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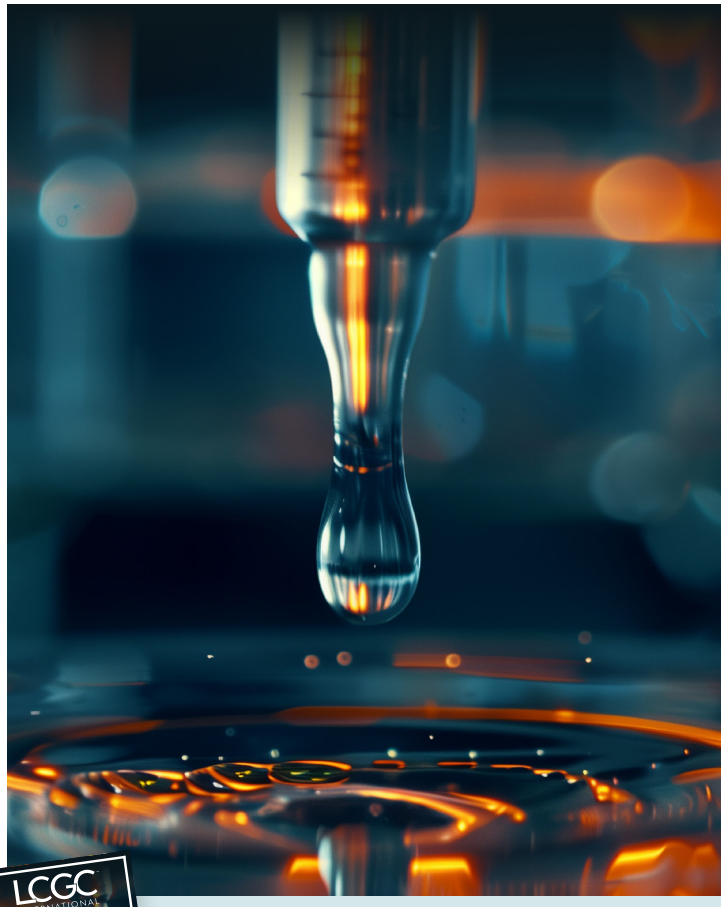

ON THE COVER

A single drop of liquid leaving a pipette tip during sample preparation.

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Around the World with The Multidimensional Chromatography Workshop

Dwight Stoll talks with Katelynn Perrault Uptmor, Pierre-Hugues Stefanuto, and Petr Vozka about the multidimensional chromatography workshop, better known as the MDCW for short.

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NOTE FROM THE CEO

WELCOME TO THE May 2024 issue of *LCGC International*!

This month, we're packed with five articles showcasing the latest advancements in LC columns and accessories, new sample preparation products, sample preparation techniques, and the latest in GC and GC–MS technology.

Kicking off this issue is the "LC Troubleshooting" column by Dwight Stoll and James Grinias titled "Treat it Like a Circuit, Part II: Applications of the Electronic Circuit Analogy to Troubleshooting Problems in LC Systems." In this article, the authors continue their exploration of the electronic circuit analogy, demonstrating how it can be used to troubleshoot common problems in LC systems. It's a practical piece with real-world applications for anyone involved in liquid chromatography.

Next, we dive into the "GC Connections" column with Nicholas Snow's article "Perspectives on the Perspectives, Part II: Impact of Sample Preparation Techniques on GC." This follow-up to his previous installment explores major trends in sample preparation based on a 2023 user survey by *LCGC Magazine*. Snow discusses common techniques like filtration and centrifugation and highlights newer trends that emphasize automation and green analysis.

Continuing with our annual reviews, this month's "Column Watch" focuses on new LC columns and accessories, and our "Sample Prep Perspectives" article features new sample prep products and accessories.. These pieces offer comprehensive overviews of the latest liquid chromatography columns and accessories, and sample preparation instruments, supplies, and accessories introduced at the 2024 edition of Pittcon and other key industry events over the past year. If you're looking to update your lab with the latest types of products, these are must-read columns.

Finally, our feature article, "Low Phase Ratio Stationary Phase Column Technology for the Characterization of Highly Volatile and Reactive Compounds by Gas-Liquid Chromatography," authored by Tetiana Davydiuk, Ronda Gras, and Jim Luong, explores the latest advancements in GC column technology. The article discusses the benefits of low phase ratio columns and their applications in analyzing highly volatile and reactive compounds, providing insights into contemporary coating techniques and 3D-printed microreactors.

We hope you enjoy this issue and find the content informative and engaging. As always, we welcome your feedback and suggestions for future topics.

Thank you for being part of our *LCGC International* community, and happy reading!

Mike Hennessy, Jr.
President & CEO, MJH Life Sciences®

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Treat It Like a Circuit, Part II: Applications and Troubleshooting

Dwight R. Stoll and James P. Grinias

The analogy that electrons flowing in wires is like water flowing through a tube can be remarkably effective for teaching and learning about fluid flow in LC systems. In this installment, we apply the concepts developed in last month's installment by demonstrating how they can be used to help troubleshoot problems in LC involving pressure and flow. We also introduce several free tools that can be used to calculate pressure drops in different elements of LC systems, including connecting capillaries and packed columns. Knowing what pressure drops to expect for system components under different chromatographic conditions is very valuable in many troubleshooting situations.

IN LAST MONTH'S INSTALLMENT of "LC Troubleshooting," we discussed the conceptual similarities between the flow of current in electrical systems and the flow of fluids in LC systems. We also discussed several basic principles and laws used to explain the behaviors of electrical systems, with the promise that similar relationships can be used to rationalize observations related to flow and pressure in LC systems, particularly as it relates to troubleshooting problems related to flow and pressure. In this installment, we continue this discussion, using several examples to illustrate how the principles discussed last month can be used to systematically explain the behaviors of flow and pressure in situations of great practical value. These include diagnosing situations where the measured pressure in a LC system appears to be too high or too low and setting up a passive flow split at different points in the flow path from pump to detector. As part of these discussions, we will also demonstrate the utility of freely available calculators that have been developed for the purpose of calculating pressure drops in LC systems.

Review of Concepts From Part I

Here, we reiterate the essential elements of the principles and relationships discussed in Part I for convenience. However, we strongly encourage readers who are unfamiliar with them and have not read Part I to pause and read through Part I before attempting to work through the examples in this installment. A major point of this two-part series is to provide the framework and principles needed to approach flow and pressure problems systematically; this is not possible without a firm understanding of the fundamental principles discussed in Part I.

In electrical systems, current flows through resistive elements (that is, wires and other electrical components that have some intrinsic resistance to the flow of electrons through them) as a result of the voltage drop across those elements. The analogous concept in fluidic systems is that fluid flows through resistive elements (that is, tubes and other fluidic components that have some intrinsic resistance to the flow of fluid through them) as a result of pressure drops across those elements. Two foundational tools used in the analysis of electrical systems are Kirchhoff's Voltage and Current Laws. The Voltage Law asserts that the sum of the voltage rises and voltage drops encountered in a loop of the circuit must be zero. The Current Law asserts that the sum of all currents flowing into a junction point in a circuit must be equal to the sum of all currents flowing out of that same junction; this is an expression of the idea that charge must be conserved in any closed system. We can apply the concepts underlying these laws to fluidic systems as well. In that case, we would say that the sum of all the pressure rises and all the pressure drops encountered in a loop in the system must be zero. Subsequently, we would say that the sum of all the flow rates of streams entering a junction point in the system must be exactly equal to the flow rates of all the streams exiting that same point; there is a conservation concept at work here as well, and that is the conservation of mass. In the next sections, we explicitly show how these ideas can be employed systematically to address practical aspects of LC systems.

Using the Concepts to Troubleshoot Common Problems Related to Flow and Pressure

Pressure That Appears to Be Too High

There are many potential causes of pressure that appears to be too high. The list of these causes, and the steps to investigate them, is well established (1). However, the major problem in troubleshooting practice is that the number of *places* where there

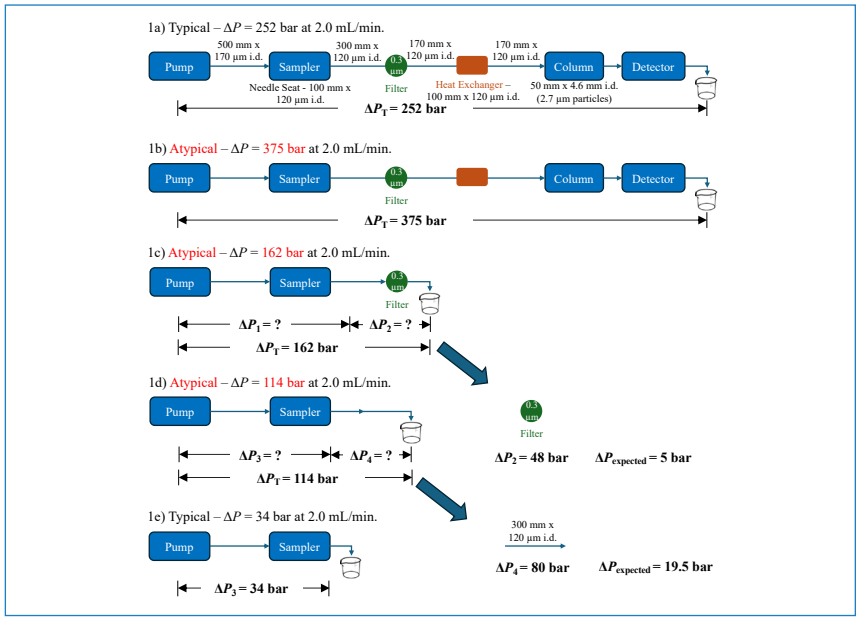

FIGURE 1: Flow diagrams for the flow path in a simple LC system at several stages of the process of troubleshooting an apparent high pressure problem. (a) Complete illustration of all the resistive elements in the flow path. (b) Pressure measured at the pump indicates a pressure that is 123 bar higher than normal. (c) The total pressure drop decreases by 213 bar when all elements after the inline filter are removed from the flow path. (d) Removing the inline filter further decreases the pressure drop by 48 bar, which is 43 bar higher than the pressure drop expected for the filter itself. (e) Removing the capillary that normally connects the sampler to the filter further reduces the pressure drop by 80 bar, which is 60 bar higher than the expected pressure drop for the capillary itself.

can be a pressure problem is also large, and identifying which is the important one, or whether there are actually multiple problems, can take some time. This is mainly because most LC systems only have one pressure sensor near the pump, which is almost always upstream from the sampler.

Figure 1a shows a schematic of a typical flow path for an LC system. The expected total pressure drop for this flow path, including the column, is 252 bar when the flow rate is 2.0 mL/min (see the next section for discussions about where this expectation comes from and free tools for calculating the pressure drops). In this case, for simplicity of demonstration, the pressure drop is calculated for a mobile phase with a viscosity of 1 cP, which is close to the viscosity of water at room temperature. Now, suppose that we encounter a situation where the pressure measured at the pump is 375 bar–123 bar higher than normal, as shown in Figure 1b. For example, this deviation is far more than we would expect to result from small changes in laboratory temperature. Again, the trouble at this point is that we don't know which of the resistive elements in the flow path is causing the higher-than-expected pressure because we only have one pressure measurement—the total drop across the entire flow path. So, we need to break down the problem, and the easiest way to do this is to remove one or more elements of the flow path and record the new pressure. Figure 1c shows that when we remove all the elements downstream from the inline filter (see reference [2] to learn more about inline filters), the pressure drops by 213 bar to 162 bar, but this alone does not tell us much about specific elements. However, if we then remove only the inline filter, we obtain a critically important piece of information. Figure 1d shows that the pressure drops by 48 bar when the filter is removed; now, we can apply the fluidic equivalent of Kirchhoff's Voltage Law, which we discussed in last month's installment. That relationship tells us that pressure drops ΔP_1 and ΔP_2 add to give the total pressure drop ΔP_T, and thus $\Delta P_2 = \Delta P_T - \Delta P_1 = 48$ bar. Now, from experience, we know that the pressure drop across this inline filter is approximately 5 bar when it is new and not at all occluded. So, the fact that the observed pressure drop across the filter is 48 bar means that the filter is partially occluded and should be replaced. We could try backflushing the filter to remove debris and decrease the pressure drop; however, in our experience, this is usually only partially successful, and usually a very short-term fix. It is far more effective to simply replace the filter.

At this point, the total pressure drop from the pump to the outlet of the capillary connecting the sampler to the inline filter is 114 bar (Figure 1d). This still seems like too much, which we might know either from experience at looking at the elements involved, reviewing prior measurements made using this system, or from calculations of the pressure drops expected for individual elements (see the next section for discussion of calculated pressure drops). In any case, we can continue checking individual elements by removing them from the system one at a time and recording the new pressure. In Figure 1e, we see that when the 300 mm x 120 µm i.d. capillary is removed from the outlet of the sampler, the pressure drops by another 80 bar. We know from our favorite calculator tool that the expected pressure drop is just 19.5 bar–60 bar lower than what we have observed. Again, we could try backflushing the capillary to recover it, but this is likely to be only partially successful, and it is usually better to simply replace the capillary.

