Life Sciences®
Mixtures of volatile organic compounds (VOCs) emanate from foods and beverages during human consumption, and these compounds blend together to create flavours and aromas. While many VOCs are present endogenously in food, others can be added intentionally as flavour enhancers, or unintentionally through contact with other materials or packaging during handling, preparation, and storage. Additionally, VOC profiles can evolve and change due to storage and handling. These VOCs then contribute to both pleasant and unpleasant or “off” flavours in foods and beverages (1). Headspace sampling techniques have been shown to be the most effective approach for the extraction of VOCs from a variety of food and beverage matrices, including wine, bread, and milk (2–4). Aroma profiles can be obtained with little disturbance to the bulk sample during headspace sampling. The most commonly employed technique for these applications is headspace solid-phase microextraction (HS-SPME) (5–7). Though widely applied for its simplicity and relatively low cost, the fibre coatings introduce selectivity into the extraction process, as the coating will preferentially sorb certain compounds over others, depending on the chemistry of the fibre and analyte. Generally, SPME also favours analytes with moderate volatilities and is highly susceptible to matrix effects. These two features of SPME can make quantification and comparison of relative abundances of different compounds between sample types challenging. Dynamic headspace (DHS) has been suggested as a powerful alternative to HS-SPME because it provides better sensitivity and has fewer concerns related to selectivity of the extraction to matrix effects (8–10). DHS was developed to overcome some limitations of static headspace sampling (11). It involves flowing a constant stream of inert gas through the headspace of a sample vial, purging volatiles that partition into the headspace onto a sorbent trap. DHS offers enhanced recoveries of analytes over other headspace extraction approaches.
techniques; however, it has multiple additional parameters that need to be optimized. DHS experimental conditions include sorbent type, sample incubation temperature, purge flow rate and volume, and sorbent trap temperature (12). To achieve optimal DHS conditions, the one-factor-at-a-time method of optimization has been popular. This classical approach is a single-factor experiment wherein each variable to be optimized is studied individually until an optimal value has been reached. Although this univariate approach has been commonly used in DHS studies, there are many disadvantages to this method. It is costly in terms of sample volume, as well as instrument and analyst time, to investigate all the variables involved in DHS individually (13). More importantly, this method is not capable of evaluating interactions among the variables and their combined effects on the process. Consequently, the optimal conditions found through a series of single-factor experiments may not be globally optimal conditions and this univariate approach is not recommended for the optimization of multiple DHS parameters (14). To the best of the authors’ knowledge, there has yet to be a report in the literature that sets out general guidelines for the optimization of DHS extractions.

Prior studies have used a multivariate design for optimization of DHS parameters, which allows several variables and their interactions to be evaluated simultaneously (15–17). A statistical approach, referred to as Design of Experiments (DoE) (18), was employed to investigate multiple DHS parameters that may collectively impact the extraction of analytes. DoE is suitable for an experimental design with the goal of obtaining sufficient information through a reduced number of experiments, thus allowing several experimental parameters to be varied systematically and simultaneously (19). In the context of DoE, independent variables or experimental parameters are called factors, and different values for each factor in designed experiments are called levels. Although DoE is a suitable approach to explore the multiple DHS experimental parameters and their interactions, the appropriate choice of factors and levels is absolutely crucial in building a DoE model, as there are sample-dependent considerations to be made (20).

The aim of this study was to develop a generalized analytical procedure to guide DHS system optimization using DoE. While previous studies have reported optimized methods for one sample type (12,14–17,19,20), the literature does not, to the best of our searching, provide an easily accessible strategy that can be applied to any sample type. Given the increasing popularity of DHS, such a strategy would benefit the community. The proposed optimization procedure involves the use of experimental designs for both liquid and solid samples. For both studies, the Box-Behnken design was selected to generate modelling of the response surface using a smaller number of required runs than other designs, such as full factorial or central composite (21). To this end, active sourdough colony and sourdough bread (made from the same colony) were chosen as representative liquid and solid samples, respectively, for developing a standardized optimization strategy for DHS extraction methods.

Sourdough is commonly added to a variety of breads to enhance its aroma and flavour (22). Due to the variety of VOCs present, sourdoughs produce an intense aroma: the influential chemical classes include alcohols, aldehydes, ketones, and esters (23). There are several factors that influence the volatile profile of sourdough colony and bread. Sourdough volatiles are affected by the type and amount of flour used, hydration, type of fermentation, how much sourdough is used, and any extra ingredients added (24). VOCs can be determined between strains of sourdough and bread through headspace extraction techniques. Here, automated DHS extraction followed by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOF-MS) was used to explore VOCs from sourdough colony and sourdough bread.

**Materials and Methods**

**Sourdough Colony Preparation for DHS Extraction:** Sourdough colony samples (100.0 mg ±1.5 mg) were weighed into 10 mL headspace vials (Chromatographic Specialties Inc.). Vials were capped tightly with magnetic screw caps (Canadian Life Sciences) with PTFE-faced silicone septa (see supplementary information; Figure S1). All sealed sample vials were placed on a heating mantle set to 70 °C for 5 min to deactivate the colonies and prevent further VOC production. After colony deactivation, samples were placed in a refrigerator at 4 °C until analysis. Details of sourdough colony preparation and maintenance are presented in the supplementary information.

**Sourdough Bread Preparation for DHS Extraction:** Sourdough bread samples were prepared by weighing 100.0 mg (±1.5 mg) of crumb or crust into 10 mL headspace vials and capping tightly (see supplementary information; Figure S1). Samples were placed in a refrigerator at 4 °C until analysis. Details of bread preparation are presented in the supplementary information.

**Experimental Design and Optimization:** A DoE model was produced in Minitab 17.0 statistical software (Minitab LLC).
for both sample types. Both models were constructed using the Box-Behnken approach with a three-factor design. In total, 15 DHS experiments were performed with three replicates on the centre point. A summary of DoE factors and levels is provided in Table 1. Values for each level were determined based on initial range finding experiments (data not shown).

The three designated factors for DoE models were chosen based on their expected impact on the amount of volatiles extracted. Though classified as a pre-extraction parameter, incubation time is a crucial step in augmenting VOCs extraction. A minimum amount of time must pass while heating the sample to facilitate pre-equilibration of analytes with the headspace prior to purging and trapping. Though there are multiple extraction parameters, purge flow rate and purge flow volume were selected for the same rationale. An optimal volume of purge gas must be flowed at an optimal rate to maximize the amount of VOCs extracted. A more thorough discussion of the rationale for excluding other parameters from optimization is included in the supplementary information.

Modelled methods were optimized based on the total number of detected compounds and the total summed peak area of analyte signals for both sample types using response surface methodology (25,26). Briefly, this statistical method fits a predictive model to optimize a response (for example, total summed peak area) affected by different quantitative factors (purge flow rate and volume) and their interactions. The result is a response surface plot, with a local maximum calculated as the optimal level for each factor.

**DHS and Sorbent Trap Desorption:** Bread samples were incubated at 80 °C; sourdough colony samples were incubated at 40 °C. A trap temperature of 25 °C was selected to trap purged volatiles on Tenax TA adsorbent tubes (Gerstel). The purging gas was high-purity (4.8) nitrogen (Praxair). DHS extraction was achieved using an Automated Dynamic Headspace Module (Gerstel). Trapped analytes were desorbed from sorbent tubes by a thermal desorption unit (TDU2, Gerstel) and cryogenically focused in a programmable-temperature vaporization inlet (PTV, model CiS4, Gerstel) prior to GC×GC separation.

Desorption of sourdough colony analytes was accomplished in solvent vent mode (at the TDU2) with the following parameters: solvent vent time 5 min, initial temperature 50 °C (hold 5 min) ramped to 250 °C at 720 °C/min and held for 10 min with a desorption flow of 75 mL/min (helium, high-purity 5.0, Praxair). Desorption of bread analytes was achieved in splitless mode (at the TDU2), and all other desorption parameters remained the same as the sourdough colony parameters. The water content of the sourdough colony samples yielded a wet headspace even when heated at 40 °C, so DHS water management steps (solvent venting) must be taken to avoid creating ice blockages between the TDU and the PTV inlet. Cryogenic focusing of analytes was maintained in the PTV, with an initial temperature of -100 °C ramped to 250 °C at 12 °C/s.

Details of other DHS parameters, GC×GC–TOF-MS analyses, and data processing are provided in the supplementary information.

### Table 1: Factors and levels selected for the two experimental designs

<table>
<thead>
<tr>
<th>Box-Behnken (Sourdough, SD)</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>Low</td>
</tr>
<tr>
<td>A Purge flow rate (mL/min)</td>
<td>20</td>
</tr>
<tr>
<td>B Purge flow volume (mL)</td>
<td>500</td>
</tr>
<tr>
<td>C Incubation time (min)</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Box-Behnken (Bread, B)</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>D Purge flow rate (mL/min)</td>
<td>50</td>
</tr>
<tr>
<td>E Purge flow volume (mL)</td>
<td>1000</td>
</tr>
<tr>
<td>F Incubation time (min)</td>
<td>2</td>
</tr>
</tbody>
</table>

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The goal of this study was to develop a cost-effective, general procedure for the optimization of DHS extraction. Although previous investigations have used the DoE approach to optimize HS and DHS parameters, the factors that were selected for optimization varied significantly across the studies and were sample-specific (27–33). On average, each study used 3–5 factors with 2–3 levels per factor in order to keep the number of experiments reasonable. Out of several variables, an effective choice of factors is critical to maintain a practical number of experiments while covering a broad range of important variables. In some studies that discuss the optimization of HS/DHS parameters, the choice of factors included in DoE were not significant enough to affect the overall procedure. For example, one study included the sorbent amount as a factor to be investigated for macroalgae volatiles (27); another assessed the effect of sample weight on the extraction efficiency of melon volatiles (28). Overall, the larger adsorbent mass increased the number of compounds extracted and the overall chromatographic peak area for macroalgae. Similarly, the largest sample size of melons yielded the highest extraction efficiency. In another study, the purge flow was kept constant while other parameters of interest were varied (29). In general, the standardization of an optimization protocol would be beneficial to offer basic guidelines for new or regular users, to help fine-tune DHS parameters through DoE for any sample type.

Prior to DoE, preliminary experiments must be performed for range finding, that is, appropriate ranges for factor levels must be determined before modelling. Some questions to consider prior to experimental design are provided in Figure 1. These questions (and their necessity) should be assessed on a per-study basis, since a priori knowledge of the sample and its behaviour during extraction will dictate how DHS experiments are designed. The number of experiments required will escalate rapidly as the number of factors or levels increases.

An entire DHS sampling procedure can be subdivided into three stages: equilibration, extraction, and injection. Table 2 summarizes the relevant parameters for each category.

Out of the three stages, the purge-and-trap step (that is, extraction of volatiles) is the most crucial step for DHS optimization. The Box-Behnken design used herein focuses on optimizing the extraction parameters. After modelling, the optimal method was assessed for extraction reproducibility and the obtained VOC profile (that is, visual assessment of chromatographic patterns).

### Design of Experiments Model

The entire DHS process was divided into three steps (Table 2), though a model was only constructed for the extraction step. A response surface plot was built; the sample regression equations output from the statistical software for sourdough bread (B) and colony (SD) are shown in equations 1–4. “TPA” refers to the total peak area or the total summed peak areas of analyte signals. Model coefficients for both sample types per optimizing metric are provided in the supplementary information.

#### Equation 1

\[
TPA_B = -2411530 + 3228 \text{FlowVolume} + 27675 \text{FlowRate} + 456586 \text{IncbTime}\]

#### Equation 2

\[
\text{PeaksSD} = 204 + 0.480 \text{FlowVolume} - 7.82 \text{FlowRate} + 60.2 \text{IncbTime} - 0.00098 \text{FlowVolume} \times \text{FlowVolume} + 0.0610 \text{FlowRate} \times \text{FlowRate} - 1.73 \text{IncbTime} \times \text{IncbTime} - 0.00030 \text{FlowVolume} \times \text{FlowRate} - 0.00169 \text{FlowVolume} \times \text{IncbTime} - 0.435 \text{FlowRate} \times \text{IncbTime}
\]

#### Equation 3

\[
TPA_{(SD)} = -133027 + 187 \text{FlowVolume} + 12840 \text{FlowRate} - 15291 \text{IncbTime} - 0.312 \text{FlowVolume} \times \text{FlowVolume} - 181.4 \text{FlowRate} \times \text{FlowRate} + 54.8 \text{IncbTime} \times \text{IncbTime} + 8.27 \text{FlowVolume} \times \text{FlowRate} + 9.6 \text{FlowVolume} \times \text{IncbTime} + 159 \text{FlowRate} \times \text{IncbTime}
\]

#### Equation 4

\[
\text{Peaks}_{(SD)} = 154.5 - 0.969 \text{FlowVolume} + 0.454 \text{FlowRate} - 5.62 \text{IncbTime} + 0.000032 \text{FlowVolume} \times \text{FlowVolume} - 0.01125 \text{FlowRate} \times \text{FlowRate} + 0.0537 \text{IncbTime} \times \text{IncbTime} + 0.000917 \text{FlowVolume} \times \text{FlowRate} + 0.00275 \text{FlowVolume} \times \text{IncbTime} + 0.0342 \text{FlowRate} \times \text{IncbTime}
\]

The DoE model was calculated using a system of linear equations to derive the model coefficients in equations 1–4, though these outputs offered no insight as to which factor was the most important in the modelled process. Similarly, the model coefficients and regression equations contain no specific
information about optimal levels for each factor (for example, 344.3 mL DHS purge volume) (16). To visualize these relationships, Pareto charts and optimization surface plots were created in the statistical software. A Pareto chart considering the TPA of sourdough colony is provided in Figure 2. It displays not only the single factor with the most impact on the model but also the interaction(s) of two combined factors.

For this model, the two most important factors affecting the DHS extraction were purge flow rate and purge flow volume. The interaction between these two parameters was similarly important—the modulation of both may enhance the abundance of analytes extracted. In this case, the incubation time did not significantly influence the outcome of the extraction. More generally, the purge flow rate and purge flow volume are the two most critical factors to optimize for any DHS extraction. Pareto charts are informative, but they provide no optimized parameters: optimization surface plots are needed. A sample of these plots obtained for the sourdough bread model considering TPA is provided in Figure 3.

The optimized conditions for both sample types were chosen based on models considering the TPA and total number of peaks. These values from the response surface plot were rounded for convenience and to account for the autosampler control software only allowing whole numbers. The optimal regions on the plots considering both total peaks and TPA for all factors were broad enough to allow the slight adjustment. For sourdough bread samples, optimal parameters were 2100 mL purge volume, 50 mL/min purge flow rate, and 10 min incubation time. Optimal parameters for sourdough colony samples were 1100 mL purge volume, 80 mL/min purge flow rate, and 25 min incubation time. Both optimized methods were reproducible; %RSD was less than 20% for living sourdough colony and less than 9% for sourdough bread for replicate samples.

VOC Profiles of Sourdough Colony and Bread: VOC profiles of sourdough colony and bread were sampled by the optimized DHS methods. A typical chromatogram obtained from each sample type is shown in Figure 4. Automatic mass spectral filtering scripts for GC×GC–TOF-MS omics data (34) were applied to rapidly characterize certain chemical classes present in all samples.

Optimized DHS methods recovered a variety of compound classes in both

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sample types. Chemical families such as alcohols, aldehydes, fatty acid ethyl esters, and terpenoids were the predominant classes identified by filtering scripts for GC × GC–TOF-MS data. In colony samples, acetoin, isoamyl alcohol, and isoamyl acetate were identified; bacterial and fermentation by-products responsible for active sourdough odour were also found (35). The presence of iso-octane esters (plasticizers) (36) solely in bread samples was most likely caused by the plastic bag used for sample storage. Interestingly, fatty acid ethyl esters were only detected in sourdough colony, while only their linear aldehyde equivalents were identified in sourdough bread. As expected, Maillard reaction products such as furfural and 5-methylfurfural were only detected in bread samples (37).

Overall, the general procedure laid out in this work can be summarized by a series of four steps for any sample type: plan, design, interpret, and apply. The first step is to plan the entirety of the DHS extraction process (factor selection), sample preparation, sample considerations, and range finding (Figure 1). Then, a DoE model must be constructed of factors and levels based on the range finding results, and the subsequent designed DHS experiments performed. The Box-Behnken approach was selected by the authors to save analysis time, though other designs are equally valid. Next, results obtained from designed DHS experiments must be optimized using at least two metrics (for example, TPA and total number of peaks) to reliably interpret the optimization plots. Once an optimized method is obtained, it must be applied to replicate samples to assess extraction reproducibility and robustness.

Conclusions

In the present study, a protocol for the optimization of dynamic headspace sampling relying on multivariate experimental design was presented. Prior understanding of the entire DHS process was required for proper selection of the most significant factors in DoE models. Troubleshooting a DHS extraction was complicated; the exhaustive

---

**TABLE 2: Summary of DHS procedure and working parameters**

<table>
<thead>
<tr>
<th>Stage</th>
<th>1. Pre-Extraction</th>
<th>2. Extraction</th>
<th>3. Post-Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation and Equilibration</td>
<td>DHS (Purge-and-Trap)</td>
<td>Sample Introduction</td>
</tr>
<tr>
<td>Purpose</td>
<td>Pre-equilibrate analytes between sample and vial headspace</td>
<td>Purge VOCs from the headspace and concentrate on a sorbent</td>
<td>Therma...</td>
</tr>
<tr>
<td>Relevant Parameters</td>
<td>• Sample volume (sample to headspace ratio) • Incubation temperature • Incubation time • Agitation</td>
<td>• Purge flow rate • Purge flow volume • Sample temperature • Trap temperature • Drying step (optional) • Sorbent chemistry</td>
<td>• Desorption temperature • Desorption time • Desorption flow</td>
</tr>
</tbody>
</table>

![Pareto Chart of the Standardized Effects](image)

**FIGURE 2**: Pareto chart for sourdough colony when considering TPA (from Minitab).

![Optimization surface plot for sourdough bread when considering TPA](image)

**FIGURE 3**: Optimization surface plot for sourdough bread when considering TPA (from Minitab).
extraction process required many considerations based on sample type and qualities prior to analyses. Initial DHS experiments must be performed to determine suitable conditions such as sample mass, that is, concentration, addition of salt or saline solution, purge flows and volumes (breakthrough prevention), and water or humidity mitigation. Sample water content was a significant consideration that should be handled through one or a combination of the following parameters: lower extraction temperatures, lower trap temperatures, solvent venting, or DHS drying (extra gas purge). Optimized methods should be assessed for reproducibility after design. Future GC(×GC) studies that require DHS sampling should adopt a similar strategy to the one proposed here.

Supplementary Information
The link to the supplementary information can be found here: http://bit.ly/3OeXrY4

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7) V. Jalili, A. Barkhordari, and A. Ghiasvand, Microchemical Journal 152, 104319 (2020).

Ryan P. Dias is a Ph.D. candidate at the University of Alberta, currently conducting research in James J. Harynuk’s laboratory and The Metabolomics Innovation Centre (TMIC). His work involves plant metabolomics and terpene profiling by two-dimensional gas chromatography for a variety of applications. Ryan completed his B.Sc. in chemistry and forensic chemistry at Towson University in Towson, Maryland, USA. Seo Lin Nam studied analytical chemistry at the University of Alberta under the supervision of James J. Harynuk and received her Ph.D. degree in January 2021. She is currently a postdoctoral fellow in the Harynuk laboratory. Her research focuses on metabolomics using GC×GC. Sheri A. Schmidt is a Masters student in the Harynuk’s lab at the University of Alberta. Her research focuses on environmental air and human breath analysis using thermal desorption and two-dimensional gas chromatography. Sheri completed her B.Sc. degree in chemistry at the University of Alberta.

A. Paulina de la Mata is an analytical chemist and the scientific coordinator for James Harynuk’s node of TMIC. Her research interests lie in chemometrics, machine learning, and developing methods for metabolomics research by GC and GC×GC.

James Harynuk is a professor of chemistry at the University of Alberta, and one of the principal investigators for TMIC. He completed his Ph.D. at the University of Waterloo in 2004, studying and developing tools in the then-emerging field of GC×GC. After completing postdoctoral research in Melbourne, Australia, he returned to Canada and joined the University of Alberta in 2007.

Current research interests include the development of new software tools for handling GC×GC data.
Several liquid chromatography (LC) troubleshooting topics never get old because there are some problems that persist in the practice of LC, even as instrument technology improves over time. There are many ways for things to go wrong in an LC system that ultimately manifest as deviations from expected retention times. Developing a short list of the likely causes of these deviations can help streamline our troubleshooting experience when retention-related problems occur.

Writing this “LC Troubleshooting” column and thinking about topics each month is interesting in the sense that there are some topics that never get old. Although certain topics or ideas become obsolete once they are displaced by newer and better ideas in the chromatography research world, there are certain topics in the troubleshooting world that have remained relevant since the first troubleshooting article appeared in this magazine (LC Magazine at that time) in 1983 (1). Over the last few years, I’ve focused several “LC Troubleshooting” instalments on contemporary trends (the relatively recent advances in our understanding of the effects of pressure on retention [2]) in liquid chromatography (LC) that are affecting the way we approach our interpretation of LC results and approach troubleshooting with modern LC instruments. In this instalment, I am continuing a series I started in January of 2022 (3) that focuses on some of the “bread and butter” topics of LC troubleshooting—those elements that are essential for any troubleshooter, no matter the vintage of the system we are working with. The topics at the heart of this series are highly related to the well-known “LC Troubleshooting” wall chart (4) that hangs in many laboratories. For the second instalment in this series, I focus on problems related to retention (too low, too high, and changing unexpectedly) in reversed-phase LC separations. I hope LC users young and old will find some useful tips and reminders related to this important topic.

Everything is Possible
More often than not, I find myself responding to troubleshooting questions with “everything is possible”, which might seem like an easy out when considering observations that are hard to explain. However, I find that this response is appropriate more often than not. LCGC’s “LC Troubleshooting” wallchart lists no less than 18 possible causes for retention to be too high, too low, or changing. One could surely add to this list if the size of the wallchart were not a constraint and if we wanted to be really thorough. The main point is that it is important to keep an open mind when thinking of reasons that the observed retention is not right.

What Is To Be Expected?
A critical step in any troubleshooting exercise—but one that I think is unappreciated—is recognizing that there is a problem to be solved. Recognizing that there is a problem usually amounts to recognizing that what is happening with the instrument is different from what is expected to happen, and our expectations are formed from theories, empirical knowledge, and experience (5).

Before getting into details about what we can expect about retention, a few words to clarify what retention is and how it is measured are warranted. First, retention time ($t_r$) is directly observable from a chromatogram, and is most commonly taken as the time corresponding to the apex of the chromatographic peak. Second, retention factor ($k$) is a commonly used measure of retention that is calculated from measurements of the retention time of a retained analyte ($t_r$) and the column “dead time” ($t_m$, or $t_0$), which is a measure of the elution time of an “unretained” marker compound.

$$k = \frac{t_r - t_0}{t_0} \quad [1]$$

Although accurate determination of $k$ is challenging because of the difficulty of obtaining accurate dead time values
(6), there are several advantages of \( k \) over \( \tau \), some of which lead to more repeatable and reproducible measures of retention over time and between laboratories and instruments:

1. \( k \) is nominally independent of parameters including flow rate, column length, column diameter, and particle size, which makes comparing retention under conditions where these parameters are varied more straightforward than directly comparing retention times.

2. Small changes in flow rate because of changes in pump performance—which do directly affect \( \tau \)—have little effect on \( k \) because the effect of a small change in the flow rate on retention will be similar to the effect on the dead time, and thus, the differences cancel out.

Finally, a third measure of retention that is common in practice is relative retention time (RRT):

\[
RRT = \frac{t_{\text{comp}}}{t_{\text{ref}}} \tag{2}
\]

The RRT carries many of the advantages of \( k \) over \( \tau \), without the difficulty associated with accurate determination of column dead time. Figure 1 shows retention data from some recent isocratic measurements under reversed-phase conditions in my laboratory. Panels A and B show retention times obtained for anisole (A) and butylphenyl ether (B) from 75 replicate injections over a period of approximately 48 h. The relative standard deviations (RSD) of the retention times are 0.11% and 0.13%, respectively. Although these RSDs are reflective of the excellent retention stability we can expect from ultrahigh-performance LC (UHPLC), panel C shows that the RRT can be even more stable. In this case, we see that the RSD drops by nearly one-half to 0.06%. Panel D shows that the \( t_r \) for the two compounds are highly correlated (the two compounds were injected as a mixture for each replicate injection), which is why the RRT is more repeatable than the \( t_r \) in this instance.