If we are interested in a comprehensive assessment of all the elements of the flow path, we would need to record the pressure drops observed after removing each element one at a time, starting with the detector and moving all the way back to the pump. In practice, we observe that most problems with high pressure because of occlusion of elements in the flow path occur between the sampler and the column inlet. Problems can occur between the pump and sampler, and downstream from the column, but these are less common because the column itself acts like a filter, and the mobile phase leaving it generally carries less debris than the mobile phase entering it.

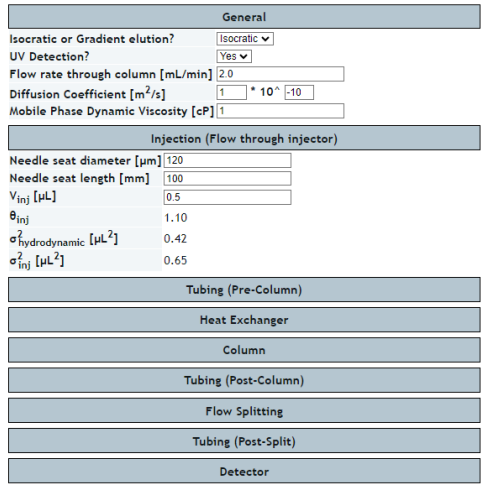

FIGURE 2: Screenshot of the input array available to users in the "Dispersion Calculator" (https://www.multidlc.org/dispersion_calculator/).

Pressure Drop	
Needle Seat Capillary [bar]	8.1
Pre-Column Tubing [bar]	171.7
Heat Exchanger [bar]	107.3
Post-Column Tubing [bar]	171.7
Post-Split Tubing [bar]	42.9
Total Tubing Pressure Drop [bar]	501.7

FIGURE 3: Screenshot of the pressure drop outputs provided by the Dispersion Calculator.

Pressure That Appears to Be Too Low

Pressures that appear to be too low almost always indicate a leak somewhere downstream from the pressure sensor in the pump. One way of thinking about this is that the leak is an unintended flow split (see the section below and Figure 4 regarding passive flow splitting). If the flow rate through the split path exiting the split junction is not zero, then the flow rate through the main path to the detector must be less than the total flow entering the split junction. The lower flow in the main path will lead to a lower-than-expected pressure drop across that part of the flow path and a lower overall pressure drop measured at the pump.

Free Calculators That Support the Use of the Concepts

Capillary Flow and Mass Rate Calculator (Pacific Northwest National Laboratory)

The functionality for calculating pressure drops across both open tubes (that is, connecting capillaries used in LC) and packed columns is available in the Molecular Weight Calculator package (developed using Visual Basic, and downloadable for location execution) developed by researchers at the Pacific Northwest National Laboratory (PNNL–https://pnnl-comp-mass-spec.github.io/Molecular-Weight-Calculator-VB6/). The open tubular column feature is most accurate and can provide the estimated pressure drop for a given capillary inner diameter and length, in addition to the flow rate through the tube. Keep in mind that the actual diameters of manufactured tubes can be slightly different from the advertised diameter. This variation becomes more important as the diameter becomes very small (for example, below 75 µm i.d.). The packed column feature gives estimates for pressure drop at a given flow rate, column dimensions, particle diameter, and interstitial porosity (that is, the fraction of the column volume that is the space between the particles) using the Kozeny-Carman and Darcy equations (3). The column dead time and volume calculations are designed for columns packed with nonporous particles, which is atypical for most commercial columns, and thus, not as accurate for

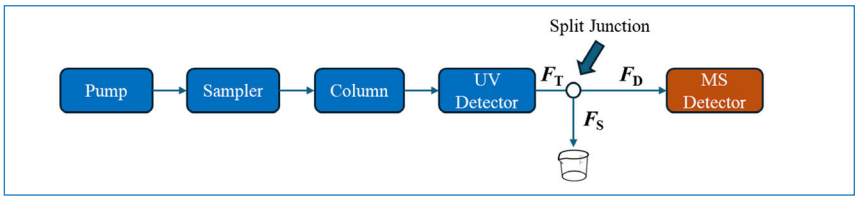

FIGURE 4: Simple schematic of a flow path commonly used for LC–MS, highlighting important details related to passively splitting the flow prior to the MS detector.

columns packed with porous particles. The most important upside of this calculator is that estimates for both connecting capillaries (that is, "open tubes") and packed columns can be made using this one easy-to-use tool.

Web-Based Dispersion and Pressure Drop Calculator (Stoll Laboratory)

The web-based tool developed by the Stoll Group mainly for the purpose of dispersion calculations (https://www.multidlc.org/dispersion_calculator/; [4–7]) also provides pressure drop calculations for open tubes. Figures 2 and 3 show screenshots of the inputs available to the user, and the pressure drops for each element of the system provided as outputs. The major upside of this tool is that pressure drops can be calculated for multiple elements at one time, and the output provides a kind of system-level view for both dispersion and pressure drop. This tool was used to calculate all the pressure drops across connecting capillaries shown in Figure 1 and discussed in the previous section.

Web-Based HPLC Simulator (Stoll Laboratory)

The web-based HPLC simulator maintained by the Stoll Group (https://www.multidlc.org/hplcsim/; see [8] for detail about how it works) also provides a means to calculate pressure drops across packed columns. An upside of this tool relative to the others already mentioned here is that the intraparticle porosity can also be specified, which affects the column dead volume estimate but not the pressure drop. The simulator calculates the mobile phase viscosity, which is needed for the pressure drop calculation, based on the temperature and mobile phase composition (that is, the organic solvent-to-water ratio) inputs, which is a convenient feature.

Application of the Concepts to Passive Flow Splitting

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one of the other places where the concepts discussed in last month's installment are often at play in the practice of LC is instances where passive flow splitting is used. By "passive," we mean that the split ratio is not actively controlled by a pump or some other mechanical device. Rather, the split ratio is simply a result of the nature of flow through two open tubes. Passive flow splitting is most commonly used when an LC method running a relatively high flow rate (for example, much higher than 500 µL/min) is coupled with mass spectrometric (MS) detection where a lower (typically lower, but sometimes much lower, than 500 µL/min) flow rate is desirable for the detector.

A simple schematic of an LC–MS system involving passive flow split prior to the MS detector is shown in Figure 4. Here, we refer to the total flow rate of mobile phase exiting the LC system as F_T, and the flow rates through the connection to the MS, and to waste, as F_D and F_S, respectively. Now, usually the outlets of both the capillary carrying the split flow F_S and the outlet of the capillary connecting to the MS are very close to atmospheric pressure, such that the pressure drops from the split junction to these points must be equal, as expressed in equation 1:

$$\Delta P_D = \Delta P_S \quad [1]$$

In each case, the pressure drop is the product of the flow rate and the flow resistance (that is, the fluidic equivalent of Ohm's Law, discussed in Part I of this series):

$$\Delta P_D = F_D \cdot R_D \quad [2]$$

$$\Delta P_S = F_S \cdot R_S \quad [3]$$

However, since the pressure drops are equal, we can relate the ratio of the flow rates to the ratio of the resistances:

$$\frac{F_D}{F_S} = \frac{R_S}{R_D} \quad [4]$$

Now, the details of the resistance of each branch are given by Poiseulle's Law (see Part I for a more detailed discussion):

$$\Delta P = \frac{8 \cdot \eta \cdot L \cdot F}{\pi \cdot r^4} \quad [5]$$

If we pull out the resistance piece, we have:

$$R = \frac{8 \cdot \eta \cdot L}{\pi \cdot r^4} \quad [6]$$

Substituting this back into equation 4, we find that only the length and radius (and therefore, diameter) of the capillaries connecting to the split point determine the ratio of the flow rates through the two branches (assuming that the viscosity of the fluid in the two branches is the same, which is correct in the vast majority of practically relevant uses of flow splitting at the inlet to a detector):

$$\frac{F_D}{F_S} = \frac{\left(\frac{8 \cdot \eta_S \cdot L_S}{\pi \cdot r_S^4}\right)}{\left(\frac{8 \cdot \eta_D \cdot L_D}{\pi \cdot r_D^4}\right)} = \frac{\left(\frac{L_S}{r_S^4}\right)}{\left(\frac{L_D}{r_D^4}\right)} \quad [7]$$

If the same tubing diameter is used for both capillaries, then equation 7 reduces even further to:

$$\frac{F_D}{F_S} = \frac{L_S}{L_D} \quad [8]$$

For example, if we wanted to split a 2 mL/min flow rate coming from the LC such that 500 µL/min goes to the MS and 1.5 mL/min goes to waste, we would simply choose lengths of the same diameter capillary to connect to the MS and to waste such that the ratio is 3:1, as shown in equation 8 (for example, 300 mm and 100 mm).

While this turns out to be beautifully simple, we should be careful. In some prior work (9), we showed that use of long tubes to connect the split junction to the MS can lead to really devastating peak broadening. Thus, in practice, we find a combination of capillaries that varies both in length and diameter while still satisfying the ratio in equation 7, such that we can make the length and volume of the capillary connecting the split junction to the MS as low as practically possible.

Summary

In this installment, we have reviewed the idea that concepts from electronics can be used to help understand the relationship between flow and pressure in LC systems, and then applied those concepts to two commonly encountered situations impacting pressure and flow. When troubleshooting higher-than-expected pressures, the idea that the pressure drops across each element of the flow path add to produce the total pressure drop measured at the pump is essential to a systematic approach to understanding which element of the flow path is producing the problem. We also briefly discussed several free calculation tools designed to calculate pressure drop across both open tubes (that is, connecting capillaries) and packed columns. These tools are very helpful in that they can help us understand what pressure drops to expect for a given set of conditions, and these expectations can help us discern whether or not there is a pressure problem at all. Finally, we've discussed a systematic approach to setting up a passive flow split that is often used to connect an LC system running at a flow rate that is too high for a MS detector. We hope that these detailed discussions will enable readers to apply the concepts relating flow and pressure to other situations they encounter in their use of LC. ■

Acknowledgements

The Pacific Northwest National Laboratory is acknowledged for developing and hosting the Molecular Weight Calculator and Capillary Flow and Mass Rate Calculator (https://pnnl-comp-mass-spec.github.io/Molecular-Weight-Calculator-VB6/).

This article has additional supplemental information only available online.
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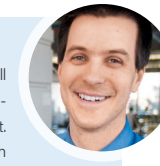

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Evolutions in Particle, Surface Chemistry, and Hardware Designs: New Liquid Chromatography (LC) Columns and Accessories for 2024

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This article covers liquid chromatography (LC) columns and accessories commercially released after Pittcon 2023 through the 2024 conference. As in the past, *LCGC International* sent out a survey in late 2023 and early 2024 asking vendors to supply information on products launched over the course of the year. Note that new products for gas chromatography (GC), LC instrumentation and software, and sample preparation are covered elsewhere. Information for this article is obtained over the course of many months, and thus, it is possible that some information could have been missed or misinterpreted. The reader is encouraged to check with specific vendor sites for additional product releases, 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 I. This year, the new products are classified by the mode of chromatography they are intended for. The largest category can be broadly classified as reversed-phase liquid chromatography (RPLC). Within this category, several subcategories are noted, including unique silica surface modifications, the ongoing trend of utilizing inert hardware, alternative column designs, and columns designed for specific target groups. Following this, and in no particular order, are products intended for other modes of separation, including hydrophilic-interaction liquid chromatography (HILIC), ion-exchange chromatography (IEX), size-exclusion chromatography (SEC), hydrophobic-interaction chromatography (HIC), chiral chromatography, and columns for preparative purposes. Accessories are a vital component to any LC system, and the last section is devoted to "non-column" new products that also contribute to successful liquid separations. It is noted here that in some cases the new products could have been classified in multiple categories.

Reversed-Phase Chromatography

Products aimed at RPLC continue to be developed and commercialized. Innovations in particle design, surface modifications, column format, and hardware all contribute to improvements in performance. These improvements are often coupled to optimize given products for the specific needs of a class of important target analytes, such as oligonucleotides.

Charged Surface

Advanced Materials Technology introduced both the HALO 160Å PCS C18 column and the HALO 90Å PCS C18 reversed-phase chromatography columns. These columns feature superficially porous particles (SPP), with a particle size of 2.7 µm and a pore size of either 160 Å or 90 Å. The uniqueness of the columns stems from the positively charged surface that is bonded with dimethyloctadecylsilane functional groups. Available in various dimensions, the column offers improved peak shape and loading capacity for basic molecules, the larger pore size intended for peptides and similarly sized analytes. The columns have been shown to be particularly beneficial in low ionic strength conditions with formic acid mobile phases. The

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TABLE I: New LC columns and accessories for 2024

COMPANY	PRODUCT	CATEGORY	COMMENTS#
Advanced Materials Technology	HALO 160 Å PCS C18	Reversed-Phase Columns, Charged Surface	SPP, 2.7 µm particles and a pore size of 160 Å. The column offers improved peak shape and loading capacity for basic peptides.
	HALO 90 Å PCS C18	Reversed-Phase Columns, Charged Surface	SPP, 2.7 µm particles and a pore size of 90 Å. The column offers improved peak shape and loading capacity for basic analytes.
Agilent Technologies	AdvanceBio Amide HILIC	HILIC Columns, Glycans	HILIC columns for glycan analysis.
	AdvanceBio Oligonucleotide Columns	Reversed-Phase Columns, Oligonucleotides	SPP with high pH stability and hybrid base material specifically designed for ion-pair reversed-phase analysis of oligonucleotides.
	InfinityLab ZORBAX Eclipse Plus C18	Preparative Columns	Now available in 30 x 150 mm dimension.
	Quick Change HPLC Inline Filters & Filter Discs	Accessories	Inline filters & filter discs suitable for UHPLC Columns up to 1300 bar.
Antec	SweetSep AEX200	Ion-Exchange Columns, Anion	This specialty column enables rapid, high-resolution separations of carbohydrates from mono- to polysaccharides.
ColumnTek LLC	Enantiocel IDC	Chiral Columns	This column can be utilized across normal phase, RP, and SFC, offering unique enantioselectivity.
Fortis	Evosphere BIO	Reversed-Phase Columns, Monodisperse	FPP, monodisperse particles with 300 Å pore size for reversed-phase separation of peptides, proteins and oligos.
	Evosphere BIOMAX	Reversed-Phase Columns, Monodisperse, Inert	FPP, 300 Å pore size monodisperse particles packed in inert hardware.
	Evosphere C12	Reversed-Phase Columns, Monodisperse	FPP, 100 Å pore size modified with C12 ligand for efficient separation of structurally similar compounds.
GL Sciences	ProteoSil 200-C18	Reversed-Phase Columns, Inert	200 Å pore size. Ideal for proteins, peptides, and oligonucleotides separations of intermediate size. Stainless steel and Bio-Inert PEEK hardware.
	ProteoSil 200-C8	Reversed-Phase Columns, Inert	200 Å pore size. Ideal for proteins, peptides, and oligonucleotides separations of intermediate size. Stainless steel and Bio-Inert PEEK hardware.
	ProteoSil 300-C4	Reversed-Phase Columns, Inert	300 Å pore size. Ideal for larger proteins, peptides, and oligonucleotides.
	ProteoSil 300-SEC	Size-Exclusion Chromatography Columns	Dihydroxypropyl modified silica gel for proteins, peptides, monoclonal antibodies, and oligonucleotides. Hardware options include stainless steel and Bio-Inert PEEK.
	ProteoSil HILIC	HILIC Columns	Amide functional group for separating highly hydrophilic compounds, peptides, glycans, and oligonucleotides. Available in stainless steel or Bio-Inert PEEK hardware.
MAC-MOD	MAC-MOD Chiral Columns	Chiral Columns	A series of amylosic and cellulosic chiral phases that provide similar selectivity to industry standard columns as well as provide some unique selectivity.
MilliporeSigma/ Merck	Ascentis Express 90 Å ES-C18	Reversed-Phase Columns	SPP columns recommended for separating cannabinoids, polyphenols, and pesticides, boasting sterically protected ligands that enhance performance under low pH conditions.
Optimize Technologies	OPTI-SOLV Reservoir Filters	Accessories	Provides an economical way to filter particles that may result from buffer salt precipitation, airborne dust, improperly cleaned glassware, or microbial contamination.
Phenomenex	Luna 3 µm Polar Pesticides HPLC Column	HILIC, Multi-Modal Columns	Robust analysis of underivatized polar pesticides in one column with fast column conditioning.

Continued on next page »

company claims that this innovation stands out for its ability to improve the separation of basic compounds without compromising the performance of neutral or acidic compounds. For more information, see https://halocolumns.com/halo-pcs/.

Inert Hardware

Restek Corporation submitted information about three new products that utilize inert hardware to reduce or eliminate undesirable nonspecific interactions with metal surfaces: Force Inert Biphenyl, Raptor Inert Biphenyl, and Raptor Inert ARC-18 reversed-phase columns. The Force columns utilize conventional fully porous particle technology modified with a biphenyl functional group. The columns come in a 3.0-µm particle size and a 100 Å pore size, in multiple dimensions. The Force Biphenyl columns are suggested

TABLE I *(CONTINUED)*: New LC columns and accessories for 2024

COMPANY	PRODUCT	CATEGORY	COMMENTS#
Restek Corporation	LC Waste Management Solutions	Accessories	Ensures safe solvent disposal with chemically resistant containers and protective features.
	Force Inert Biphenyl	Reverse-Phase Columns, Inert	Suggested for analyzing mycotoxins, steroid sulfates, and drugs of abuse, offering improved response of metal-sensitive compounds due to inert hardware coating.
	Raptor Inert ARC-18	Reverse-Phase Columns, Inert	SPP boasts a unique feature of low pH (pH 1) stablility, making it particularly suitable for challenging acidic environments.
	Raptor Inert Biphenyl	Reverse-Phase Columns, Inert	Featuring SPP and a unique biphenyl functional group, these columns offer improved responses for metal-sensitive compounds like mycotoxins and phosphorylated steroids.
SIELC Technologies, Inc	BIST A, BIST A+, BIST B, BIST B+	Specialty Charged Columns	Columns developed for Bridge Ion Separation Technique (BIST) for charged molecule separation.
SiliCycle Inc.	C18 Silica Gel Designed for Peptide Purification	Preparative Columns	C18 phase was specifically designed to withstand washing cycles carried out at pH up to 12-13 to help recover full column capacity after repeated injection.
	SiliaSphere PC C18 SMB	Preparative Columns, SMB	Specifically designed for industrial scale simulated moving bed (SMB) and continuous chromatography, providing reproducible purifications for compounds of varying polarity without the backpressure and cost of preparative HPLC.
Thermo Fisher Scientific	µPAC Neo High Throughput	Micro-Pillar Array Columns	Superficially porous pillars for high throughput proteomics: up to 300 samples per day.
	µPAC Neo Low-Load	Micro-Pillar Array Columns	Non-porous pillar base material with a C18 functional group that excels in handling the smallest proteomic sample amounts, enhancing chromatographic performance.
	Hypersil GOLD Peptide Columns	Reversed-Phase Columns	Columns offer superior consistency and are specialized for peptide mapping applications.
	ProPac 3R SCX and SAX HPLC Columns	Ion-Exchange, Anion and Cation	Columns excel in charge variant analysis of therapeutic proteins and fill state analysis of AAV particles, offering unrivaled reproducibility, outstanding resolution, and improved robustness.
Tosoh Bioscience	TSKgel HIC-ADC Butyl	HIC Columns	Hydrophobic interaction chromatography (HIC) columns for analyzing antibody-drug conjugates (ADCs).
Waters Corporation	MaxPeak Premier 3.5 µm HPLC Columns	Reversed-Phase Columns, Hardware	Hardware that reduces unwanted metal adsorption and results in improved peak shapes, increased sensitivity, and faster analysis.
	ACQUITY Premier Glycoprotein BEH Amide Columns	HILIC Columns, Inert	Suitable for released N-glycan analysis and intact glycoprotein or glycopeptide analysis.
	ACQUITY Premier Protein SEC Columns	Size-Exclusion Chromatography Columns, Inert	Designed for size exclusion chromatography (SEC) in protein analysis.
	XBridge Premier GTx BEH SEC 450 Columns	Size-Exclusion Chromatography Columns, Inert	Ideal for aggregate and heterogeneity analysis of viral vectors and small nucleic acids via size-exclusion chromatography.
	Ultra-short ACQUITY Premier Oligonucleotide BEH C18 Columns	Reversed-Phase Columns, Oligonucleotides, Inert	Columns offer ultrafast oligonucleotide separations without sacrificing resolution.
YMC Co.	YMC Accura BioPro IEX QF	Ion-Exchange, Anion, Inert	Anion exchange specialty column designed for applications such as oligonucleotides, antibodies, and LC–MS analyses.
	YMC Accura BioPro IEX SF	Ion-Exchange, Cation, Inert	Designed for cation exchange, the column delivers exceptional benefits including high recoveries without preconditioning, sharp peak shapes, reproducibility, and rapid throughput analyses.

Source: # Comments supplied by vendors

for analyzing mycotoxins, steroid sulfates, and drugs of abuse, offering improved response of metal-sensitive compounds because of the inert hardware coating. The Raptor Inert Biphenyl columns are built on SPP technology with a particle size of 2.7 µm and pore size of 90 Å. The surface is modified with a unique biphenyl functional group. These columns are stated to offer improved responses for metal-sensitive compounds like mycotoxins and phosphorylated steroids. The Raptor Inert ARC-18 columns are constructed on the same SPP technology but carry a sterically protected C18 functional group. The company claims the columns are stable at low pH, making them particularly suitable for challenging acidic envi-

ronments. The suggested applications include organophosphorus and acidic pesticides. In each case, the inert hardware coating is stated to ensure accurate analysis of metal-sensitive compounds. For more details see https://www.restek.com/enews/view/?id=73967.

Waters Corporation provided information on its MaxPeak Premier 3.5 µm HPLC Columns. These columns, using Waters' MaxPeak HPS Technology for mitigating non-specific adsorption (NSA), feature multiple phases and are available in various dimensions. The company claims the inert hardware technology offers significant benefits, such as improving peak shape and reproducibility, as well as eliminating analyte loss. With suggested applications (including method development and quality assurance–quality control), the columns offer scalability and compatibility with any HPLC system. More details can be found on the product webpage (https://www.waters.com/tothemax).

Micro-Pillar Array

Thermo Fisher Scientific continued to expand its product line of micro-pillar array columns by introducing both the µPAC Neo High Throughput and the µPAC Neo Low-Load reversed-phase chromatography columns. The High Throughput columns exhibit a superficially porous layer modified with a C18 functional group on 3 µm x 75 µm pillars and pore sizes ranging from 100-300 Å. The columns offer improved resolution, robustness, and reproducibility and, as the name indicates, particularly beneficial for high-throughput proteomics applications handling up to 300 samples per day. The µPAC Neo Low-Load reversed-phase chromatography columns feature a non-porous pillar base material with a C18 functional group and a pillar size of 2.5µm x 16µm. The company notes that these columns offer improved resolution, robustness, and reproducibility, and they are particularly suitable for single-cell proteomics applications. For further product details, see thermofisher.com/LowFlowHPLCColumns.

Monodisperse, Fully Porous

Fortis Technologies introduced Evosphere BIO and Evosphere BIOMAX reversed-phase chromatography columns. These columns feature 300 Å, monodisperse, fully porous silica. The columns are available in 5 µm or 3 µm particle sizes, and with C4, C12, and diphenyl surface chemistries. The monodisperse characteristics combined with the high surface area of the particles offer high efficiency and sensitivity, low backpressure, and high loading capacity. The BIOMAX line offers the same features and characteristics as the BIO line, but with the added feature of inert hardware. The suggested applications include proteins, peptides, and oligonucleotides.

Fortis also introduced the Evosphere C12 reversed-phase chromatography column as an extension of its 100 Å, monodisperse, fully porous particles (FPP) line of columns. The new column features an alkyl chain C12 functional group that offers high pH mobile phase resistance and efficient separation of structurally similar compounds. More details can be found at www.fortis-technologies.com. For further insight into the attributes of monodisperse, fully porous particle technology, see a recent "Column Watch" article entitled "The Effect of Particle Monodispersity in HPLC Column Performance (1)."

Oligonucleotides, Proteins, and Peptides

Agilent Technologies introduced the AdvanceBio Oligonucleotide Columns. The columns feature SPP technology with high pH stability because of a hybrid base material. These columns, with particle sizes of 2.7 µm and 4 µm, boast a pore size of 100 Å and various dimensions ranging from 2.1 mm to 21.2 mm i.d. Specifically designed for ion-pair, reversed-phase analysis of oligonucleotides, the columns provide high resolution separation and are particularly beneficial for distinguishing full-length oligonucleotide products from closely related sequence impurities. Notably, the columns facilitate analytical characterization, semi-preparative, and preparative purification processes relating to oligonucleotides. More information on the product can be found at https://www.agilent.com/en/product/advancebio-oligonucleotide-columns.

GL Sciences provided information on its new ProteoSil 200-C18 and ProteoSil 200-C8 reversed-phase chromatography columns. With particle sizes ranging from 1.9 to 5 µm and internal column dimensions of 2.1 mm and 4.6 mm, these columns are said to be ideal for mid-sized protein, peptide, and oligonucleotide separations. The company notes that the unique 200 Å pore size promotes an "easy-clean" feature ensuring faster analysis over 100 Å based columns. GL also introduced ProteoSil 300-C4, a reversed-phase chromatography column tailored for larger protein and peptide analysis, particularly hydrophobic peptides. The column, with a 5 µm particle size and a 300 Å pore size, offers shorter retention times than many columns, and is suitable for proteins with moderate retention needs. The primary benefits include facilitating analysis and enhancing efficiency compared to existing products. All the columns are available in both stainless steel and Bio-Inert PEEK hardware. For more information, visit https://www.glsciences.com/product/lc_columns/bio_column/02862.html.