Bearing in mind these details about the different ways of measuring retention, it is important to understand that there is no single standard we can use to judge whether the retention we see in a routine assay today is reasonable relative to prior work. Different applications with different conditions run on different instruments and in different laboratories will be characterized by different levels of variability in retention. Although the repeatability of retention shown in Figure 1 is excellent, it will not always be this good, even when using the finest equipment. The user must be aware of what constitutes “typical” performance. In the best-case scenario, a control chart or some other indicator of retention over time (days, weeks, or months) is used. It is only against this background that one can assess whether a retention observation today seems too low or high, or is varying too much.

**Situations Involving Retention That Is Decreasing or Lower Than Expected**

There are a few common potential causes of retention that either appears to be too low or decreasing over time. *Loss of Stationary Phase*: Harsh mobile phase conditions can lead to loss of the component of the stationary phase that imparts retentive properties to the packing material. In reversed-phase LC, we frequently refer to this as the “bonded phase”, and for silica-based materials, this bonded phase is a ligand covalently bonded to the silica surface through siloxane (Si–O–Si) bonds. These bonds are susceptible to hydrolysis under acidic conditions (\( \text{pH} < 2 \)), and once the bond is hydrolyzed, the ligand can be washed out of the column, leading to a decrease in retention (7). This process manifests as a slow loss of retention over days or weeks, rather than a sudden, abrupt decrease in retention.
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Confirming that this is, in fact, happening is difficult. However, if such phase loss is suspected, it is best to adjust the mobile phase conditions to a less acidic pH, use a different, more chemically stable stationary phase, or both.

**Mass Overload:** If gradually decreasing retention is observed as the mass of analyte injected is increased (but the injection volume is constant), this could be a sign of mass overload (8). Mass overload can be a complex phenomenon, but one mechanism that seems to occur for charged analytes involves mutual repulsion of injected analytes by charged compounds already adsorbed to the stationary phase surface. One potential solution to this problem is to increase the ionic strength of the mobile phase; ionized mobile phase components (for example, sodium or ammonium ions) can interrupt the charge-repulsion mechanism and minimize the effect of the injected mass of analyte on retention time.

**Volume Overload:** The composition of the matrix of the injected sample can also have a dramatic effect on apparent \( t_r \). For example, in reversed-phase LC, if the sample contains more organic solvent than the mobile phase, this can lead to a decrease in retention (9). In hydrophilic interaction liquid chromatography (HILIC) separations, the same effect can be observed, but in this case, samples that contain more water than the mobile phase lead to a decrease in retention (10). Whereas the effect of mass overload on retention tends to be gradual, with volume overload the change in retention can be sudden and dramatic because of a “breakthrough” of the analyte band as some of it travels with the band of sample solvent through the column (11). This effect is relatively easy to study by varying the volume and composition of the injected sample over ranges of interest to the application at hand and looking for a correlation with the observed \( t_r \). If volume overload appears to be a problem, a variety of approaches can be used to mitigate this effect, including decreasing the injection volume, preparing the sample with less “strong solvent” (10), or installing a mixer between the injector and the column (12).

**Stationary Phase Dewetting:** Most stationary phases designed for reversed-phase LC are, by design, quite hydrophobic. When highly aqueous mobile phases (<2% organic solvent) are used with the phases, the mobile phase can actually be physically expelled from the narrow pores of the particles because of the low thermodynamic favorability of the highly aqueous phase interacting with the highly hydrophobic stationary phase (13, 14). This process leads to an apparent loss of retention because analytes simply cannot diffuse into pores that have no solvent in them. This type of retention loss is usually sudden. The good news is that it is reversible by simply flushing the column with a few column volumes of mobile phase rich in organic solvent, and that is usually sufficient in restoring retention (13). This problem can be avoided by not using purely aqueous mobile phases with highly hydrophobic reversed-phase phases (for example, C8, C18, or phenyl). If highly aqueous mobile phases must be used, then more hydrophilic reversed-phase phases should be considered (for example, C1 or CN).

**Situations Involving Retention That Is Increasing or Higher Than Expected**

There are a few common potential causes of retention that either appears to be too high or increases over time. **Decreasing Flow Rate:** If the flow rate through the LC column has decreased, or is slowly decreasing over time, the \( t_r \) of an analyte will be higher than expected or slowly increase. Such a decrease in flow rate could be because of leaks outside of the pump (for example, connection to the column, sampler needle seat, sampler valve rotor seal), in which case the pump is working properly but not all of the mobile phase actually goes through the column. Also, the pump itself may not be producing the flow rate indicated by the software because of a leaky check valve or piston seal (3). These decreases in flow rate could be sudden or gradual depending on the root cause of the change. If decreasing flow rate is the suspected cause of increasing retention, it is helpful to compare both \( t_r \) and retention factor (or RRT) to historical values. Whereas \( t_r \) is dependent on flow rate, retention factor and RRT should be independent of flow rate. If a difference in the dependence of \( t_r \) and \( k \) on flow rate is observed, this difference is an important clue.

Resolving the problem may or may not be tricky depending on the exact cause. Leaky check valves do not produce leaks that we can see with our eyes in a way that a leaky needle seat or column connection would (see [3] for suggestions about troubleshooting leaky check valves). If the nominal flow rate is 2 mL/min, then a leak of 5% (100 µL/min.) because of a poor connection should be easy to spot. However, if the flow rate is 50 µL/min, then a leak rate of 5% only corresponds to 2.5 µL/min, which is harder to spot.

**Changing Mobile Phase Composition:**

Given the strong dependence of retention on the mobile phase composition in reversed-phase LC, small changes in the mobile phase delivered by the pump can significantly increase or decrease retention. Changes in the composition delivered by the pump could be because of a leaky check valve in just one of the two channels of a binary pump. For example, if the acetonitrile channel is leaky,
then the contribution of organic solvent to the mobile phase will be lower than expected, and retention will increase. In pumps (usually quaternary) that use electric solenoids to open and close the flow path between a solvent bottle and the pump, a valve that fails to open correctly will lead to changes in the composition of the mobile phase delivered to the column (see [15] for differences between these pump designs). In this case, both \( t_r \) and retention factor would be affected, but RRT would not be as affected. Comparing all three values to historical data may provide important clues.

The software used with modern LC instruments has built-in diagnostic tests that can be used to determine whether or not the pump is delivering the expected mobile phase composition both accurately and precisely (16).

### Situations Involving Retention That Appears to Be Fluctuating

The “LC Troubleshooting” wallchart lists 11 different potential causes for fluctuating retention. Here, I discuss the two that I see causing problems most frequently.

**Inadequate Buffering of the Mobile Phase pH:** Retention of ionogenic compounds that have a \( pK_a \) in the range of pH values normally used in reversed-phase LC (that is, from 2–10) can be sensitive to small changes in mobile phase pH. When working with these compounds, it is important to use a buffer with a good buffer capacity (typically more than 10 mM of the buffering agent, such as phosphate or formate), and under conditions where the target pH is within 1 pH unit of the \( pK_a \) of the buffering agent. For example, the \( pK_a \) of formic acid is approximately 3.8 in water, so formate buffers should only be used in the range between 2.8–4.8. Working outside of this range, or with too low a concentration of the buffering agent, would hinder the ability of the buffer to do its job. In other words, it would not be a buffer at all, and it would not be able to stabilize the mobile phase pH.

**Uncontrolled Column Temperature:** The retention of most compounds analyzed by reversed-phase LC decreases with increasing column temperature, thus the stability of retention over time is dependent on the stability of the column temperature over time. Most modern LC instruments have the capacity both to preheat the mobile phase before it enters the column and to control the temperature of the column environment (17). However, some instruments are used without these features, and this can be a source of variability in retention. For some highly sensitive compounds, retention can vary because of temperature fluctuations even with state-of-the-art LC equipment. In these cases, controlling the room temperature as tightly as practically possible is helpful (18).

### Summary

In this second installment on essential topics in LC troubleshooting, I discussed situations where the observed analyte retention is somehow different from what is expected or normal. Effective troubleshooting for this type of problem begins with a sense for what the expected retention behaviour is, so that a deviation from these expectations is noticeable. There are many different potential causes of retention related problems (too low, too high, or varying). In this instalment, I discussed some of the causes I see most frequently in detail. Understanding these details provides a good place to start troubleshooting but does not capture all possibilities. Readers interested in learning about a deeper list of causes and solutions are referred to the “LC Troubleshooting” wallchart.

### Acknowledgements

I would like to thank Tina Dahlseid and Carter Henning for their effort in collecting the data shown in Figure 1.

### References


### ABOUT THE COLUMN EDITOR

Dwight R. Stoll is the editor of “LC Troubleshooting.” Stoll is a professor and the co-chair of chemistry at Gustavus Adolphus College in St. Peter, Minnesota, USA. His primary research focus is on the development of 2D-LC for both targeted and untargeted analyses. He is also a member of LCGC’s editorial advisory board. Direct correspondence to: amatheson@mjlifesciences.com
Is the Solution Dilution? Hidden Uncertainty in Gas Chromatography Methods

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Used by chemists and nonchemists alike, gas chromatography (GC) is considered mature and among the most widely used instrumental techniques for chemical analysis. Because instruments have become both more sensitive and easier to use, columns have achieved higher resolution and stationary phases have greater selectivity. As a result, gas chromatographs have taken on a “black box” view. With greater sensitivity, resolution, and advanced data handling capabilities, a new set of experimental uncertainties emerge that may not be apparent to most users, especially those who were not formally trained as analytical chemists. In this instalment, we examine these uncertainties in typical GC methods, especially as they relate to quantitative analysis. We look at hidden experimental uncertainty, especially in the glassware used for sample preparation. We also comment on injection and detection with an eye towards understanding the sources of the errors. It is important to understand that experimental error and uncertainty are inherent in all analytical techniques; they can be reduced but cannot be eliminated.

An increasing portion of the chromatographic literature of today describes applications and quantitative analysis rather than fundamental advances in chromatographic techniques and principles. I am not offering an opinion about whether this is good or bad, but it is apparent. Articles describing chromatographic methods show quantitation at parts per billion (ppb) and lower levels are now commonplace. However, much of this literature shows common mistakes and problems with experimental uncertainty in quantitative analysis. Most commonly, uncertainties, usually in the form of standard deviations, are presented with too many significant digits. Uncertainty should be expressed in the least significant digit. The uncertainty then determines the number of significant digits in the result regardless of the number of digits provided by the data system. Often, I see both uncertainties and quantitative results presented with too many significant digits.

In addition to this first challenge, the basic reporting of results often confuses the presentation of both the experimental result and the uncertainty. Classically, results larger then 10 and smaller than 0.1 should be reported using scientific notation. This guideline is often stretched to 0.01–100, but it should not be stretched further. Strict adherence to this rule by itself reduces confusion in result reporting and significant figures.

An additional challenge relates to the common use of the standard deviation and relative standard deviation (RSD) as a figure of merit for the precision of chromatographic results. As we know from basic population statistics, one standard deviation from the mean indicates that approximately 68% of results should be expected to be in that range. The true experimental uncertainty is larger, typically around two standard deviations, which would include approximately 95% of the results, or three standard deviations, which would include about 99.7% of expected results. However, most quantitative analytical chemistry experiments do not generate enough results for population statistics, so these generalizations do not hold. Classically, as taught in quantitative and instrumental analysis texts for decades, a 95% confidence interval about the data is best used as the experimental uncertainty, which for small populations of data would be significantly wider than one standard deviation. In much of the chromatography literature, this additional step is not performed. In short, much of the literature underestimates experimental uncertainty and overestimates significant figures.

This observation is not to denigrate the work of the many scientists developing and optimizing gas chromatography (GC)
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methods. Because GC has become increasingly popular over the years, it has gone from a technique largely performed by formally trained analytical chemistry specialists to a much broader range of scientists, who may not have had formal training in quantitative analysis and analyzing experimental error and uncertainty.