Thermo Fisher Scientific submitted information regarding its new Hypersil GOLD Peptide columns. These RPLC columns feature FPP silica with a C18 functional group, 1.9 µm particle size, and 175 Å pore size. Column dimensions range from 2.1 mm x 50 mm to 2.1 mm x 150 mm. The primary benefits include increased retention of hydrophilic peptides and simultaneous separation of deamidated species, ensuring minimal variability and high lot-to-lot consistency crucial for biopharmaceutical development. Notably, these columns offer superior consistency and are specialized for peptide mapping applications. For more information, see https://www.thermofisher.com/order/catalog/product/26002-152130?SID=srch-srp-26002-152130.

Waters reported on its ultra-short ACQUITY Premier Oligonucleotide BEH C18 columns that are designed for RPLC.

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The columns offer ultrafast oligonucleotide separations without sacrificing resolution. They utilize BEH hybrid particle technology for maximum pH and temperature stability, along with MaxPeak HPS High Performance Surface Technology to minimize non-specific adsorption. Available in 130 Å and 300 Å pore sizes and dimensions of 2.1 mm x 20 mm, these columns cater to chromatographers seeking efficient oligonucleotide analysis. For further details on specifications and applications, see https://www.waters.com/nextgen/us/en/shop/columns/186011021-acquity-premier-oligonucleotide-beh-c18-300-a-17-mm-21-x-20-mm-1.html.

Additional RPLC Columns

MilliporeSigma/Merck provided information about the Ascentis Express 90 Å ES-C18 RPLC columns. These columns feature an SPP base material modified with a sterically protected C18 functional group. The columns are offered in various column dimensions and are available with particle sizes of 2.7 µm and 2 µm, and a pore size of 90 Å. The company notes that the columns exhibit improved stability at low pH mobile phase conditions, making them suitable for long-term use with acidic hydrolysis protection. They are recommended for separating cannabinoids, polyphenols, and pesticides. For more information, see https://www.sigmaaldrich.com/US/en/substance/ascentisexpress90esc1827umhplccolumn1234598765.

Hydrophilic Interaction Liquid Chromatography (HILIC)/Multi-Modal

RPLC struggles to retain hydrophilic analytes. For such cases, HILIC and multi-modal modes of chromatography can save the day. Interestingly, this year it is the amide surface chemistry for HILIC that dominates new products in this category.

Agilent Technologies introduced AdvanceBio Amide HILIC columns. These HILIC columns offer increased peak capacity, charge group selectivity, temperature stability, and longevity compared to existing Agilent glycan columns. A notable feature, according to the company, is the unique capability to modulate charge group selectivity by adjusting mobile phase ionic strength. The columns are available in dimensions of 2.1 mm x 100 mm and 2.1 mm x 150 mm, a particle size of 1.8 µm and a pore size of 300 Å. For more details, visit https://www.agilent.com/en/product/biopharma-hplc-analysis/glycan-analysis/glycan-analysis-columns/advancebio-glycan-mapping.

GL Sciences submitted information on its new ProteoSil HILIC chromatography columns. The columns feature amide functional groups, are available in particle sizes of 1.9, 3, and 5 µm, and exhibit a pore size of 100 Å. These columns, available in stainless steel or Bio-Inert PEEK hardware, excel in separating highly hydrophilic compounds, peptides, glycans, and oligonucleotides. Find more details at https://www.glsciences.com/product/lc_columns/bio_column/02862.html.

Waters Corporation provided information about its ACQUITY Premier Glycoprotein BEH Amide columns. The columns are described as a specialty column suitable for released N-glycan analysis and intact glycoprotein or glycopeptide analysis. With a rapid analysis time of under 7 min, the columns offer advantages in large biomolecule analysis, clone identification in bioprocessing, real-time insights in downstream bioprocessing, and drug discovery. These columns are built using inert hardware that minimizes non-specific binding. See https://www.waters.com/nextgen/us/en/shop/columns/186011017-acquity-premier-glycoprotein-beh-amide-300-a-17--m-21-x-20-mm-co.html for additional details.

Multi-Modal Chromatography

Phenomenex submitted information on the Luna 3 µm Polar Pesticides HPLC Column. The column is described as providing robust analysis of underivatized polar pesticides in one column. The product offers fast conditioning, versatile retention for anionic and cationic pesticides, high sample loading, and 100% aqueous and organic stability. The proprietary surface chemistry, built on a 380 m^2/g porous silica, provides a multi-modal retention, according to the company. For more information, see www.phenomenex.com/LunaPolarPesticides.

Ion-Exchange Chromatography

Where analytes differ in their charge state or degree of ionization, ion-exchange is a powerful means of separation, and is an indispensable tool for most practicing chromatographers. Based on the number of new products in this category this year, it is apparent end-users are finding growing utility in applying IEX.

Antec Scientific recently launched its SweetSep AEX200 column designed for high-performance anion-exchange chromatography (HPAEC). This specialty column, with a base material of poly(divinylbenzene-co-ethylvinylbenzene), features a unique agglomerated pellicular resin coated with quaternary amine functionalized nanoparticles, enabling rapid, high-resolution separations of carbohydrates from mono- to polysaccharides. According to the company, what sets this column apart is its ability to operate at significantly lower back pressures, making it compatible with a wide range of chromatography instrumentation, including older models. It boasts features like multi-purpose analysis capability and compatibility with electrochemical and mass spectrometry detection. For more information, see https://antecscientific.com/products/columns/sweetsep/ and a recent article in the Column Watch series about HPAEC (2).

Thermo Fisher Scientific now offers specialty ion-exchange columns known as ProPac 3R SCX and ProPac 3R SAX HPLC columns for strong cation-exchange (SCX) and strong anion-exchange (SAX) modes of operation. These columns, available in various dimensions and featuring sulphonic acid (SCX) and quaternary amine (SAX) functional groups, boast spherical, monodisperse and non-porous particles. The columns excel in charge variant analysis of therapeutic proteins and fill state analysis of AAV particles, offering what the company notes as unrivaled reproducibility, outstanding resolution, and improved robustness. For additional information, visit

https://assets.thermofisher.com/TFS-Assets/CMD/brochures/eb-001998-ccs-ProPac-3R-SCX-SAX-eb001998-na-en.pdf.

YMC introduced two new ion-exchange columns of its own: Accura BioPro IEX QF and Accura BioPro IEX SF. The QF is an anion-exchange specialty column designed for applications such as oligonucleotides, antibodies, and general LC–MS analyses. The SF columns are designed for cation-exchange and are also applicable to antibodies, proteins, and peptides. Both columns boast exceptionally high recoveries without preconditioning, sharp peak shapes, superior reproducibility, and rapid throughput analyses, making them ideal for LC–MS. Both columns also feature inert hardware. For more information, see https://ymc.eu/d/brDpV or https://ymc.eu/bioinert-columns.html.

Bridge Ion Separation Technique (BIST)

SIELC Technologies introduced the Bridge Ion Separation Technique (BIST) and the columns developed to take advantage of the technique, including BIST A, A+, B, and B+. According to the company, BIST involves the adsorption of doubly charged ions onto a surface of the opposite charge in high organic systems. The result is an excess charge from the doubly charged ions that can then interact with oppositely charged target analytes. Since the excess charge from the double layer requires low water in the system to reduce ion solvation, the potential advantage of the technique lies in the ability to switch polarity solely by changing the water level in the mobile phase. There is thus the potential to retain and separate analytes of opposite charge on a single column. The columns noted above are specifically developed for BIST separation, and may present a unique offering in chromatography. More details about the products and the BIST technique can be found at https://sielc.com/wp-content/uploads/2022/05/BIST-Short-05.12.2022.pdf.

Size-Exclusion Chromatography

Size-exclusion chromatography (SEC) has become a crucial tool for the characterization of large molecule therapeutics and for industrial polymer analysis and design. Improvements in the control of particle pore structure, surface chemistry, and column hardware design have all contributed to new products in this important realm of chromatography.

GL Sciences now offers the ProteoSil 300-SEC column for SEC for large molecule analysis and dialysis. The columns feature dihydroxy propyl ligands bonded to 5µm silica, and are available in both stainless steel and Bio-Inert PEEK hardware with dimensions ranging from 2.1 mm to 7.6 mm IDs. The columns are suitable for protein, peptide, monoclonal antibodies, and oligonucleotide applications. For more information, see https://www.glsciences.com/product/lc_columns/bio_column/02862.html.

Waters Corporation launched ACQUITY Premier Protein SEC columns. The columns, designed for SEC of proteins, feature a Bridged Ethylene Hybrid (BEH) surface. The columns are available in a pore size of 250 Å and particle size of 1.7 µm. Notably, the columns deliver fast analyses (< 3 min) for large biomolecules such as monoclonal antibodies (mAbs), facilitating rapid identification of clones in bioprocessing and real-time insights into mAb quality attributes. The key advantages include reduced development time, minimized non-specific binding, and suitability for high throughput screening. For additional information, see https://www.waters.com/nextgen/ie/en/shop/columns/186011018-acquity-premier-protein-sec-column-250-a-17--m-46-x-100-mm-1-pk.html.

Waters Corporation also provided information on its XBridge Premier GTx BEH SEC 450 chromatography columns. These columns are specifically designed for SEC, and are ideal for aggregate and heterogeneity analysis of viral vectors and small nucleic acids. The columns feature a diol bonded to ethylene bridged hybrid substrate, providing higher resolution and low multi-angle light scattering (MALS) noise compared to current products. Available in various dimensions, such as 4.6 mm x 150 mm and 7.8 mm x 300 mm, and 2.5 µm particle size, these columns offer unique benefits, including low binding adsorption hardware and minimal secondary interaction. More information about these columns can be found at https://www.waters.com/nextgen/se/en/products/columns/gtx-columns.

Hydrophobic Interaction Chromatography

Tosoh Bioscience highlighted its new product, TSKgel HIC-ADC Butyl, for hydrophobic-interaction chromatography (HIC) analysis of antibody-drug conjugates (ADCs). The columns feature a 5 µm, non-porous polymeric spherical base material. Noteworthy benefits include superior separation performance, fast analyses (such as the determination of antibody drug ratio in 7 min), and highly reproducible data with long column lifetime. For more information, visit https://www.separations.eu.tosohbioscience.com/products/hplc-columns-uhplc-columns/hydrophobic-interaction/tskgel-hic-adc-butyl.

Chiral Chromatography

ColumnTek continues to add to its line of chiral columns with the new Enantiocel IDC. The new columns boast an immobilized cellulose tris(3,5-dichlorophenyl carbamate) as its functional group, enabling high column efficiency and expanding solvent compatibility over its coated predecessor. According to the company, the columns can be utilized across normal phase, reversed-phase, and supercritical fluid chromatography. For more information, see www.columntek.com.

MAC-MOD Analytical now offers its own line of chiral columns under the brand MAC-MOD Chiral Columns. The new line contains a variety of amylose and cellulose materials, providing diverse selectivity. In some cases, these columns offer similar selectivity to industry standard columns and in other cases provide unique selectivity. For further details, visit https://www.mac-mod.com/brands/mac-mod-chromatography-solutions/mac-mod-chiral/.

Preparative Chromatography

Agilent Technologies added a new dimension of 30 mm x 150 mm to its InfinityLab ZORBAX Eclipse Plus C18 chromatog-

raphy column line. The preparative columns, with a base material of 5 µm silica, are noted for their high pH resistance (from 2.0 to 9.0), making them suitable for moderately high pH applications. The columns offer a unique endcapped phase ensuring superior peak shape for basic compounds alongside a scalability feature from sub-2 µm column dimensions to prep HPLC column dimensions. Further details on the product can be found at https://www.agilent.com/store/productDetail.jsp?catalogId=575150-902&catId=SubCat1ECS_1642312. For an interesting article comparing the use of SPP and FPP particles for preparative efforts, the reader is referred to "Developing a Fast Purification Method for a Natural Product with a Preparative LC Column Packed with Superficially Porous Particles" from a recent *LCGC* supplement (3).

SiliCycle introduced C18 Silica Gel Designed for Peptide Purification that the company notes is ideal for chromatographers aiming for high-quality peptide purification. This RPLC column boasts high pH tolerance and coverage, making it suitable for a range of applications, including the purification of GLP-1 receptor agonists, such as liraglutide and semaglutide. Notably, its proprietary grafting and endcapped stationary phase characteristics ensure longevity and efficiency, even after repeated injections and rigorous washing cycles up to pH 12–13. More details can be found at https://www.silicycle.com/products/c18-silica-gel-designed-for-peptide-purification.

SiliaSphere PC C18 SMB, a RPLC column with high coverage of C18 monomeric functional groups, was also introduced by SiliCycle. This silica-based column, with particle sizes ranging from 200 to 500 µm and a pore size of 100 Å, is specifically designed for industrial scale simulated moving bed (SMB) and continuous chromatography, providing reproducible purifications for compounds of varying polarity without the backpressure and cost of preparative HPLC. For further information, see https://www.silicycle.com/products/siliasphere-pc-c18-smb.

Accessories

Agilent Technologies offers Quick Change HPLC Inline Filters & Filter Discs, suitable for UHPLC columns up to 1300 bar. As particles can enter the system from a variety of sources, inline filters can be used to protect valuable columns by capturing the particles before they cause irreparable damage. The new inline filter design features finger-tight and tool free replacement of filter discs. Multiple dimensions are available. For more information, see https://www.agilent.com/en/product/liquid-chromatography/hplc-supplies-accessories/pump-degasser-supplies-for-hplc/infinitylab-quick-change-inline-filter.

Optimize Technologies recently introduced the OPTI-SOLV Reservoir Filters. The product uses a conical flow path design to prevent air bubbles from being trapped and interfering with analyses. Utilizing reservoir filters is an essential means of filtering particles that may come from buffer salt precipitation, airborne dust, improperly cleaned glassware, or microbial contamination. More information can be found at https://www.optimizetech.com.

Restek Corporation reported on the launch of LC Waste Management Solutions, a series of products for ensuring safe solvent disposal with chemically resistant containers and protective features. The newly released products include safety spouts for decanting or emptying of waste, chemically resistant carboy waste collection containers, and waste funnels with a protective lid and sieve for catching stir bars. Each product prioritizes safety and convenience in laboratory solvent management. For additional information, see https://www.restek.com/search/solvent-waste-management/_/N-236285123?Ns=restekProduct.x_productBadge%7C0.

Conclusions

Developments in particle design, improvements in surface chemistry modification, column hardware advances, and the combination of these technologies continues to produce new products in the realm of liquid chromatography. The largest number of new products can be classified broadly as intended for RPLC. New surface modifications, alternate particle architecture, and further products centered around micro-pillar array formats are highlighted. It is also clear from the abundance of new products launched aiming at HILIC, IEX, SEC, HIC, and chiral chromatography that alternative modes of retention are being demanded and utilized by practicing chromatographers. In addition, columns for preparative purposes and the need for quality accessories continue to be developed and introduced.

There continue to be evolutionary improvements made in all aspects of the column design, from particle to hardware to format. Several of the new products are new combinations of existing technologies, often targeted for a particular area of focus. In many cases this year, new products have been developed that utilize advances in inert hardware, a trend observed consistently over the past several years. ■

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ABOUT COLUMN EDITOR

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Reflecting on the Influence of the Current State of Sample Preparation on GC, Part 2: Techniques

Nicholas H. Snow

Recently, we examined major trends in sample preparation and how they relate to gas chromatography (GC), based on a 2023 user survey by *LCGC International*. In Part 2 of *LCGC*'s survey of sample preparation users, the prevalence of sample preparation techniques, from the most common, such as weighing, to the most complex, such as online extractions, was discussed. In this installment, we examine trends in the use of sample preparation techniques through the lens of instrumental analysis by gas chromatography (GC) and GC–mass spectrometry (GC–MS). We see that, although common and classical techniques, such as filtration and centrifugation, are still generally the most widely used, newer techniques that allow automation, simplification of analysis steps, and green analysis are gaining traction.

HIGH RESOLUTION and sensitivity instrumental techniques, including gas and liquid chromatography (LC), are unable to perform as expected without effective sample preparation. As seen in a recent survey conducted by *LCGC International* and discussed by Raynie, there are myriad techniques available for sample preparation in conjunction with gas chromatography (GC) (1). This survey is a follow-up to previous surveys, and looking at all of them provides interesting perspectives about the evolution of sampling and sample preparation for chromatography (2). Sample preparation techniques for GC range in complexity from simple dilutions and "neat" liquid injections to complex online automated extraction systems. Not surprisingly, the simpler and more fundamental chemical techniques are reported by users are being used the most often. Although the survey includes all areas of chromatography and sample preparation, we can see trends and interests that directly relate to GC.

Users were surveyed first about the instrumental techniques they use. In 2023, GC holds its place along with high performance liquid chromatography (HPLC) dominating the users' responses. HPLC users are now almost evenly split between traditional HPLC and ultrahigh-pressure liquid chromatography (UHPLC). In GC, a new response category for GC with headspace sampling was added in 2023, and a similar number of respondents reported using GC with headspace sampling as GC alone and more than gas chromatography–mass spectrometry (GC–MS). The most recent (and now classical) literature discussing the basics of headspace sampling with GC is the text by Kolb and Ettre, published nearly 20 years ago (3). With so many laboratories performing headspace analysis, managers should consider additional training, and instrument vendors should increase support and knowledge sharing in this area.

Sample Mass and Volume

The data presented on the volume and mass of collected samples and samples following sample preparation show interesting trends. The survey showed that most chromatographers' initial samples have volumes between 0.5–20 mL of liquid or gas samples or initial mass of 0.05–5 g for solid samples. The roughly identical initial volume of liquid or gas samples is not as surprising as it may seem. Initial liquid samples of up to 20 mL are likely to undergo additional sample preparation, such as solid-phase extraction (SPE) or liquid–liquid extraction (LLE), prior to injection, resulting in a lower final sample volume prior to injection. The survey data reflects this as the majority of all liquid samples are prepared to a final volume of 1–2 mL prior to injection.

Gas samples have a much larger volume for the same mass as liquid samples. So, in GC, gas samples are generally directly injected using a sample valve or syringe that accommodates the larger volume.

Solid samples are generally dissolved in a liquid or undergo an extraction procedure prior to gas chromatographic analysis, so it is not surprising that the mass used varies significantly.

Interestingly, for all three initial sample phases, the volume or mass used is generally convenient for the user and is reminiscent of sample volumes used over the decades of my own career. Final sample volumes, most of which are in the 1–2 mL range for liquids, match closely with the classical 2 mL auto-injector vials used for decades in most autosamplers. In GC, there is much room for reducing final sample volumes prior to injection, as the typical injected volume of a liquid sample is only 1 µL. The vast majority of all final samples in the vial for gas chromatography are eventually disposed as waste. Finally, these initial and final sample volumes are easy for users to handle with standard glassware and equipment. With the increasing interest in sustainability and green chemistry, we can expect initial and final sample volumes to decrease over time, but this may require some rethinking of glassware, sample handling, and methods to limit the expected increases in experimental uncertainty that come with smaller sample volumes (4).

Techniques

Turning to the techniques used by chromatographers, we see that classical glassware handling and wet chemical techniques continue to outpace instrumental and automated techniques, in addition to those that require phase changes or alter the chemical nature of the analyte. Raynie notes that the order of the techniques may have shifted, with biological techniques, such as centrifugation and sample cooling, now occupying spots near the top of the most commonly used techniques. Table I shows the techniques discussed in the survey, separated into classical or wet chemical and phase change, and they are listed in the popularity order presented in the survey, with the most popular at the top of the list. With the exception of pressurized fluid extraction, which somehow shows up at second on the overall list, the classical techniques that involve manipulating or transferring a sample rather than a phase change or an instrument are generally more popular; techniques that were introduced more recently or those requiring instrumentation were less popular.

TABLE I: Popularity of sample preparation techniques separated by classical versus instrumental. Listed in order from most to least popular.

Classical	Phase Change/Instrumental
Centrifugation	Pressurized fluid extraction
Cooling	Column chromatography
Filtration	Liquid-liquid extraction
Dilution	Solid-phase extraction
Concentration	Protein precipitation
Internal standard addition	Headspace
Evaporation	Solvent exchange
Weighing	Dialysis
Digestion	Stir-bar sorptive extraction
pH adjustment	Solid-phase microextraction
Drying	Matrix solid-phase dispersion
Grinding/milling	Ultrasound assisted extraction
Sonication	Microwave assisted extraction
Derivatization	Soxhlet extraction
Mixing	Cloud point extraction
Blending	Precipitation
Heating	Purge and trap
Vortexing	Supercritical fluid extraction
Reagent addition	QuEChERS
Lyophilizing/freeze drying	
Beta-glucoronidase removal	
Reconstitution	
Large volume trace enrichment	
Cell disruption	
Phospholipid removal	
Ultrafiltration	

Classical Techniques

Looking more closely at Table I, some interesting trends are seen, with the observation that sample preparation methods for all chromatographic techniques are included, not just those primarily used with GC. As Raynie observes, centrifugation and cooling, which are more commonly used with biological sample analysis, usually with LC-related techniques, were the most popular classical methods in the survey. These are followed by filtration, dilution, concentration, internal standard addition, and weighing, a set of techniques used in most chromatographic methods across the entire field.

The top half of the classical group of methods in Table I reminds us that the

fundamental chemical techniques are still performed the most. Although these may seem simplest as well, they are often rushed, and their impact on overall method performance is underestimated. Mistakes or experimental uncertainty in these techniques can have an outsized impact on method performance, and are one of the first places I look when consulting on method optimization, especially if reproducibility is not satisfactory. These are areas in which it is very common to see analyst-to-analyst variability in technique and performance. In the research community, these techniques are often considered "mature," "fundamental," or "basic," so there is often little emphasis in ensuring correct technique in teaching and training.

Instruments and Phase Changes

Looking at the more instrumental and phase change-related techniques in Table I, we surprisingly see pressurized fluid extraction (PFE) at the top of the list. PFE is usually used to transfer organic contaminants from solid matrices, such as soil, into liquid solvents, using high pressure and often heating (5). The high pressure and temperature force the solvent into small pores in the solid, increasing the surface area exposed to the extraction, and therefore the recovery. High pressure and temperature also make these extractions relatively fast, on the order of 30 min, and the instrumentation is usually automated, with the capability to extract samples in batches. PFE is often considered "green," as using elevated temperature and pressure can allow better extraction kinetics and performance with lower solvent volume and more benign solvents.

Again, among the top five techniques we now see two, column chromatography and protein precipitation, that are more used for purification rather than traditional chromatographic analysis. This is further evidence that the analytical chemistry landscape is changing, with reduced emphasis on small molecule analysis and more on large molecules and biologics, either as a sample matrix or as the analytes themselves.

Classical liquid-liquid extraction (LLE), solid-phase extraction (SPE), and static headspace extraction (SHE) highlight the next several entries. These are all staple sample preparation techniques for GC, and each depends on a phase equilibrium, very similar to chromatography itself, with LLE involving analyte transfer between two immiscible liquid phases, SPE between a liquid and solid phase, and back to a liquid phase, and SHE between a liquid or solid phase and the vapor phase.

Although GC has been automated for decades through autosamplers, the most popular sample preparation techniques are still analyst-intensive and hands-on. There have been many recent advances in automation, instrument operation, and control, yet we still inject using similar syringes to those used fifty years ago, and sample preparation is still generally based on the same techniques in place for decades. Interestingly, as instruments and autosamplers have become more reproducible, this has placed more emphasis on reproducible sample preparation.

As we proceed down the list of phase transfer techniques, we see several that are more instrumental and automated. Over the decades, the route to fully automated sample preparation for GC has been a bumpy one. Automation requires a significant up-front investment, and may not immediately return benefits in reproducibility over manual methods. Furthermore, operation of the automated system itself requires care, maintenance, and troubleshooting, often by a specially trained operator.

Sample preparation methods are becoming more complex and being performed in large batches, as seen in the surveys over time. While there is significant variability, users report about four steps in a typical sample preparation and sample batches commonly ranging over 50 samples. Thinking further about this and the most popular techniques, we see that a typical sample batch can easily include over 200 sample preparation steps, not including standards, blanks, and quality control samples. Again, this points to an ever-increasing need for skilled analysts performing fundamental tasks and processes in analytical laboratories.

Finally, the survey addresses users' perceptions about the needs and future of sample preparation. Not surprisingly, the most common desires were for more green and miniaturized methods. This may prove challenging, as, while the survey indicates satisfaction with the analytical performance of current methods, the most popular current methods, such as LLE and SPE, may not lend themselves easily to miniaturizing or "greening" while maintaining performance including detection limits, reproducibility, ruggedness, and simplicity.

Summary

Recently, *LCGC International* has surveyed subscribers on their use and perceptions of sample preparation for chromatography. While GC and LC share roughly equal proportions of the respondents, we see the biggest increases in use of techniques related to biological analysis, most likely for LC. Classical chemistry techniques, including dilution, weighing, pipetting, glassware handling, SPE, and LLC, continue to dominate chromatographic methods, with further automation and miniaturization on the horizon. Sample preparation continues to be analyst intensive, requiring training and practice. ■

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New Sample Preparation Products and Accessories for 2024

Douglas E. Raynie and Mary Ellen McNally

This yearly report on new products introduced in the preceding year, since March 2023, covers sample preparation instrumentation, supplies, and accessories.

OUR ANNUAL REVIEW of sample preparation products covers the previous year, since March 2023. It is becoming increasingly more difficult to identify new products. The trend of vendors avoiding exhibiting at general analytical conferences in favor of more focused meetings continues, and they are increasing their marketing via social media or other digital means. In late 2023, the *LCGC* staff submitted a survey to vendors of sample preparation products. Responses to this survey are compiled in this review, as are new product introductions during the past 12 months noted via direct mailings, email, and other means. Additionally, vendors at the 2023 Eastern Analytical Symposium and Pittcon 2024 were perused. Themes that emerged during sample preparation product development include headspace sampling, solid-phase extraction (SPE) techniques, and approaches toward greener analysis.