In the rest of this instalment, we examine some common cases where experimental uncertainty arises in GC methods.

**Dilute-and-Shoot**

Even simple “dilute-and-shoot” methods have hidden experimental uncertainties. We start with this case because nearly all other sample preparation techniques and methods involve dilution in the preparation of samples and standards. Many analysts make the incorrect assumption that volumetric glassware is perfect. Figure 1 shows a close-up look at a typical “Class A” 100 mL volumetric flask, with the class indication circled. For any volumetric glassware, it is best to use Class A glassware and avoid any glassware that does not indicate class or has the markings worn off. If you look closely, you can note the uncertainty value of ±0.08 mL provided in the printing on the flask. Although the uncertainty seems small, the rules for propagation of errors tell us that each additional dilution step will add to the experimental uncertainty of the result.

Table 1 shows the uncertainties involved with some Class A volumetric flasks and transfer pipettes. It illustrates one of the interesting problems in analytical method development. Because we are pressured to reduce the use of solvents, smaller volumes introduce higher relative experimental errors in each step. Table 1 shows that there is flask-to-flask and pipette-to-pipette experimental uncertainty involved with volumetric glassware that must be considered when developing methods. Drawing a sample to the mark in a pipette or filling to the mark in a flask may not deliver the exact volume stated on the device. To this end, experimental procedures for gravimetrically verifying the volume delivered by a pipette or contained in a flask have been published by the National Institute of Standards and Technology (NIST) (1).

Consider the experimental uncertainty involved in a 1:100 dilution of a pre-prepared stock standard solution by two different procedures. In the first procedure, a 1-mL class A transfer pipette is used to deliver 1 mL of the stock solution into a 100-mL class A volumetric flask and the flask is filled to the mark with the dilution solvent. In the second procedure, a 1-mL class A transfer pipette is used to deliver 1 mL of the stock solution into a 10-mL class A volumetric flask that is filled to the mark with the dilution solvent. Using a second 1-mL class A transfer pipette, 1 mL of the diluted solution is then transferred to a second 10-mL Class A volumetric flask, which is then filled to the mark with the dilution solvent. In procedure 1, a total of about 100 mL of dilution solvent is used; in procedure 2, the amount of dilution solvent is reduced by 80% to about 20 mL. Table 2 shows the propagation of errors comparison of procedures 1 and 2. Detailed equations and discussion of propagation of errors are not provided here but can be referenced in nearly any textbook on analytical chemistry or instrumental analysis (2). In the chapter in reference 2, I share a specific example of a propagation of error analysis applied to a pharmaceutical analysis method. Table 2 shows that the serial dilution procedure increases the uncertainty from 0.6% to 0.9% over the single dilution, which is an increase of 50% in the uncertainty.

As seen in Table 2, the serial dilution procedure, which most analysts would prefer today because it uses far less solvent, has significantly higher experimental uncertainty, and this experimental uncertainty would then be added to any uncertainty in the stock standard. Note that when making extremely low concentration standards, which is a common need with the extremely sensitive methods and instruments of today,
the experimental uncertainty involved in using even the most precise available glassware to prepare standards and samples in multiple dilution steps can lead to relatively large uncertainties that may reduce the number of significant figures in the experimental results. As a result, it is likely that any determinations at sample concentrations lower than parts per million (ppm) level should be reported with no more than one or two significant figures.

**Uncertainty In Sample Injection**

The inlet and injection process in GC is well-known to provide hidden uncertainty, as discussed in two recent “GC Connections” instalments and a classic book (3–5). As discussed above, as pipettes and sample delivery system volumes get smaller, relative experimental uncertainty in the delivered volume increases. Most microlitre-volume syringes are accurate to approximately 1% of their nominal maximum volume. A typical 10 μL syringe has graduations representing 0.1 μL. Interestingly, it is common to inject 1 μL of liquid using a 10 μL syringe, providing a nominal error of up to 10% in the accuracy of the delivery. Furthermore, the graduations on the syringe barrel do not account for the internal volume of the syringe needle, which is approximately 0.6 μL. Therefore, our 1 μL injection using a 10 μL syringe may be an injection of 1.5–1.7 μL, depending on the accuracy of the syringe.

With the use of an auto-injector and proper maintenance, a syringe can remain highly reproducible for hundreds or possibly thousands of injections. Syringe manufacturers provide quick guides on how to take care of and perform maintenance on syringes (6) that include suggestions for extending syringe lifetime. With the high precision of auto-injectors, users can typically expect injections with

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Glassware Used</th>
<th>Glassware Percent Uncertainties (%)</th>
<th>Uncertainty in Final Dilution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 × 100 mL volumetric flask 1 × 1 mL volumetric pipette</td>
<td>0.08% (flask) 0.60% (pipette)</td>
<td>0.6%</td>
</tr>
<tr>
<td>2</td>
<td>2 × 10 mL volumetric lask 2 × 1 mL volumetric pipette</td>
<td>0.20% (flask) 0.60% (pipette)</td>
<td>0.9%</td>
</tr>
</tbody>
</table>
less than 1% RSD in the resulting peak areas from injection to injection. Difficulty can arise when the syringe is changed; you can expect peak areas to vary by several percentage points up or down from the original syringe. With effective calibration, and either internal or external standard, this change may not be noticed unless working at or near the limit of detection (LOD) or limit of quantitation (LOQ), where a few percent of reduced injected sample volume might move results lower than the LOD or LOQ.

### Uncertainty in Detection and Quantitation

In a recent installment, we discussed several hidden challenges in measuring the LOD for an instrument or as part of the method validation process (7). We saw that the classical International Union of Pure and Applied Chemistry (IUPAC) calculation for LOD includes terms only related to uncertainty in the measured signal, not in the calibration curve. We saw an alternate calculation, based on propagation of errors, for determining the LOD that includes terms for uncertainty in both the slope and y-intercept of the calibration curve. Earlier in this column, we discussed uncertainty in simple “dilute-and-shoot” procedures, which are often similar to the procedures used to generate calibration curves.

In general, greater increases in experimental uncertainty from calculations occur when subtraction and division are used in equations and formulas. In both cases, the calculation result becomes smaller while the uncertainty becomes larger. Table 3 illustrates this principle with some simple calculations, in which the values 1.100 × 10^4 ± 0.2 and 1.000 × 10^2 ± 0.2 are combined by addition, subtraction, multiplication, and division. Note that both initial values would be seen as highly precise.

As seen in Table 3, the original data have a relative uncertainty of 0.2%. When the data points are multiplied or divided, the uncertainty is determined from the relative uncertainties, so the final uncertainty is the seam on a relative basis for both. Since experimental uncertainties are additive for addition and subtraction, adding the two data points results in a decreased relative uncertainty as the result increases faster than the uncertainty. However, subtracting them results in a much greater relative uncertainty, since the result is now smaller and the uncertainty larger. In this case, the four significant figure original data are reduced to three in the result. The relative experimental uncertainty increases from a fraction of a percent to nearly 3% in the subtraction case. When I examine GC methods with unsatisfactory reproducibility, the first places to look for the source of the problem are calculations and glassware choices, followed by injection and detection.

Error analysis and the consideration of experimental uncertainty may seem like a chore. However, it is one of the most important aspects of method development, optimization, and validation. GC instruments and data systems are highly precise, and they generate raw data that may have three, four, or more significant figures. In the past, we would look first at potential instrumental challenges when troubleshooting and optimizing problems with precision and accuracy. Today, I look first at the glassware and calculations, followed by the instrument. Chromatographers should be cautious and recognize that there are hidden uncertainties in almost all quantitative chromatographic methods that may increase experimental uncertainty, reduce the number of significant figures, and make the data coming from the data system look more precise and accurate than it really is.

### References


### ABOUT THE AUTHOR

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**LCGC Europe** May 2022
New Liquid Chromatography Columns and Accessories for 2022

David S. Bell, Column Watch Editor

This article covers liquid chromatography (LC) columns and accessories commercially released after Pittcon 2021 through to this year’s virtual conference. Like in the past, LCGC sent out a survey in late 2021 and early 2022 asking vendors to supply information on products launched over the past year. Note that new products for gas chromatography (GC), LC instrumentation and software, and sample preparation are covered elsewhere. The information for this article was obtained over several months, therefore, it is possible that some information was missed or misinterpreted. The reader is encouraged to check with specific vendor sites for additional products as well as more detailed information on product usage and attributes. Links to vendor sites are provided where applicable.

The vendors that responded to the survey and their new liquid chromatography (LC) products are listed in Table 1. The products vary in targeted analyte type as well as in mode of chromatographic operation. The columns were initially categorized as addressing small molecule or large molecule separations. Within these categories, the products were further separated based on the mode of separation, including reversed-phase LC, hydrophilic-interaction LC (HILIC), chiral, size-exclusion chromatography (SEC), and affinity chromatography. Two additional subcategories extracted this year are treated as separate entities—new nano- and micro-column format offerings and columns targeting oligonucleotides are also discussed.

The products launched over the past year revealed that there was an emphasis in the industry to reduce or eliminate analyte–metal interactions. Most of the categories this year include some type of advance in metal passivation or new uses of established “non-metal contact” hardware, such as PEEK (polyetheretherketone)-lined stainless steel. Unlike in previous years where there were enough new products in given categories to generate illustrative sub-tables, the new products introduced this year are spread evenly across the categories, leaving only a few in any given section.

Small Molecule Reversed-Phase: Selectivity and retention alternatives for small molecule separations continues to be a market need. Fortis introduced two new surface chemistries on their 2.6- and 5-µm superficially porous particles (SPP) platforms. The SpeedCore C18-PFP is a C18 and pentafluorophenyl combination phase that continues a recent trend of “enhanced C18 chemistries” intended to improve the resolution of diverse compound sets and provide alternate selectivity when compared to the traditional alkyl chain stationary phases. The second phase introduced by the company in this category is the SpeedCore Aqua phase. This stationary phase is described as a proprietary polar-endcapped phase intended for polar analytes not suited to hydrophobic C18 columns. More information on these columns may be obtained on Fortis’s website (https://fortis-technologies.com/core-shell-physicals/).