In this review, we will discuss instrumentation for sample preparation, SPE, and sample preparation consumables. Tabular summaries for each product category are provided.

Sample Preparation Instrumentation and Accessories

Perhaps the most extensive new product introduction was an extension of green headspace analysis by Entech Instruments, building off its Sorbent Pen line and vacuum-assisted headspace sampling. These are marketed as Vacuum Assisted Sorbent Extraction (VASE), Flash-VASE, and Full Evaporative Vacuum Extraction (FEVE). Combined with the rest of the Entech family of products, the company claims to optimize recovery of volatile compounds (that is, gas chromatography [GC]-compatible compounds) based on the sample matrix of interest (both liquids and solids) and the volatility range of the compounds of interest. VASE allows extractions from ambient temperatures up to 70 °C under static vacuum conditions that are often at full equilibrium. Vial sizes from 20 to 125 mL are used. Meanwhile, Flash-VASE extends the extraction temperature range up to 280 °C and uses 2–20 mL vials to achieve a more rapid extraction. Both techniques may extract compounds up to a boiling point of 500 °C. This analyte boiling range is extended to 600 °C with the FEVE technique. In FEVE, smaller vials (2–6 mL) are utilized with extracts at 30–40 °C to remove the volatile matrix, followed by extraction at 50–280 °C. These conditions allow FEVE to accommodate volatile liquid matrices with low solid content. Depending on the choice of extraction mode, an analyte volatility range of -50 to 600 °C is attained, greater than that found with other headspace methods, with higher recoveries. However, it should be noted that headspace composition varies with applied temperature and pressure, so analysts must diligently consider the goals of their analysis, for example flavor aromas versus more exhaustive compositional analysis. Getting back to Entech's roots, a SkyCan Autosampler was also introduced for canister sampling in air monitoring studies. Part per trillion detection of airborne compounds may be achieved with 1.4-L canisters.

Continuing with the theme of greener (solventless) thermal desorption and headspace techniques for analysis of volatile and semivolatile organic compounds are new product releases from Markes International and Gerstel. The Markes TT24-7 NRT is introduced for the near real-time determination of airborne chemical warfare agents and toxic industrial chemicals. Two focusing traps in the system work in tandem to create conditions for 100% data capture. Trapping at temperatures as low as -30 °C combine with backflushing and an optimized flow path for quantitative determination of nerve and blister agents and related compounds. The cryogen-free system combines with most standard GC and GC–Mass Spectrometry

(GC–MS) systems for monitoring in remote, mobile, or discreet locations. Gerstel also offers cryogen-free trapping, called Dynamic Focusing, with its thermal desorption unit to provide improved accuracy and precision in the analysis of very volatile organic compounds (VVOCs), volatile organic compounds (VOCs) and semivolatile organic compounds (SVOCs). The trap liner of the Dynamic Focusing system uses a weak sorbent at -10 °C for trapping. VVOCs are very weakly retained to allow focusing as a sharp injection band in subsequent GC. VOCs and SVOCs require heating of the trapping sorbent in the second stage of desorption. The Gerstel PYRO-Core system accommodated sample pyrolysis at temperatures up to 1000 °C. Ramped pyrolysis provides additional chemical information from solid and liquid samples.

A more comprehensive sample preparation workstation is delivered in the ePrep ONE system. The workstation provides most liquid handing functions, including sample aliquoting, addition of diluents, reagents, surrogates, and standards, serial dispensing and dilution, and volume adjustment, as well as magnetic stirring, SPE, and liquid-liquid extraction (LLE). The system handles five to 200 samples with transfer to autosampler racks.

Ancillary sample preparation accessories are also featured in 2023. Two systems address sample comminution. The newest in Fritsch's Pulverisette cutting mills, the Pulverisette 29 Mini Cutting Mill, is designed for fast milling of small sample volumes (up to 100 mL) down to 0.25 to 6 mm. Easy cleaning helps maintain sample integrity for processing of medium-hard, soft, brittle, tough, fibrous, tough-elastic, and temperature-sensitive samples. The PreOmics BeatBox provides tissue homogenization and cell lysis of 1–50 mg samples in a 96-well format. Maximum protein release in ten minutes renders the system compatible with subsequent liquid chromatography–MS (LC–MS) analysis. A BeatBox Bead Remover accessory removes the GYUTO beads for downstream liquid handling.

Table I summaries the newly introduced sample preparation instrumentation and accessories.

Solid-Phase Extraction

Since last year's review of solid-phase extraction products, the largest focus of new developments has been in the direction of preparation techniques for proteomics and biological samples.

Last year, CDS Analytical introduced micropipette tips using the Empore technology. Since then, the product line has expanded significantly, not only offering the pipet tips in 10- and 200-µL sizes but also a 500-µL spin column, a 3-mL cartridge, and a 1.2-mL 96-well plate. These products offer the diversity to handle a wide variety of sample types in terms of volume, quantity, and size. Labeled as the E3 series,

TABLE I: Sample preparation instrumentation and systems.

Vendor	Product Name	Description
Entech International	Vacuum Assisted Sorbent Extraction (VASE)	Combines Sorbent Pen technology with vacuum headspace in a manner which minimizes interference from aerosols. Application of vacuum allows headspace equilibrium to be reached faster.
	Flash-VASE	The proximity of the sample to the collection sorbent more completely recovers a wide volatility range of analytes. Intended for low moisture samples.
	Full Evaporative Vacuum Extraction (FEVE)	Unique configuration of sorbent and vacuum extracts both polar and nonpolar analytes with equal recovery. Complete delivery of extracted compounds to GC or GC–MS.
	SkyCap	Automated sample canister analysis system places canisters on top of GC–MS for improved reliability.
Markes International	TT24-7 NRT	Cryogen-free operation with a twin-trapping configuration allows efficient characterization of hazardous airborne compounds.
Gerstel	Dynamic Focusing	A two-stage trapping and desorption mechanism without valves or transfer lines allows determination of VVOCs, VOCs, and SVOCs with improved quantification and minimal system downtime.
	PYRO-Core	Pulsed sample pyrolysis up to 1000 °C with capacity for 120 samples. Calibrates sample temperature using traceable thermocouples.
ePrep	ONE Sample Preparation Workstation	Increases efficiency and accuracy of sample preparation workflow, allowing reduced cost and lover environmental footprint.
Fritsch	Pulverisette 29 Mini Cutting Mill	Stainless Steel knife mill for laboratory grinding of small volumes of grains, food pellets, seeds, spices, certain plastics, and a range of other samples.
PreOmics GmbH	BeatBox Tissue Homogenizer	Homogenization of milligram quantities of wet tissues with minimal heat induction and cross contamination.

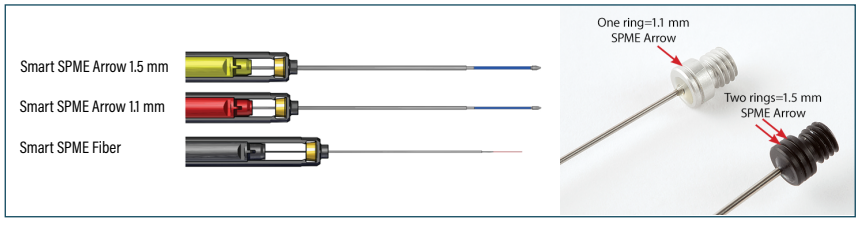

FIGURE 1: Smart SPME Arrows and fibers from Restek with a smart chip which tracks usage history, parameters, and ranges. SPME Smart Arrows are available in two sizes, 1.1 and 1.5 mm, distinguishable by color.

the glass bead-based technology has the capability to do sample preparation in a single vessel, including filter-based sample processing, general shotgun proteomics, clinical proteomics, and biomarker discovery. CDS Analytical also announced a new centrifuge-ready spin column series for proteins and peptide applications, consistent with their other Empore products. They are made of a low-binding polypropylene material and are chemically resistant to organic solvents as well as acid and base conditions. The sorbents are high density C8 and C18 phases, cation and anion exchange phases, and mixed-mode styrene-divinyl benzene phases.

MAC-MOD Analytical launched a new line of SPE products for omics clean-up, LC-UV background improvement, and high-throughput assays, called MemBrain. These products also come in cartridges, extraction tips, disks, and 96-well extraction plates. A touted feature of these products is that there is no silica bed to disrupt. Instead, because these are membranes, uniform distribution of analytes of interest is achieved. The membranes can be mixed and matched to optimize the extraction for a particular application, and the plate format allows for high-throughput screening for unknown mixtures. The choice of bonded phase is broad; beyond reversed-phase and ion-exchange, chelating phases are also available. PreOmics GmbH launched sample preparation kits for proteomic sample preparation for high through-put LC–MS analysis. This new line of products includes ENRICH-iST 8x, ENRICH-iST 96x, and ENRICH-iST 96x HT. These ENRICH-iST kits contain all the core components to perform a complete proteomic sample preparation: protein enrichment, alkylation, reduction, digestion, and peptide clean-up. The enrichment phase of the workflow uses EN-Beads, a paramagnetic bead, to take low-abundant proteins while conserving proteome coverage. The kits are focused on the use of mammalian plasma or serum samples and are optimized for low input, 20-µL samples, large-scale proteomic studies.

Two new SPE products for proteomics and biologicals were announced by Waters Corporation. The first, developed to purify, desalt, and buffer exchange proteins above MW 5000, is designed especially for desalting of denatured, reduced, and alkylated proteins prior to tryptic digestion. These conventional SPE cartridges have a cross-linked dextran base and can be used in a manual or automated workflow stream. The cartridges, part of the well-known and established SEP-Pak family of cartridges, are simply called SEC Desalting Cartridges, are 1 cc in size, and have been quality tested to obtain greater than 80% protein recovery. The second product introduced by Waters is the OligoWorks SPE Microplate Kit. This kit is in a standard 96-well plate format and is a detergent-free proteinase K sample pretreatment system which uses a weak anion exchange extraction phase. The resultant concentrated low-volume eluate produced can be directly injected into an LC–MS.

From a sustainable perspective, CTC Analytics has developed a new PAL system for Micro-SPE cartridges, a miniaturized and robust automated SPE system that uses less sample and less solvent with equivalent performance to conventional-size SPE cartridges. The PAL tool which sits on top of the cartridge consists of a syringe and a needle guide. The cartridge is two parts, the inert polymer material and the sorbent material. The bottom outlet of the cartridge is designed to penetrate pre-slit septa of a GC or deliver directly into an LC injection port. The cartridge can withstand high pressure, up to 10 bar, and prevent leakage around the syringe needle. Currently, it is only designed to inject into the PAL system liquid injection ports. The applications of this can be widespread. However, as of this writing, only QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) and strong anion-exchange phase µSPE cartridges are available.

As an alternative to traditional QuEChERS technology use of dispersive solid-phase extraction (dSPE) clean-up, Biotage advanced the methodology with the use of column solid-phase extraction (cSPE). Compared to QuEChERS clean-up, these cSPE cartridges offer a simplified procedure with fewer manual steps, cleaner extracts, and improved reproducibility along with automated processing options for improved accuracy. Since the QuEChERS method is considered landmark in pesticide residue analysis, it should come to no one's surprise that developments will continue to be made to make it faster and more reliable. These cSPE cartridges are registered under the name ISOLUTE. Also, from Biotage, the Extrahera HV-5000 is a high-volume sample prep workstation, Good Laboratory Practice (GLP)-capable, and positive pressure-driven. It is dedicated to column-based SPE and supported-liquid extraction (SLE) consumable formats, and therefore goes hand in hand with the cSPE for QuEChERS clean-up described above. It caters to large sample volume cartridges of 3, 6, 10, and 15 mL and is capable of dispensing volumes from 250 to 5000 µL (0.25 to 5 mL) through four individual pipet tips. From a sustainability viewpoint, the instrument offers three-channel waste segregation for organic, aqueous, and chlorinated solvents. The instrument supports 12, 24, and 48 sample cartridge positions and has a collection carousel that allows for not only multiple waste, but also multiple elution, positions. Manual refilling can be eliminated with the five automatically refillable 40-mL solvent reservoirs. The applications of this system are broad; beyond QuEChERS, the application of EPA Method 1633 for 40 PFAS (per- and polyfluoroalkyl substances) in various environmental matrices via liquid-solid extraction analysis has also been demonstrated.

Another automated solid phase extraction elution unit, the EL870, has been presented this past year by GL Sciences. This instrument is equipped with a high-precision and reliable syringe pump that improves efficiency and accuracy via automation. It is equipped with a user-friendly key controller and accommodates up to six SPE columns while compatible with most organic solvents. This simple instrument aids in the obtaining high precision analysis in SPE for effective clean-up of biological, food, and

TABLE II: Solid-phase extraction products.

Vendor	Product Name	Description
CDS Analytical	Empore] Micropipette Tips	Expanded offerings to include to a 500-µL spin column, a 3-mL cartridge, and a 1.2-mL 96-well plate.
	Centrifuge Ready spin Column Series	For protein and peptide applications, high density C8 and C18 phases, cation- and anion-exchange phases and mixed-mode styrene divinyl benzene phases.
MAC-MOD Analytical	MemBrain	SPE products made with a membrane instead of a silica bed for Omics clean-up, LC-UV background improvement and high-throughput assays
PreOmics GmbH	ENRICH-iST 8x, ENRICH-iST 96x, ENRICH-iST 96x HT kits	ENRICH-iST kits contain all the core components to perform a complete proteomic sample preparation: protein enrichment, alkylation, reduction, digestion, and peptide clean-up using a paramagnetic bead.
Waters Corporation	SEP-Pak SEC Desalting Cartridges	These cartridges are designed especially for desalting of denatured, reduced, and alkylated proteins prior to tryptic digestion.
	OligoWorks SPE Microplate Kit	The kit is in a 96-well plate format and is a detergent free proteinase K sample pretreatment system.
CTC Analytics	PAL System for Micro-SPE Cartridges	A sustainable alternative, this miniaturized and robust automated SPE system uses less sample and less solvent.
Biotage	ISOLUTE Cartridges	An SPE cartridge offering a simplified procedure with fewer manual steps, cleaner extracts, improved reproducibility for the traditional QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure.
	Extrahera HV-5000	A high-volume sample prep workstation, GLP capable, and positive pressure-driven, dedicated to column-based SPE and SLE consumable formats.
GL Sciences, Inc.	EL870	An automated solid-phase extraction elution unit, using syringe pumps accommodating up to six SPE columns.

TABLE III: Sample preparation accessories and related products.

Vendor	Product Name	Description
Agilent	Viral DNA/RNA Wastewater Prep Kit	Powered by microbubbles from Akadeum Life Sciences, which break down the cell walls using nucleic acid binding. The shell of the microbubbles can be made of polymers, lipids, or proteins, with a gas center.
Waters	Glycoworks RapiFluor-MS N-glycan Eco Labeling Kit	This kit replaces N,N-dimethylformamide (DMF) with dimethyl sulfoxide (DMSO) to label N-glycans.
	PeptideWorks Tryptic Protein Digest Kits	Kits for peptide mapping of biotherapeutic proteins.
Sartorius	Safetyspace Filter Tips	By adding a larger space between the sample and the filter, the risk of sample absorption and cross-contamination can be eliminated with these filter tips.

environmental matrices prior to analysis by GC or LC.

The new product introductions in SPE are summarized in Table II.

Sample Preparation Consumables

Powered by microbubbles, the new Agilent Viral DNA/RNA wastewater prep kit has been created specifically for the purpose of the analysis of viral DNA and RNA in wastewater samples. The functionalized microbubbles break down the cell walls using nucleic acid binding to extract nucleic acids. No pasteurization or filtration is required with the use of the microbubbles, and shorter processing times can be achieved. The microbubbles themselves, provided by Akadeum Life Sciences, can be made of a variety of materials but generally have a shell constructed of polymers, lipids, or proteins, with a gas center. Microbubbles are nanometers, nm, to micrometers, µm, in size, so they are small, simple, and efficient. Using the principles of physics, microbubble sorting relies on buoyancy. The microbubbles allow for gentle transport of extremely small cells or particles. Where other sorting methods can alter or damage cells, microbubbles do not. Advantages of this microbubble technology include increased sensitivity to track a viral load within a community, smaller sample volumes which limit the infectious agents in the laboratory, and quicker turnaround times. The Viral DNA/RNA wastewater prep kit can capture SARS-CoV-2 viral RNA.

A released N-glycan labeling kit was created by Waters called the GlycoWorks RapiFluor-MS N-glycan Eco Labeling Kit. An application note defines the objective of this kit is to replace N,N-dimethylformamide (DMF) with the more environmentally friendly dimethyl sulfoxide (1). This has been suggested by the 2023 European Chemical Agency (ECHA) because of the detrimental effects of DMF on both the analyst and the environment. The report demonstrated a one-to-one equivalence of DMSO for DMF both as a solvent and a co-solvent in a manual and automated procedure of the GlycoWorks RapiFluor-MS N-glycan Eco Labeling Kit.

PeptideWorks Tryptic Protein Digest Kits were also presented by Waters since the last new product review. These kits for peptide mapping of biotherapeutic proteins can be used in both manual and automated procedures, yielding peptide fragments to further characterize specific peptide regions and modifications. They yield high-efficiency, reproducible peptide maps in less than 2.5 h. They can achieve a 78% reduction in missed cleavages as well as a 98% reduction in contaminated autolysis peaks. In addition, the kits aid in maximizing sensitivity while minimizing metal adsorption of acidic deamidated peptides in liquid chromatography with both UV and MS detection.

New from Sartorius are Safetyspace filter tips. Their uniqueness is simply the larger space between the sample and the filter than

conventional filter tips. This space prevents the risk of sample absorption into the filter and avoids cross-contamination between samples and the pipette. Compatible with most commercial pipettes, they are certified to be free of DNase, RNase, and endotoxins. The tips are made of virgin polypropylene and the filters are polyethylene without any self-sealing additives. They are available in 10 to 5,000 µL volumes.

Smart SPME (solid-phase microextraction) Arrows and fibers introduced this past year by Restek offer the same application, performance and longevity of other SPME formats (non-Smart Arrows and conventional fibers) with the added feature of a smart chip which tracks usage history, parameters, and ranges. While not all instruments are compatible with this new Smart SPME technology, they are however backward compatible with any generation of PAL3 systems. The Smart SPME Arrows and fibers require a different manual holder then the holder for non-Smart SPME devices. There are two diameter sizes for the SPME Smart Arrows, 1.1 mm, and 1.5 mm, distinguishable by color, as represented in Figure 1. Table III summarizes these sample preparation accessories.

Conclusions

In our recent survey of the state of sample preparation (2), we noted a strongly increasing trend toward greener approaches to sample preparation. This trend is observed in the new products introduced this year. Thermal desorption and headspace analysis eliminate the need for potentially hazardous solvents, while other improvements minimize solvent use or replace particularly objectionable solvents. When applied to emerging areas in bioanalysis and environmental analysis, we look for future sample preparation innovations to continue this trend. ■

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FEATURE ARTICLE

Low Phase Ratio Stationary Phase Column Technology for the Characterization of Highly Volatile and Reactive Compounds by Gas-Liquid Chromatography

Tetiana Davydiuk, Ronda Gras, and Jim Luong

Recent advances in coating techniques and improved polymers employed as gas chromatographic stationary phases have resulted in commercializing low phase ratio capillary column technology with respectable chromatographic efficiency and inertness. Highly volatile compounds that are critical in challenging industrial applications such as alkanes, alkenes, arsine, phosphine, oxygenated, and sulfur compounds have been successfully analyzed with respectable chromatographic performance and resolution using a low phase ratio column such as a 60 m × 0.32 mm id × 8 µm polydimethylsiloxane (PDMS) with a β value of 10. An R value of 5.6 was achieved for the separation of hydrogen sulfide and carbonyl sulfide without the use of cryogenic chromatography. In addition to improved retention for highly volatile organic compounds, a low phase ratio column can be employed effectively to enhance chromatographic inertness for reactive analytes. The augmentation of a 3D-printed two-stage microreactor for use with flame ionization detection enabled carbon-compound independent response, thereby lower cost-of-ownership and improved lab safety.

WITHOUT ANY DOUBT, gas chromatography (GC) is the ideal analytical technique for characterizing highly volatile and volatile organic compounds in various matrices (1–3). Despite advances gained through decades of research, challenges remain to improve the performance of this ubiquitous technique.

One of the critical areas of research focuses on expanding the role of GC for the characterization of highly volatile analytes encountered in several sectors, including petrochemical, pharmaceutical, environmental pollution, and scientific research. Gas-solid chromatography (GSC) has been proven to be a viable solution to provide high retention for the analytes (4,5). In adsorption chromatography, the compounds to be separated interact with the stationary phase composed from adsorbents such as aluminum oxide, silica, molecular sieve, carbon molecular sieve, or polymer materials such as divinyl benzene and its variants (6). Each compound adsorbs to the surface of the adsorbent to a greater or lesser extent, and, therefore, moves through the entire column at a different speed. Ideally, the compounds separate before they exit from the column. While this approach is perceived as an adequate solution, several constraints are encountered. In adsorption chromatography, chemical reactivity between the analytes and the adsorbent can cause the loss of analyte along the flow path, undesired catalytic reaction, peak tailing, and low solute capacity, leading to asymmetric chromatographic peak profile (7). Another concern is the potential of shedding of the adsorbents that can clog chromatographic components along the analyte flow path, such as metal unions, diffusion bonded plates used for switching or splitting, or a jet orifice employed by the flame ionization detector (FID).

Gas-liquid chromatography (GLC) is another viable option (8,9). Here, analytes partition between the mobile phase and a liquid. The compounds have different partition coefficients (the extent to which they dissolve in both phases which determines the

time required for the compounds to travel through the column). The advantages of GLC include substantial improvements in inertness when compared to GSC, improved chromatographic peak symmetry, and potentially faster analysis due to lower analyte capacity factor (k'). GLC have been challenging when it comes to compounds with high volatility, due to limited interaction of these analytes with the stationary phase. Analyte retention can be increased by using cryogenic chromatography (10,11). However, the need for the cryogen increases method complexity and ownership cost. Until recently, common columns with β > 63 (0.25 mm id, 1 µm) or (0.32 mm id, 1 µm) do not provide sufficient retention for highly volatile compounds such as C1 to C6 alkanes and alkenes, carbon dioxide, methanol, and formaldehyde, to name a few.

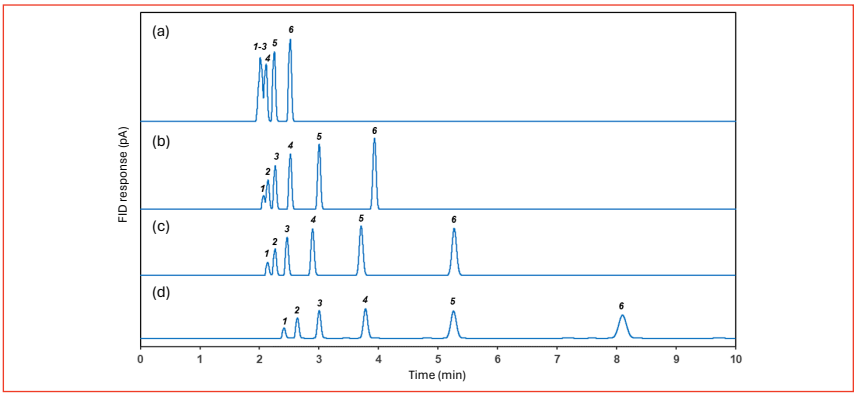

FIGURE 1: Overlay of chromatograms of 1000 ppm (v/v) each of n-alkanes (C1–C6) in nitrogen on 60 m × 0.32 mm id PDMS columns with different film thickness: (a) 1 µm (β = 80); (b) 3 µm (β = 27); (c) 5 µm (β = 16); (d) 8 µm (β = 10).

Attempts to lower β value by increasing film thickness can lead to a substantially reduced chromatographic coating efficiency using the conventional static

TABLE I: Summary of the characteristic parameters for n-alkanes (n-C_1-C_6, at 1000 ppm each) separation on four PDMS columns with different phase ratio. All the experimental parameters except for the film thickness were maintained constant across the experiments. Hold-up time (t_M) value of 1.8559 min (calculated by the software) was used for the retention factor (k) calculations.