MilliporeSigma introduced a new monolithic column for small molecule analysis. The Chromolith HR, 2 mm internal diameter (i.d.) C18 column features a macropore (through-pore) size of 1.15 µm and a mesopore (internal pore) size of 150 Å. The column provides rapid separations at low back pressure and cost-savings because of its longer column lifetimes, as the unique macropore size prevents column fouling due to matrix contaminants. The company also claims that a high plate count, which rivals sub-2-µm ultrahigh-performance LC (UHPLC) columns, can be achieved. For additional information, the reader is directed to the following website: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/analytical-chemistry/small-molecule-hplc/chromolith-hplc-and-uhipc-columns.
<table>
<thead>
<tr>
<th>Company Name</th>
<th>Product Name</th>
<th>Category</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced Materials Technology</td>
<td>Halo 1.5 mm columns</td>
<td>Micro- and nano-column hardware formats</td>
<td>The Halo 1.5 delivers increased sensitivity and reduced solvent consumption with UHPLC and UHPLC–MS systems, allowing scientists to experience the benefits of capillary columns without the pain of specialized microflow systems.</td>
</tr>
<tr>
<td>Agilent</td>
<td>AdvanceBio SEC PEEK-lined Stainless Steel columns</td>
<td>Size-exclusion chromatography (SEC)</td>
<td>Hydrophilic coating on silica ensures low secondary interactions leading to good peak shape with low tailing and high resolution; Biolert metal-free flow path eliminates concern for metal-sensitive biologics.</td>
</tr>
<tr>
<td>ColumnTek LLC</td>
<td>Enantiocel A8</td>
<td>Chiral</td>
<td>Unique enantioselectivity, high column efficiency, and excellent peak shape</td>
</tr>
<tr>
<td>Daicel Corporation</td>
<td>Chiralpak IK</td>
<td>Chiral</td>
<td>Expended solvent compatibility; new chiral selector</td>
</tr>
<tr>
<td>Fortis</td>
<td>SpeedCore Aqua</td>
<td>Small molecule reversed-phase</td>
<td>Retention and separation of polar analytes</td>
</tr>
<tr>
<td></td>
<td>SpeedCore C18-PFP</td>
<td>Small molecule reversed-phase</td>
<td>Retention and separation of diverse compound sets. Orthogonal selectivity over other alkyl chain L1 columns.</td>
</tr>
<tr>
<td>Hilicon AB</td>
<td>iHILIC-(P) Classic</td>
<td>HILIC</td>
<td>PEEK (polyetheretherketone)-lined stainless-steel column hardware takes both advantages of biocompatibility of PEEK column and mechanical strength of SS column at higher temperature.</td>
</tr>
<tr>
<td></td>
<td>iHILIC-Fusion(P)</td>
<td>HILIC</td>
<td>PEEK-lined stainless steel column hardware takes both advantages of biocompatibility of PEEK column and mechanical strength of SS column at higher temperature.</td>
</tr>
<tr>
<td></td>
<td>iHILIC-Fusion(+)</td>
<td>HILIC</td>
<td>PEEK-lined stainless steel column hardware takes both advantages of biocompatibility of PEEK column and mechanical strength of SS column at higher temperature.</td>
</tr>
<tr>
<td></td>
<td>iHILIC-Fusion</td>
<td>HILIC</td>
<td>PEEK-lined stainless steel column hardware takes both advantages of biocompatibility of PEEK column and mechanical strength of SS column at higher temperature.</td>
</tr>
<tr>
<td>MilliporeSigma</td>
<td>Chromolith WP300 Epoxy, 2 mm i.d.</td>
<td>Affinity</td>
<td>Column provides a “blank slate” for end users to immobilize ligand of choice.</td>
</tr>
<tr>
<td></td>
<td>Chromolith WP300 Protein A, 2 mm i.d.</td>
<td>Affinity</td>
<td>Rapid mAb titer determination with long column lifetimes</td>
</tr>
<tr>
<td></td>
<td>Chromolith HR, 2 mm i.d.</td>
<td>Small molecule reversed-phase</td>
<td>Rapid separations at low back pressure, Cost-savings are achieved as the column lifetimes are much longer than for particulate HPLC columns.</td>
</tr>
<tr>
<td></td>
<td>Supel Carbon LC</td>
<td>Small molecule reversed-phase</td>
<td>Unique mechanism enables retention of very polar compounds without the need for HILIC mobile phase conditions; complete pH range available for method developers to employ.</td>
</tr>
<tr>
<td></td>
<td>Chromolith WP300 C18, 2 mm i.d.</td>
<td>Large molecule reversed-phase</td>
<td>Rapid separations at low back pressure that can minimize artifact generation with especially fragile biomolecules</td>
</tr>
<tr>
<td>Phenomenex</td>
<td>Biozen Nano LC columns</td>
<td>Micro- and nano-column hardware formats</td>
<td>High efficiency, excellent inertness, increased sensitivity, and exceptional quality and robustness</td>
</tr>
<tr>
<td>Regis Technologies</td>
<td>Whelk-O1 Core columns</td>
<td>Chiral</td>
<td>Faster more efficient separations than conventional fully porous chiral stationary phase materials</td>
</tr>
<tr>
<td></td>
<td>Reflect l-Cellulose Z Polysaccharide columns</td>
<td>Chiral</td>
<td>Immobilized phase extends mobile phase compatibility and application range</td>
</tr>
</tbody>
</table>

*: Comments supplied by vendors

Continued on Page 184
MilliporeSigma also recently introduced a new porous graphitic carbon (PGC) phase, Supel Carbon, as an alternative column choice for polar compound analysis via reversed-phase LC. The company notes that a unique mechanism enables retention of polar compounds without the need for HILIC mobile phase conditions. In addition, the complete pH range is available for method developers to employ. The company states that the patent-pending synthetic procedure enables columns to operate up to 12,000 psi, which was never possible with prior generation carbon particle-packed columns. More information is available at https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/analytical-chemistry/small-molecule-hplc/supel-carbon-lc-hplc-columns.

HILIC: The introduction of HILIC phases has decreased over the past several years. However, with the attention focused on metal elimination and passivation, it is not surprising to witness a bit of a resurgence in this category. The small, polar molecules most often analyzed in HILIC are notorious for interactions with metal surfaces that can result in poor peak shapes and also low recovery.

Hilicon introduced a number of their HILIC phases in PEEK-lined stainless steel to eliminate or limit nonspecific binding of analytes with metal surfaces. The iHILIC-Fusion(P) is described as a modulated amide with phosphate and quaternary ammonium moieties built on a polymeric support. The iHILIC-(P) Classic is a modulated diol HILIC column with sulphate and quaternary ammonium attachments. This phase is also based on a polymer substrate. The iHILIC-Fusion and iHILIC-Fusion(+) are silica-based HILIC stationary phases that are described as modulated hydroxyethyl amide columns. The former contains the quaternary ammonium moiety as well as both sulphate and phosphate groups, whereas the latter contains only the quaternary ammonium and sulphate groups. All of the columns are said to provide retention and selectivity for a wide range of polar analytes. More information is available at https://www.hilicon.com/ihilic/.

Waters developed Atlantis Premier ethylene bridged hybrid (BEH) Z-HILIC columns for HILIC separations. The sulfobetaine zwitterionic bonded phase chemistry, built on 1.7-µm, 2.5-µm, and 5-µm BEH particles, provides a wide range of polar analyte retention at an extended pH range, according to the company. The new
offering in this chemistry utilizes Waters metal passivation technology, which significantly reduces unwanted interactions between analytes and metal surfaces in the column hardware. The result is improved peak shape and increased sensitivity for untargeted metabolites as well as other target analytes, such as aminoglycosides, nucleosides, nucleotides, and nucleobases, among others. See the Waters website (https://www.waters.com/nextgen/global/products/columns/atlantis-premier-columns.html) for further details.

**Chiral:** New offerings in chiral separations continue the recent trend of expanding on polysaccharide-based supports and taking advantage of SPP technology.

ColumnTek LLC introduced the Enantiocel A8, which is described as an amylose tris(3-chlorophenyl carbamate)-coated phase that is available on both 5- and 3-µm fully porous particles (FPP). The new phase is noted as providing unique enantioselectivity, high column efficiency, and excellent peak shape. According to the company, the coated amylose tris(3-chlorophenyl carbamate) selector can provide better enantioselectivity than the corresponding immobilized version. Further details can be obtained by emailing info@columntek.com.

Daicel Corporation introduced Chiralpak IK, immobilized cellulose tris(3-chloro-5-methylphenylcarbamate) in both 5- and 3-µm formats. Immobilization is stated by the organization to expand solvent compatibility. The new chiral selector is a cellulotic version of Chiralpak IG. A media release statement and further information can be accessed at https://chiraltech.com/daicel-chiral-technologies-announces-the-launch-of-chiralpak-ik/.

Regis Technologies, Inc, launched Reflect I-Cellulose Z Polysaccharide columns for chiral analysis in normal phase, reversed phase, and supercritical fluid chromatography (SFC). The phase is based on cellulose tris(3-chloro-4-methylphenylcarbamate). The phase is reported to extend the range of selectivity available in the line of Reflect Polysaccharide-based chiral stationary phases (CSPs). More on this line of chiral columns may be viewed at https://www.registech.com/reflect-polysaccharide-phases/.

Regis Technologies also introduced Whelk-O1 Core columns to the market. Built on SPPs, the columns are reported to provide faster, more efficient separations than conventional 5- or 3-µm fully porous chiral stationary phase materials. One advantage of the synthetic CSP is that it is available in two different absolute configurations: R,R and S,S, which can be important when peak elution order matters. Applications and other supporting information can be found at https://www.registech.com/whelk-o1/.

**TABLE 1 (CONTINUED): New LC products**

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Product Name</th>
<th>Category</th>
<th>Comments*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tosoh</td>
<td>TSKgel FcR-III-A-5PW</td>
<td>Affinity</td>
<td>The only analytical affinity column that can analyze mAb glycoforms.</td>
</tr>
<tr>
<td>Waters</td>
<td>Acquity and XBridge Premier Protein SEC 250Å columns</td>
<td>Size-exclusion chromatography (SEC)</td>
<td>Columns combine innovations in column hardware and SEC particle technology that work synergistically to minimize undesired ionic and hydrophobic secondary interactions between proteins and the column, affording chromatographers the flexibility to use generic or platform SEC methods for size-based separations of biotherapeutic proteins.</td>
</tr>
<tr>
<td></td>
<td>Atlantis Premier BEH Z-HILIC columns</td>
<td>HILIC</td>
<td>Increase polar analyte retention across a wide pH range (2–10), increased retention and different selectivity, reduced unwanted interactions between analytes and metal surfaces</td>
</tr>
</tbody>
</table>

* Comments supplied by vendors

**Large Molecule Selectivity and Performance**

**Reversed-Phase:** Fortis launched a new product intended for the reversed-phase separations of large molecules. The SpeedCore Bio Phenyl column is available as a 2.6- or 5-µm, 300 Å wide pore bio phenyl phase that has been optimized for peptide and protein separations. The column can be used in conjunction with Bio C18 to run complex protein and tryptic digests, according to the manufacturer. For additional information, see https://fortis-technologies.com/core-shell-physicals/.

MilliporeSigma released a wide-pore monolithic column, called Chromolith WP300 C18, in 2 mm i.d. hardware. The macro pore structure of 2 µm and mesopores of 300 Å allow rapid separations at low back pressure, which can minimize artifact generation with fragile biomolecules. The company notes that for biomolecule analysis, the unique macro pore size prevents column fouling because of matrix contaminants. The company also notes that the high plate count provided by the new monoliths rivals sub-2.0-µm
Size-Exclusion Chromatography (SEC): SEC resolves analytes based on molecular size and is often used as a complementary technique to reversed phase analyses of proteins. Columns utilized in SEC are often characterized by their strict control of pore size and by their inert surface chemistry. Because inertness also includes the column hardware, it is not surprising that the new products in this category include different approaches towards metal passivation.

AdvanceBio SEC PEEK-lined stainless-steel columns from Agilent Technologies provide aggregate analysis of metal-sensitive biologics owing to the use of their Biolinert metal-free flow path, which is reported to eliminate the concern for metal-sensitive biologics. The columns come with 1.9-μm particles with both 120 Å and 200 Å pores. As stated by the company, a hydrophilic coating on silica ensures minimal secondary interactions leading to good peak shape with low peak tailing and high resolution. For a more detailed description of the Agilent offering, see https://www.agilent.com/cs/library/brochures/brochure-bioInert-columns-5994-2983en-agilent.pdf.

Phenomenex introduced Biozen dSEC-2 SEC columns to the market. The Biozen dSEC columns are packed with low pore volume silica with a proprietary hydrophilic diol-type bonded surface chemistry. The silica provides mechanical strength to improve column packing. The stationary phase modification prevents the silica surface from interacting with the protein samples. The company claims column stability exceeding 100 h even with harsh mobile phase and high flow rates. More information can be found at https://www.phenomenex.com/products/detail/biozen/dSEC-2.

Waters recently commercialized Acquity and XBridge Premier Protein SEC 250 Å columns. The columns are said to provide an accurate determination of protein aggregates, monomers, and small-sized fragments in proteins that range in size from 10,000 to 650,000 daltons. The columns are intended for the characterization and monitoring of biotherapeutic product size variants. The Acquity column utilizes Waters hybrid (BEH) particle technology in a 1.7-μm particle size, whereas the XBridge sports a 2.5-μm particle bed. In both cases, the BEH particles are modified with hydroxy-terminated polyethylene oxide (PEO) and utilize Waters metal passivation technology. The columns are reported to combine innovations in column hardware and SEC particle technology that work synergistically to minimize undesired ionic and hydrophobic secondary interactions between proteins and the column. Further details and offerings can be found at www.waters.com/sec.

Affinity: Affinity chromatography is a separation technique based on highly specific interactions between two partners. Through the immobilization of one partner to a solid surface, the other partner can be effectively “fished” out of a complex mixture with high specificity. Affinity chromatography is yet another complementary tool employed to fully characterize large molecules.

MilliporeSigma introduced the Chromolith WP300 Epoxy, 2 mm i.d. column that can be customized by the user to immobilize their own ligand to
The monolithic structure provides long column lifetimes and consistent binding efficiencies of mAbs even at high flow rates, according to the company. For further information on wide-pore monolith offerings, see https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/analytical-chemistry/large-molecule-hplc/chromolith-widepore-300-hplc-columns.

To be consistent with other products in the company’s monolith line, Chromolith WP300 Protein A, for rapid monoclonal antibody (mAb) titer determination. 

**Micro and Nano Column Hardware Formats**

Advanced Materials Technologies introduced a number of their Halo columns in a new 1.5 mm i.d. format. Four new product offerings, including a 90 Å C18, 160 Å ES-C18, and two 1000 Å phases (C4 and diphenyl) in column lengths of 50–150 mm, are now available. According to the company, the 1.5 mm i.d. delivers increased sensitivity and reduced solvent consumption, allowing scientists to experience the benefits of capillary columns without the pain of specialized fittings and techniques as it feels and installs like an analytical 2.1-mm i.d. column. Separations utilizing the 1.5-mm format consume half the solvent compared to 2.1-mm columns. In addition, twice the signal intensities in comparison to 2.1-mm columns are reported. A number of applications, as well as a brochure, are available at https://halocolumns.com/introducing-the-new-halo-1-5/.

Phenomenex introduced Biozen Nano LC columns (https://www.phenomenex.com/info/page/21resourcesnano). According to the developers, the 75-µm i.d. columns utilize core–shell particles with a highly consistent morphology. The format minimizes band broadening associated with diffusion and mass transfer, leading to higher efficiency. The company also claims minimal peak widths, which enhances the separation for ‘omics analysis. The columns also feature a SecurityLink finger-tight fitting system that simplifies the connection process with technology that prevents column damage because of overtightening. Stationary phases that are available in this format include Biozen Peptide PS-C18 (charged surface ligand/ C18 ligand), Biozen Peptide XB-C18 (di-isobutyl C18), and Biozen Polar C18 (“enhanced selectivity” C18). Each of the stationary phases is available in 150- and 250-mm columns lengths.

**Oligonucleotides**

Like polar molecules and proteins, oligonucleotides are also notorious for binding to metals. Phenomenex commercialized bioZen Oligo columns that utilize organo-silica core–shell particles with a highly consistent morphology that minimizes band broadening associated with diffusion and mass transfer. According to the company, this leads to higher efficiency and minimal peak widths, which is critical for the separation of closely eluting impurities associated to synthetic oligonucleotides. The columns are constructed with BioTi hardware, which reduces sample loss because of metal adsorption, and core–shell particles, which provide for high efficiency. The company also claims robustness at high pH and temperature. For further information, see https://www.phenomenex.com/Products/detail/biozen.

**Conclusions**

From small polar molecules to proteins and oligonucleotides, different approaches to minimizing undesired metal interactions are being applied. Although the new chromatography column products launched in 2021 and early 2022 were evenly spread across many categories, they did have one common theme—metal passivation. There was some type of metal passivation or elimination that was apparent in each of the categories except “Chiral”. Whether metal-free chromatography is enacted via PEEK or PEEK-lined stainless-steel hardware, selected metal alloys, or passivated stainless-steel approaches, it will be interesting to see if the trend continues.

**Acknowledgements**

The author would like to acknowledge and thank Brian J. Murphy and Michael Dong for their review of the draft manuscript and valuable feedback.
New Sample Preparation Products and Accessories 2021–2022

Douglas E. Raynie, Sample Preparation Perspectives Editor

Our annual report on new products introduced in the preceding year (since March 2021) covers sample preparation instrumentation, supplies, and accessories.

As the world emerges from the pandemic, assessing new products remains challenging. Although print and electronic materials abound, professional meetings and exhibitions have been hit or miss, and have been a combination of virtual, hybrid (a mix of virtual and in person), and, on the rare occasion, in person. Personal visits with sales representatives came to a near grinding halt.

Similar to the 2021 new product review and presumably because of the pandemic, the number of new sample preparation technologies introduced in the past year appear to be down compared with previous years, though it is hard to assess without the personal visits to Pittcon vendors.

Our annual review of sample preparation products covers the previous year. In late 2021, the LCGC staff submitted a survey to vendors of sample preparation products. The responses to this survey are compiled in this review. In total, there were fewer responses than in previous years and less new product introductions during the past 12 months. Most product introductions were aimed at improved performance of existing technologies, especially solid-phase extraction (SPE), including sorbents and accessories.

This review is presented in three sections. First, SPE sorbents and products are discussed. Next, other sorbent-based sample preparation technologies are presented. Finally, attention is turned to other sample preparation accessories and supporting technologies. To assist the reader with some of the details behind these new products, each section presents a tabular summary of the associated products. In all cases, the new products we uncovered are presented in the annotated table, while the text highlights particularly worthwhile products.

### Solid-Phase Extraction (SPE)

United Chemical Technologies, or UCT, led the way in developing new SPE products in 2021. The company introduced a new polymeric SPE in the 96-well plate format with Micro-Prep. The Micro-Prep utilized disk technology with dense packing of the sorbent particles to generate the uniform flow needed for sample clean-up and analyte enrichment. Four different chemistries, reversed-phase hydrophilic-lipophilic balance (HLB), mixed-mode strong cation exchange, strong cation exchange, and strong anion exchange are available in a single package that includes the extraction plate, collection plate, and sealing film. Next, UCT brought to market a silver ion-functionalized silica gel cartridge, the Enviro-Clean Fusion Ag⁺. The sorbent, packed as 500 mg or 1 g in 6-mL cartridges or 2 g in a 15-mL cartridge, is not deactivated by water and is used for the fractionation of aliphatic and aromatic hydrocarbons because the aromatics form a charge-transfer complex with silver ions. To address environmental friendliness even further, acetone can be used in place of dichloromethane as the elution solvent. The final product entry by UCT was the HydraFlow vacuum-controlled SPE workstation. The four independent channels can extract samples independently or simultaneously in an environment free of cross-contamination. The sample pathways are coated to minimize corrosion from exposure to organic solvents or acids. Channel switching accommodates the collection of the sample eluate and separation of aqueous and organic wastes.

Two cartridge products from Restek focused on sample clean-up. The Resprep CarboPrep Plus SPE cartridge is a carbon-loaded device specifically designed for the clean-up of water...
and soil samples for the analysis of organochlorine pesticides. Meanwhile, in both cartridge and plate formats, the Resprep PLR SPE is intended for phospholipid and protein removal from plasma, serum, and whole blood prior to chromatographic analysis, avoiding signal suppression in subsequent mass spectral characterization. A top frit captures proteins, and a sorbent bed of a porous polymer sintered with silica particles retains phospholipids. Another product aimed at protein or peptide desalting and fractionation is the Empore C18 Stage tips from CDS Analytical. The pipette tips feature chemically resistant C18-treated silica gel placed into micropipette tips. The chemical resistance allows use of organic solvents and acidic or basic conditions for protein and peptide desalting and fractionation.

Speciality SPE phases are featured in two new SPE devices. The CDS Empore 6080 HLB Standard 96-well plate features a proprietary prefilter followed by a mixed-mode hydrophilic-hydrophobic balance styrene-divinylbenzene material for extraction of serum, plasma, and whole blood. The pyrrolidone and urea HLB sorbent bed is claimed to allow high product consistency. Hilicon AB introduced the iSPE HILIC, featuring hydroxyethylamide, sulfate, and quaternary ammonium silica in both cartridge and 96-well plate formats with high retention capacity for sample preparation of hydrophilic compounds. Suggested applications include the purification of glycopeptides, glycans, hydrophilic peptides and metabolites, and related biomolecules. Table 1 provides a summary of each of these SPE products.

**TABLE 1: Solid-phase extraction products**

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Product Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Chemical Technologies</td>
<td>Micro-Prep</td>
<td>A 96-well micro-elution plate featuring disk technology with dense sorbent particle packing for uniform sorbent distribution with minimal diffusion distance. Available with reversed-phase, mixed-mode strong cation exchange, strong cation exchange, and strong anion exchange formats.</td>
</tr>
<tr>
<td></td>
<td>Enviro-Clean Fusion Ag*</td>
<td>Silver ion-functionalized silica gel available in 500 mg or 1 g sorbent bed in 6-mL cartridge or 2 g sorbent bed in 15-mL cartridge. Designed for fractionation of aliphatic and aromatic hydrocarbons.</td>
</tr>
<tr>
<td></td>
<td>HydraFlow</td>
<td>Vacuum-controlled SPE workstation with four independent channels. Sample pathways are coated to prevent corrosion from exposure to organic solvents and acids.</td>
</tr>
<tr>
<td>Restek</td>
<td>Resprep CarboPrep Plus SPE Cartridge</td>
<td>Carbon sorbent bed (95 mg) packed into 3-mL cartridge for sample clean-up to minimize plugging of systems during analysis of organochlorine pesticides</td>
</tr>
<tr>
<td></td>
<td>Resprep PLR SPE Cartridges and Plates</td>
<td>Available in cartridge (25 mg sorbent in 1-mL cartridge) or 96-well plate (25 mg sorbent) for removal of phospholipids and proteins prior to GC–MS or LC–MS/MS</td>
</tr>
<tr>
<td>CDS Analytical</td>
<td>Empore C18 Stage Tips</td>
<td>Micropipette tips (200 μL) with two layers of Empore membrane. C18 tips developed for protein and peptide desalting and fractionation. Also available in C8, SDB-XC, SDB-RPS, SAX, SCX formats.</td>
</tr>
<tr>
<td></td>
<td>Empore 6080 HLB Standard 96-well Plate, 1.2 mL</td>
<td>Mixed-mode styrene divinylbenzene and pyrrolidone and urea 96-well plates. A prefilter prevents particulates and macromolecules from interrupting solvent flow. A patented collar design prevents cross-contamination. High flows, high capacity, and reduced elution volumes.</td>
</tr>
<tr>
<td>Hilicon AB</td>
<td>iSPE-HILIC</td>
<td>High purity silica (50 μm, 60 Å) functionalized with hydroxyethylamide, sulfate, quaternary ammonium for universal preparation of hydrophilic biomolecules. 1-, 3-, and 6-mL cartridges with 25–2000 mg HILIC material or 96-well plates with 200 mg HILIC material per plate.</td>
</tr>
</tbody>
</table>

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TABLE 2: Sorbent-based sample preparation products

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Product</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenomenex</td>
<td>Verex Filter Vials</td>
<td>Filtration and vial technology combined in polypropylene vials ready for standard autosamplers. High sample recovery achieved with low sample dead volume (30 μL) in 12 × 32 mm vials. Five polymeric membrane options allow a variety of application areas.</td>
</tr>
<tr>
<td></td>
<td>Novum Pro SLE</td>
<td>Upgraded synthetic sorbent for clinical assays by simplified liquid extraction.</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Thermo Scientific QuEChERS</td>
<td>Pouches and pre-filled tubes for the original QuEChERS collection. Available in pouches and pre-filled tubes, the product collection accommodates the original QuEChERS methods, as well as the Association of Official Analytical Chemists and European methods.</td>
</tr>
<tr>
<td>International</td>
<td>HiSorb</td>
<td>High capacity sorptive extraction for trace-level immersives and headspace sampling of volatile and semivolatile organic compounds. Four sorptive phases available.</td>
</tr>
</tbody>
</table>

are reapplied in several other sample preparation modalities. This review period featured four of these. Phenomenex claims an all-in-one sample storage, transfer, and filtration process with Verex filter vials. The polypropylene 12 × 32 mm vials incorporate a syringe, filtration membrane, vial, cap, and septa into a single product, as depicted in Figure 1. Liquid sample is placed into the reservoir of the external vial, then the internal plunger is compressed to push the sample through the membrane, which traps particulates to produce a particulate-free sample. Equipped with a pre-slit PTFE/silicone septa, the vial can be placed into most chromatography autosamplers. A variety of filtration media are available for a range of environmental, food, and bioanalysis applications. The sole hydrophobic membrane, polytetrafluoroethylene (PTFE), is suitable for filtration of organic, highly acidic, or basic solvents. Four hydrophilic membrane options exist including regenerated cellulose for aqueous and organic solutions, nylon for bases, solvents, alcohols, and aromatic hydrocarbons, polyethersulfone for critical biological samples, tissue culture media, additives, and buffers, and polyvinylidene fluoride for alcohols or biomolecules.

QuEChERS (quick, easy, cheap, effective, rugged, and safe) and simplified liquid extraction (SLE) are two sorbent-based extraction technologies that are developing a following in the field. In response, new product offerings addressed these techniques. Thermo Scientific refined their QuEChERS collection to include pre-weighed high-quality salts and sorbents designed to minimize clumping of salts to provide reliable and reproducible results. Available in pouches and pre-filled tubes, the product collection accommodates the original QuEChERS methods, as well as the Association of Official Analytical Chemists and European methods. Phenomenex developed a cleaner version of their synthetic SLE sorbent for the Novum Pro SLE to achieve lower detection levels for clinical assays.

Markes International introduced the HiSorb sorptive extraction system for the analysis of volatile and semivolatile organic compounds in liquids and solids by thermal desorption-gas chromatography–mass spectrometry (GC–MS). High capacity immersive or headspace sampling accommodates trace analysis with the use of four available sorbents; polydimethylsiloxane (PDMS) for volatiles and nonpolar semivolatiles; PDMS/carbon wide-range (CWR) for low-molecular-weight compounds; PDMS/divinylbenzene (DVB) for volatiles, amines, and polar compounds; and DVB/CWR/PDMS for volatiles, semivolatiles, and flavour compounds. Inert-coated probes are available for reactive systems. When used with Markes’ thermal desorption system, trapping is achieved with electrical cooling rather than cryogens.

Table 2 provides a summary of these sorbent-based technologies.

Sample Preparation Accessories and Related Products

The field of sample preparation is so broad, and there are a large number of established and emerging technologies identified as sample preparation, that commercial developments and new product introductions are often in seemingly scattered areas. An attempt is made here to unify these varied product offerings.

Gas Sampling: Markes International created a compact radial diffusive sampler, the Pocket Diffusive (POD) Sampler, compatible with thermal desorption. The sampler has a large diffusive surface area for adsorption and a short diffusive pathway. After the POD Sampler is deployed, the cover is removed for sampling. After the suitable sampling period, the cover is replaced. Volatile compounds as low as C4 are sampled. Two valve systems were introduced for gas sampling. ASDevices presented the S4 Sample Stream Selection System. Two, four, six, and eight inlet versions are available, with a proprietary purge leap sealing valve to preserve sample integrity by eliminating contamination by ambient air. The Restek Air Valve Excellence
(Rave+ ) air diaphragm valve possesses durable, roller-hardened valve port threads and an all-metal seat for long service life. These valves are standard in the Restek line of air sampling canisters.

**Solvent Evaporation:** Two evaporation systems came to the market this year. The Porvair Sciences Ultravap Mistral XT150 is a blow down solvent evaporator capable of drying at temperatures up to 80 °C. Programmable evaporation methods are allowed and 15-cm vials and microplates are accommodated. Biotage presented the TurboVap Dual automated single- and dual-channel well-plate evaporation system. Preheated gas and convection heating speeds the evaporation process and improves consistency. Energy and gas consumption are monitored.

**Homogenization/Milling:** PreOmics GmbH introduced the BeatBox sample homogenizer for rapid (less than 10 min) and scalable treatment of tissues and cells from 96-well plates.

The Frontier Laboratories IQ Mill-2070 cryogenic mill is a benchtop grinding device that can handle up to three samples at a time. The impact and shear crushing mechanism comes from a high-speed figure eight motion.

**Enrichment:** YMC developed a N-Rich impurity amplification system for the isolation of product-related impurities from active pharmaceutical ingredients via a fully automated process.

**Glassware:** Thermo Scientific assisted analysts with the Sure Start collection of vials, caps, inserts, kits, mats, and well-plates. High-quality materials minimize analyte adsorption, pH shifting, and contamination. Table 3 provides a summary of these sample preparation accessories and related products.

**Conclusion and Future Directions**

Although it seems as if new sample preparation product offerings have slowed since the pandemic, vendors must continue to innovate to address market needs. As the pandemic winds down and conferences and expositions become live events again, awareness of new product developments should rebound. As a result, breakthrough technologies should follow.

### Table 3: Sample preparation accessories and related products

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markes International</td>
<td>Low-volume sampler for short-term radial diffusive sampling of low atmospheric concentrations of C4-C9 pollutants. Compatible with all commercial thermal desorption systems. Developed in collaboration with the European Commission’s Joint Research Centre in Ispra, Italy.</td>
</tr>
<tr>
<td>ASDevices</td>
<td>Manual, automatic, or remote control and a proprietary purge leak sealing valve accommodates a number of gas sampling situations. Avoids sample contamination by atmospheric air.</td>
</tr>
<tr>
<td>Restek</td>
<td>A positive stop prevents over-opening and strengthened threads upgrade the performance of the Restek line of air sampling canisters.</td>
</tr>
<tr>
<td>Porvair Sciences</td>
<td>Programmable blow down evaporation methods up to 80 °C. Designed for 15-cm vials and microplates.</td>
</tr>
<tr>
<td>Biotage</td>
<td>Well-plate evaporator with single- and dual-functionality. Designed to prevent well-to-well cross-contamination. Preheating from the top and convection heating reduces time and gas consumption. 13 × 14-in footprint. Energy and gas use are monitored.</td>
</tr>
<tr>
<td>PreOmics GmbH</td>
<td>Homogenization of tissues and cells in less than 10 min in 96-well plates. Completely shears most DNA with minimal protein release.</td>
</tr>
<tr>
<td>Frontier Laboratories</td>
<td>Benchtop cryogenic mill of up to three samples at a time. Figure-8 impact and shear crushing with less nitrogen consumption.</td>
</tr>
<tr>
<td>YMC</td>
<td>Automated multistep process to isolate impurities from active pharmaceutical ingredients</td>
</tr>
</tbody>
</table>

**ABOUT THE COLUMN EDITOR**

Douglas E. Raynie is Department Head and Associate Professor at South Dakota State University, USA. His research interests include green chemistry, alternative solvents, sample preparation, high-resolution chromatography, and bioprocessing in supercritical fluids. He earned his Ph.D. in 1990 at Brigham Young University under the direction of Milton L. Lee. Raynie is a member of LCGC’s editorial advisory board. Direct correspondence about this column via e-mail to amatheson@mjhlifesciences.com
Q. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) has become ubiquitous for the quantitative determination of small molecules in food and feed samples. However, according to your 2020 paper (1), matrix effects continue to be an issue for those using an LC–MS/MS method—in particular for multi-analyte approaches. What are the issues surrounding matrix effects in this area?

Rudolf Krska: Matrix effects are an issue in various analytical techniques. For LC–MS using both high-resolution (HR)MS and tandem MS, matrix components coeluting with analytes of interest influence the ionization efficiency of the latter. This decreases—and on rare occasions increases—their analytical response in the sample extract compared with the same analyte concentration in a neat solvent standard, and thus compromises the accuracy of the method. The classical approach of dedicated sample clean-up (such as by an immune-affinity clean-up) to remove the matrix components is limited to single target analytes or a group of analytes, although there are approaches involving combining different antibodies (2,3). An example of this would be for the mycotoxins addressed by regulatory limits. The same is true for stable isotope labelled internal standards that compensate losses during ionization and are therefore the preferable option for official control analysis, but they are available for only approximately 20 compounds (4). This leaves the option of matrix matching, which can be defined as preparing the calibration curve in sample extracts instead of solvent-based standards for methods that target a broader range of compounds. However, there are some difficulties in executing this approach, including the lack of samples that are true blanks for all the analytes, or accommodating samples from different matrices in one analytical sequence.

Q. The method you published in your 2020 paper was a “dilute-and-shoot” method and covered more than 500 secondary metabolites, including all mycotoxins addressed by regulatory limits as well as emerging and masked mycotoxins in seven different food matrices. What did the obtained data involving combining different antibodies (2,3). An example of this would be for the mycotoxins addressed by regulatory limits. The same is true for stable isotope labelled internal standards that compensate losses during ionization and are therefore the preferable option for official control analysis, but they are available for only approximately 20 compounds (4). This leaves the option of matrix matching, which can be defined as preparing the calibration curve in sample extracts instead of solvent-based standards for methods that target a broader range of compounds. However, there are some difficulties in executing this approach, including the lack of samples that are true blanks for all the analytes, or accommodating samples from different matrices in one analytical sequence.

Q. The method you published in your 2020 paper was a “dilute-and-shoot” method and covered more than 500 secondary metabolites, including all mycotoxins addressed by regulatory limits as well as emerging and masked mycotoxins in seven different food matrices. What did the obtained data
indicate about the applicability and practicability of the current guidelines for method validation on such a broad method?

Michael Sulyok: Following non-LC–MS-specific guidelines results in a considerable consumption of time for data analysis, particularly for levels close to the estimated limit of detection/limit of quantification (LOD/LOQ), which requires manual inspection of each peak. As it took close to two years to evaluate and compile all data for our 2020 publication, it was our aim to come up with a proposal to reduce this burden without compromising the overall assessment of the method performance. We believe that spiking experiments at low concentration levels are not essential as we found recoveries to be independent of the concentration level, and also it is feasible to come up with a conservative estimation for LOD/LOQ that is independent of the matrix. Instead, the focus should rather be on absolute and relative matrix effects, which can be studied at high concentrations that result in peaks that are reliably processed by automatic peak integration. In addition, our data did not support the concept of matrix groups mentioned in some guidelines. Differences in apparent recovery between the individual nut, grain, and dried fruits matrices indicated that matrix effects should be characterized for each new matrix, even if it is similar to another commodity that has already been evaluated.

Q. What conclusions were reached on the current guidelines and what were the recommendations for improvement?

RK: There is a lack of recommendation to what extent matrix effects are acceptable, which is true both for the absolute extent, namely the difference in signal intensity to the identical concentration of a given analyte in neat solvent, as well as for the difference in matrix effects between different individual samples, such as different varieties, brands, and so forth of a given matrix, which is known as relative matrix effects. Even extreme absolute matrix effects may be compensated for in principle by matrix matching, but it may be questioned whether a method can be considered valid in such a case. In contrast, the issue of relative matrix effects cannot be overcome by matrix matching and is neglected in many publications focusing on method validation. This may be partially explained by the fact that many guidelines foresee determination of repeatability on technical replicates, such as “identical test items” or “18 aliquots of a blank material”, which implies to some extent that any individual sample is representative for all samples of the same matrix. In our work, we emphasize that different individual samples should be included in the validation set.

Q. A further paper published in 2020 presents a comprehensive quantitative LC–MS/MS approach for the analysis of agrocontaminants in animal feeds. What challenges exist in the analysis of animal feeds and why are they important?

MS: Animal feed is particularly challenging because the fractions of the main ingredients vary to a large extent, thus emphasizing the issue of relative matrix effects. In addition, finding samples that are true blanks for all analytes of interest is close to impossible, which makes it difficult to find a suitable sample set for method validation. We have proposed to prepare model samples from the related single feed ingredients mirroring the common ranges of the exact composition.

Q. The paper mentions the construction of a prevalence database. What is the function of this database?

RK: The database intends to link occurrences of mycotoxins and fungal metabolites with complementary data, such as origin, weather, or agricultural practice. This serves to identify particular hot spots of mycotoxin contamination in the global supply chain of feed ingredients and finished feed, and also to monitor changes in the pattern of mycotoxins/fungal metabolites on a larger time scale due to global warming.

Q. Did you encounter any challenges in carrying out this research with regards to the instruments and methodologies available?

MS: In our multi-class paper, we explored the limits of data acquisition in tandem mass spectrometry, which is strictly consecutive, meaning that for a given time period (dwell time) on the scale of tens of milliseconds, only one MS/MS signal of one given analyte is scanned. This runs into the problem of not having to sequentially scan all signals for the analytes of interest.
sufficient acquisition time in the case of hundreds of analytes, even if the “scheduled multiple reaction monitoring mode” is applied, namely scanning a particular analyte only within a pre-defined period at the expected LC retention time. We have shown that reducing the number of data points per peak while keeping the dwell time as high as possible results in an acceptable data quality. However, we think our method is close to the limit of what is feasible in terms of number of analytes—if an even faster scanning technology is not feasible in tandem mass spectrometry, methods comprising even more analytes need to be transferred to HRMS in full-scan mode.

Q. Does the method have applications in other areas of research?

RK: Our multi-class paper has proven that our “dilute-and-shoot” approach is not limited to mycotoxins, although other contaminants might pose problems, such as the solubility and stability issues of certain sub-classes of veterinary drugs. We believe that our considerations on method validation for such broad methods can be extrapolated to LC-MS-based methods dealing with other contaminant classes and matrices.

Q. What are you currently working on?

MS: Our current work in the area of LC-MS/MS method development focuses on various aspects. We are investing a lot of time and effort in the validation of a further extended multi-class method, including some toxic metabolites such as byssochlamic acid, in processed grain-based products. Considering the plethora of secondary metabolites of plants and fungi present in food crops, even the 1250+ method does not suffice. We are also studying the metabolization of mycotoxins by insects and plants by utilizing a stable isotope-assisted LC–HRMS metabolomics-based approach in a functional genomics context.

References
3) V.M.T. Lattanzio et al., J. Chromatogr. A 1354, 139–143 https://doi.org/10.1016/j.chroma.2014.05.069
4) www.romerlabs.com/shop/inter_en/reference-materials/biopure-standards/mycotoxins
**Head Space Sampler**

2t sampler is a portable manual system for static head space sampling and is suitable for quantitative analysis. According to the company, the sampler does not block any injection port and can be used with any GC instrument in a economical and easy way.

www.teknokroma.es/en  
Teknokroma Analítica S.A. Barcelona, Spain.

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**Method Development Kits**

Avantor ACE Method Development Kits offer carefully selected column chemistries that are grouped to offer combinations of different retention mechanisms to maximize selectivity, with options including Avantor ACE novel phases, such as C18-PFP and C18-AR. Kits available cover reversed-phase, HILIC, and bioanalysis, with a choice of column lengths and internal diameters.

www.vwr.com/ace  
Avantor, Radnor, USA.

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**Chiral Phase**

Daicel’s immobilized chiral selector, ChiralPak IK, builds on the ChiralPak IG, an effective chiral chromatography phase used in life science laboratories. ChiralPak IK uses the same chiral derivative as ChiralPak IG but with a cellulose polymer, offering the opportunity to re-evaluate and discover new chiral separations not previously separated on other polysaccharide-based phases. Available in 3 and 5 micrometers.

www.chiraltech.com  
Daicel Chiral Technologies Europe, France.

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**Nitrogen Generator**

The VICI DBS HP Tower Nitrogen Generator produces a 24/7 on-demand supply of high-purity nitrogen with flow rates from 500 to 4000 mL/min, purity up to 99.999% and less than 0.1 ppm, and THC pressure up to 5 bar. The generator can be placed close to the instrument, which eliminates the need for long gas lines from external cylinder supplies.

www.vici-dbs.com  
VICI AG International, Schenkon, Switzerland.

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**UHPLC–MS Columns**

1 mm YMC-Triart UHPLC high-performance columns are designed for UHPLC–MS applications. According to the company, they provide high sensitivities with very low sample amounts/concentrations, while the high precision column hardware ensures reliable and reproducible results. These columns are dedicated for labs that routinely use LC–MS.

https://ymc.eu/d/brDmC  
YMC Europe GmbH, Dinslaken, Germany.
**Sample Preparation**

Nexera Prep is the ideal tool for preparative fractionation of complex sample mixtures, according to the company. Separation methods developed in analytical-scale can be easily scaled up using the integrated method transfer software, to allow for seamless preparation of high-purity substances. Fraction purity can then be confirmed by re-injection of the collected sample and re-analysis on the same system.

https://www.shimadzu.eu/better-prep

Shimadzu Europa GmbH, Duisburg, Germany.

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**Deep Well Plate**

The KF deep well plate and tip comb plate combo from Porvair Sciences has been shown to improve the yield and quality of the isolated protein or nucleic acid when used on the Thermo Scientific KingFisher range of nucleic acid purification systems. Manufactured in a cleanroom production environment using ultrapure polypropylene that has the lowest leachables, extractables, and is free from DNase and RNase allows SARS-CoV-2 test samples to be purified with confidence.

www.microplates.com/draft/kingfisher-compatible-96-well-microplate/
Porvair Sciences Ltd, Wrexham, UK.

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**High-Performance Pulse Dampers**

Baseline fluctuation is a thing of the past. Knauer’s Pulse Dampers provide excellent damping performance whilst remaining simple to integrate into HPLC systems due to their membrane-free assembly, according to the company.

https://www.knauer.net/
Knauer Wissenschaftliche Geräte, Berlin, Germany.

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**EAF4 System**

Postnova’s simultaneous electrical and asymmetrical flow field-flow fractionation (EAF4) system is designed to enhance separation and characterization of biopharmaceutical, environmental, and nanomaterials. In an EAF2000 system, electrical and cross-flow fields are applied simultaneously, enabling separations by particle size and particle charge based on electrophoretic mobility to characterize complex proteins, antibodies, and viruses, as well as environmental and charged nanoparticles or polymers.

www.postnova.com
Postnova Analytics GmbH, Landberg, Germany.

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**LPGC–MS Column Kit**

Multiresidue pesticides can be analyzed by LPGC–MS in a third of the time required for conventional methods, but LPGC–MS can be difficult to implement because manual connections between different tubing diameters are prone to leaks. Restek’s factory-coupled, low-pressure GC column kit makes getting set up as simple as a column change, and ensures consistent, leak-free performance, according to the company.

www.restek.com
Restek, Bellefonte, Pennsylvania, USA.

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**UHPLC Columns**

The new Halo 1.5-mm-i.d. column bridges a gap between analytical and microflow. According to the company, this innovative dimension delivers up to 2× the sensitivity and 50% less solvent consumption than a 2.1 mm i.d. column. Designed for use with UHPLC and LC–MS systems, it can replace current analytical dimensions to provide more performance.

https://halocolumns.com/introducing-the-new-halo-1-5/
Advanced Materials Technology, Delaware, USA.
HISSS 2022 and HPTLC 2022

The 26th International Symposium on Separation Sciences (ISSS 2022) and The 25th International Symposium for High-Performance Thin-Layer Chromatography (HPTLC 2022) will be held from 28 June–1 July 2022 in Ljubljana, Slovenia. ISSS 2022 is organized jointly with HPTLC 2022 to allow scientists to attend both symposia. This will be a special opportunity to join the two symposia, meet more speakers and participants, and gain access to more workshops and content. Several renowned keynote speakers from 23 countries have already confirmed their participation.

ISSS 2022 will cover new advances and challenges in all fields of separation sciences, and includes a scientific programme that will encompass everything from the fundamentals of separation science to metabolomics and biomarker discovery, as well as applications in all fields. A special scientific section will be dedicated to Professors Pavel Jandera and Milan Hutta to memorialize their contributions to the field of separation sciences.

HPTLC 2022 will cover new advances and challenges in the field of high-performance thin-layer chromatography (HPTLC). Scientific contributions will include all areas of HPTLC such as fundamentals, advances in instrumentation, hyphenated techniques, and their applications in all fields. A special scientific section will be dedicated to Professors Rudolf E. Kaiser and Joseph Sherma to memorialize their contributions to the field of HPTLC.

The programme of both symposia will consist of invited lectures, oral and poster presentations, young scientist oral presentations, and workshops. Each symposium will also conduct a Best Poster Award competition that all poster presentations will enter. The presenting author of the winning poster at ISSS 2022 will be awarded with free participation at ISSS 2023. Additionally, in recognition of important contributions to the development of separation science, the Central European Group for Separation Sciences Awards will be presented at ISSS 2022. The events will also feature a comprehensive exhibition of key sponsors and exhibitors.

For the first time all participants will have the opportunity to take part in a special creative competition called CHROM-ART. Interesting HPTLC plate images, chromatograms of unusual shapes, and other images created during chemical analysis will be presented in a CHROM-ART gallery from an artistic point of view. One creative CHROM-ARTWORK will receive the CHROM-ART Award.

The series of ISSS and HPTLC symposia have historically been an excellent means for young researchers to expand their knowledge about state-of-the-art methods and approaches used in separation sciences, as well as build vital social connections. An attractive and rich social programme will create the perfect atmosphere to connect with old acquaintances and build long-lasting friendships that may blossom into fruitful joint adventures. The organizers hope to inspire future research, innovation, and scientific collaboration, as well as offer participants a taste of Slovenia’s natural beauty, intriguing history, culture, and cuisine.

The organizers invite researchers from all relevant areas to submit abstracts to both ISSS 2022 and HPTLC 2022.

For more information and registration, visit the ISSS 2022 and HPTLC 2022 webpage at: https://issss2020.si & https://hptlc2020.si • E-mail: info@issss2020.si & info@hptlc2020.si
The Chromatographic Society (ChromSoc) has announced the winners of their 2022 Martin Medal and Jubilee Medals as Luigi Mondello, University of Messina, Italy, Sebastiaan Eeltink, Free University of Brussels, Belgium, and Martin Gilar, Waters Corporation, Massachusetts, USA, respectively.

“...the Chromatographic Society is delighted to be able to acknowledge the work of these eminent scientists and the impact they are having in the field of separation science!” said Tony Edge, President of ChromSoc.

The Martin Medal is named after Professor A.J.P. Martin who, together with Richard Synge, received the Nobel Prize for Chemistry in 1952 for their seminal work on partition chromatography. The award represents the highest honour that the Chromatographic Society confers. The award has been made in recognition of Luigi Mondello’s enormous contribution to high-resolution chromatography techniques, as well as the development of hyphenated and multidimensional “comprehensive” techniques, applying them to the study of naturally complex matrices. He is the author of over 500 publications and the editor/co-editor of many prominent chromatography books. His work in the organization of chromatographic symposia is also notable, with the International Symposium on Capillary Chromatography (ISCC) being inextricably linked to his name. He has also been involved with numerous other symposia including many in South America. Indeed, Mondello’s commitment to sharing his scientific knowledge has led to him amassing a total of 841 conference presentations, and guiding many students and collaborators towards positions of seniority within the field.

Luigi Mondello was proud to be awarded the Martin Medal and told LCGC Europe: “I feel immense pleasure and pride to get this honour from the prestigious The Chromatographic Society. The seminal work conducted by Professor Martin has profoundly inspired my work since the early stages of my career, and this award represents a mark of acknowledgement bestowed for my efforts in the field of high-resolution and multidimensional chromatography. This achievement will further motivate my future work and is also a chance to preface my feelings of gratitude to those who supported me through this journey.”

The Jubilee Medal was created in 1982 to mark the 25th anniversary of The Chromatographic Society, with the intention of recognizing up-and-coming separation scientists who have made major use of separation science in their own field or important contributions to a particular area of separations science. This year the society has decided to announce two winners.

Sebastiaan Eeltink was awarded the medal in recognition of his ability to apply his theoretical knowledge to the design and manufacture of highly novel technology. His laboratory focuses on the design, development, and application of modern analytics for high-resolution profiling of biomolecules, which has led to three patent applications for spatial three-dimensional liquid chromatography and more than 100 scientific publications on which he is first or senior author.”

Eeltink also has a strong academic record and currently holds a position at the Department of Chemical Engineering at the Free University of Brussels, where he teaches courses in the field of separation sciences.

Upon receiving the award, Eeltink praised his colleagues stating: “This award is in fact a recognition for teamwork driven by many postdocs and PhD students working in my team since 2010.”

Martin Gilar was awarded the medal in recognition of his ability to drive cutting-edge research in the field of separation science. The award committee further recognized his passion for supporting the next generation of scientists, noting that he can often be seen at conferences running tutorials, or, most recently, being actively involved in the organization of events, such as the recent HPLC 2018 Symposia held in Washington, D.C, USA. Gilar is a scientific fellow of the separations R&D group within Waters Corporation, where he has worked for the past 23 years. Over this time, he has published hundreds of papers in a wide variety of separation science fields, which has led to him being attributed to 30 patents. The industrial environment in which he operates gives his research a very practical edge.

“It is an honour to receive the Jubilee Medal from the Chromatographic Society and become part of the community of distinguished researchers who have received this award. For me, chromatography has always been an exciting science and I get as much fun out of solving separation problems as I do in solving brain teasers, riddles, and other logic puzzles,” said Gilar.
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