Column Parameters	Retention Time (min)	k'	Area (pA x s)	Height (pA)	Width (min)	Resolution^{PeakNo}	Selectivity	Symmetry
	2.01	0.0	8580.0	1904.8	0.08	0^{1-3}	0	0.87
d_f = 1 µm β = 80	2.11	0.1	6099.3	1712.5	0.06	0.8^{3-4}	2.8	1.40
	2.25	0.2	7122.1	2087.8	0.06	1.4^{4-5}	1.9	1.34
	2.52	0.3	8521.4	2458.4	0.06	2.7^{5-6}	1.9	1.33
	2.07	0.1	1276.4	417.0	0.06	-	-	1.31
	2.15	0.1	3012.9	874.2	0.06	0.8^{1-2}	1.7	1.22
d_f = 3 µm β = 27	2.27	0.2	4293.7	1297.5	0.06	1.3^{2-3}	1.7	1.20
	2.52	0.3	5590.5	1659.3	0.06	2.7^{3-4}	1.8	1.23
	3.01	0.5	6867.9	1949.1	0.06	5.0^{4-5}	1.9	1.10
	3.93	1.0	8240.4	2133.6	0.06	9.1^{5-6}	1.9	1.03
	2.14	0.1	1336.6	383.8	0.06	-	-	1.02
	2.26	0.2	2740.0	788.0	0.06	1.3^{1-2}	1.7	1.12
d_f = 5 µm β = 16	2.47	0.3	4068.1	1138.0	0.06	2.0^{2-3}	1.7	1.10
	2.89	0.5	5302.6	1378.8	0.06	4.2^{3-4}	1.8	1.00
	3.71	0.9	6515.8	1475.4	0.07	7.2^{4-5}	1.9	0.95
	5.27	1.7	7854.4	1408.4	0.09	11.6^{5-6}	1.9	0.96
	2.42	0.2	113.9	309.2	0.06	-	-	1.00
	2.64	0.4	2299.4	611.9	0.06	2.2^{1-2}	1.5	1.05
d_f = 8 µm β = 10	3.00	0.5	3420.5	826.5	0.07	3.4^{2-3}	1.5	0.97
	3.78	0.9	4450.6	880.1	0.08	6.3^{3-4}	1.7	0.96
	5.26	1.7	5472.7	811.6	0.11	9.4^{4-5}	1.8	0.97
	8.10	3.1	6547.8	689.2	0.15	13.1^{5-6}	1.9	0.97

coating technique and severe bleeding at elevated temperature. Other techniques of coating that have been reported in the literature can be considered. For instance, with the standard dynamic coating technique, a very thick film can be easily generated if the viscosity of the coating liquid and the plug speed is sufficiently high. However, such a film is unstable and can rearrange to form droplets due to drainage and Raleigh instability (12,13). Therefore, there is a need for a new alternative or combination of alternatives. A potential solution can be employing instant thermal fixation technique. Instant thermal fixation involves an immediate fixation of the film directly behind the meniscus of the coating plug. The fixation can be implemented by heat-accelerated cross-linking using an appropriate polymer. Recent advances in coating techniques and the synthesis of polymers with higher diffusion constant resulted in the successful manufacturing and commercialization of columns with β as low as 10 without significant loss in chromatographic performance and with respectable bleed profiles.

In this work, we explore the strategy of employing a low phase ratio column with an appropriate stationary phase that has high diffusion coefficient. This strategy offers a credible alternative to GSC with the increase in retention for highly volatile analytes, and improved analyte loading with more symmetric peak. Overall inertness is also improved, since the thicker film decreases the possibility of analytes interacting with the inner surface of the fused silica column. The use of low phase ratio can help enhance sample throughput. We also demonstrate the strategy with analytes frequently encountered in challenging industrial applications. The augmentation of post-column reaction with catalysis for use with flame ionization detection enabled carbon-compound independent response, thereby lower cost-of-ownership and improved lab safety.

Experimental

Four analytical platforms were employed for the study. All the platforms were equipped with a split/splitless inlet (SSL). The first one is an Agilent 7890A series gas chromatograph (Agilent Technologies) equipped with a 7693A autosampler, a Polyarc (Activated Research Corporation) 3D-printed post-column two-stage microreactor, and a flame ionization detector was used (GC/Polyarc/FID). The second is an Agilent 8890A series gas chromatograph (Agilent) with a flame ionization detector (GC-FID). The third is a 7890B series gas chromatograph (Agilent) equipped with a sulfur chemiluminescence detector in tandem with a flame ionization detector (GC-SCD-FID). Finally, the fourth is a 7890B series gas chromatograph (Agilent) equipped with a 5977B Mass Selective Detector (GC–MSD). Helium was used as a carrier gas for all the experiments.

For the first analytical platform, namely GC-Polyarc-FID, GC inlet was at 250 °C with a split ratio of 5:1, at a split flow rate of 25 mL/min helium for 2 min and the gas saver flow of 15 mL/min starting at 2 min. The liner was a 4 mm id quartz wool, straight borosilicate glass. For the comparison of the four columns with different film thickness, the oven temperature program was 40 °C (0.5 min) to 260 °C (10 min) at 6 °C/min with the FID temperature at 250 °C, hydrogen flow rate at 2 mL/min, air at 350 mL/min, and nitrogen make-up gas flow rate at 15 mL/min. For all other experiments, the ramp rate was increased to 15 °C/min, with the initial temperature kept at 40 °C (1 min), and final temperature reduced to 230 °C. The split ratio was set to 100:1, unless specified otherwise.

Four columns, each coated with 100% polydimethylsiloxane (PDMS) (Agilent) with different phase ratios with dimensions of a) 60 m × 0.32 mm × 1 µm, b) 60 m × 0.32 mm × 3 µm, c) 60 m × 0.32 mm × 5 µm, and d) 60 m × 0.32 mm × 8 µm, were used for the study. Polyarc reactor was at a nominal temperature of 293 °C (450 °C actual). Air flow was set at 2.5 mL/min and hydrogen flow at 35 mL/min. Chromatographic data were processed with an Agilent ChemStation B.04.03SP2 software.

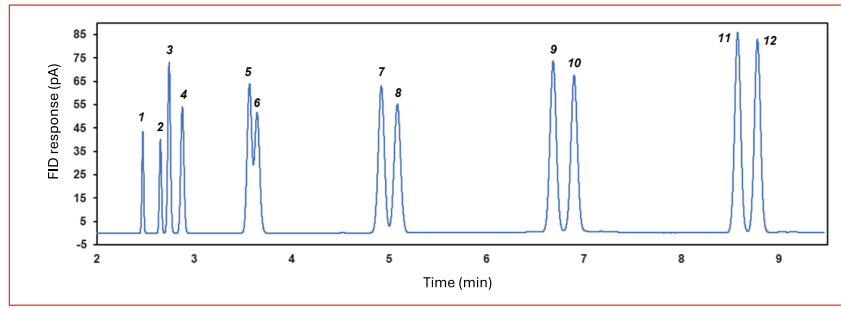

FIGURE 2: Separation of n-alkanes (C_1-C_6), alkenes (C_2-C_6) and carbon dioxide on 60 m × 0.32 mm × 8 µm PDMS column. Peak identity: 1) methane; 2) carbon dioxide; 3) ethylene; 4) ethane; 5) propylene; 6) propane; 7) butene; 8) butane; 9) pentene; 10) pentane; 11) hexene; 12) hexane.

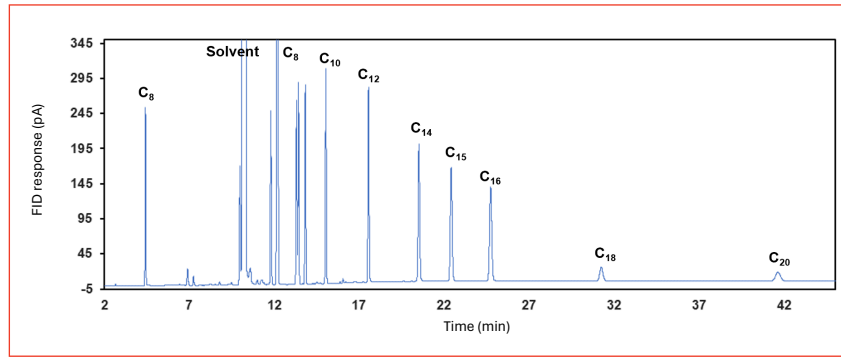

FIGURE 3: Separation of longer chain n-alkanes on PDMS 30 m × 0.25 mm × 5 µm. Standard with 1000 ppm w/w C8, C10, C12, C14, C15, C16, and 200 ppm w/w C18, C20, C22, C24 in cyclohexane was used.

For the second platform (GC/FID), inlet temperature was set at 250 °C with the flow split 100:1 and on-column carrier gas flow rate of 2 mL/min. Two GC columns a 30 m × 0.25 mm × 5 µm PDMS (Quadrex) and a 30 m × 0.25 mm × 0.25 µm 5%-phenyl PDMS (HP-5, Agilent) were used. For thermal bleeding profile evaluations, the temperature program was set at 20 °C/min with initial temperature at 40 °C (1 min) and final temperatures at 150, 200, 225, and 250 °C with 15 min hold time. Same temperature programming was used for alkane/alkene analysis with final temperature set to 220 °C. For the analysis of C8-C24 hydrocarbon mixture, the final temperature and the rate were increased to 230 °C and 30 °C/min respectively. For sulfur compound mix analysis, oven temperature was increased from 40 °C (4 min) to 230 °C (20 min) at 20 °C/min. The FID gas flow rates were set at 35 mL for hydrogen, 350 mL/min for air, and 28 mL/min for the make-up gas (N_2).

For the third analytical platform (GC-SCD-FID), two GC columns were employed, a 40 m × 0.32 mm × 3 µm DB-Sulfur and a 60 m × 0.32 mm × 8 µm PDMS. GC inlet was 250 °C with a split ratio of 5:1 and a split flow rate of 20 mL/min with a column flow rate of 4 mL/min. The liner was a 4 mm id, single taper ended. The effluent of the column was split with an Ultimate three-way splitter (Agilent) with a ratio of 2:1 between the FID and the SCD. A 2.2 m × 0.15 mm id fused silica, deactivated, but uncoated was installed for the SCD. A 0.5 m × 0.32 mm fused silica tubing was installed for the FID. SCD burner temperature was 800 °C, oxidant at 60 mL/min, upper hydrogen was 38 mL/min and lower hydrogen was 8 mL/min.

The FID temperature was 250 °C. Fuel gases for FID include air at 350 mL/min, nitrogen at 25 mL/min, and hydrogen at 30 mL/min. For all experiments, the oven temperature was 40 °C (5 min) to 220 °C (5 min) at 15 °C/min.

For the fourth analytical platform (GC–MSD), GC inlet was at 250 °C with a split flow ratio of 10:1, at a split flow rate of 20 mL/min for 2 min and the gas saver flow of 15 mL/min starting at 2 min. The liner was a 4 mm id quartz wool, straight borosilicate glass. For all experiments, the oven temperature program was 40 °C (0.5 min) to 260 °C (10 min) at 6 °C/min. A 60 m × 0.32 mm × 8 µm PDMS column at a flow rate of 2 mL/min helium was used. The mass spectrometer ion source was 230 °C, quadrupole filter was 150 °C, gain factor of 1, scan range from 20 Da to 300 Da having a threshold at 150. Data were collected with Agilent Mass Hunter version 10.2.489.

Utility gases such as helium and hydrogen were obtained from Linde. Air and nitrogen were generated onsite. Standard materials were purchased from Sigma-Aldrich.

Results and Discussion

Chromatographic Performance

A column phase ratio (β) is a dimensionless value, as described by equation 1:

$$\beta = d/4d_f \quad [1]$$

Where d is the internal diameter of the column, and d_f is the film thickness of the stationary phase.

If the same stationary phase and column temperature of either isothermal or program are maintained, the change in the phase ratio can be used to accurately predict the change in a solute's retention via distribution constant K_c in equation 2, where:

$$K_c = C_s/C_m = k \times \beta = k \times (d/4d_f) \quad [2]$$

Where C_s and C_m are the concentrations of the analytes in the stationary phase and mobile phase, respectively, and k is the retention factor (14).

Hence, an increase in the phase ratio results in a decrease in retention (k) since K_c is constant. Conversely, a decrease in the phase ratio results in a corresponding increase in retention k which can be advantageously applied to tackle highly volatile analytes. As an illustration, Figure 1 shows an overlay of four chromatograms with the separation of n-alkane homologues from *n*-C1 to *n*-C6, at 1000 ppm (v/v) each in nitrogen, with four different β values of 10 (8 µm), 16 (5 µm), 27 (3 µm), and 80 (1 µm) under the same gas chromatographic conditions. Table I summarizes the t_R (min), and k obtained. The retention time of hexane increases from t_R of 2.5 min to t_R of 8.1 min as β decreases from 80 to 10. Similarly, the R value between methane and ethane increases from 0 to 2.2. The results clearly demonstrated that an increase in retention and resolution can be achieved by using low phase ratio column for highly volatile compounds.

With the large amount of stationary phase in the column, one of the main concerns is the excessive level of bleed at elevated temperature. Supplementary Material (SM) 1 (available through the QR code at the end of the article) shows thermal bleed profiles at different final temperatures for the column with 5 µm film thickness with a maximum operating temperature at 280 °C. The bleed increased by only about 4 pA at 250 °C. Similarly, only 3 pA increase in bleed was observed at 250 °C for the column with 8 µm film thickness with a maximum temperature of 300 °C. A further increase in the final temperature to 270 °C resulted in 6 pA. The resulting bleed was much lower than expected which demonstrates the effectiveness of cross-linking of the polymer and the bonding of the polymer to the inner surface of the fused silica to immobilize the stationary phase.

One of the benefits of low β column is increased solute loadability. Table I lists the peak symmetries of the alkane and alkene probe compounds, each at a 1000 ppm (v/v) level. Peak symmetries ranging from 0.87 to 1.34 were obtained, which are quite respectable for open tubular, wall-coated capillary gas chromatography. Yet another benefit of having thicker film thickness is the potential improvement on inertness as the possibility of solute-inner fused silica interaction is minimized. Supplemental Material 2 shows a chromatogram of 500 ppm (v/v) of formaldehyde in nitrogen with respectable symmetry.

Industrial Applications

The analytical strategy of using low phase ratio column was illustrated with challenging industrial applications.

Volatile Hydrocarbons

Volatile hydrocarbons, including alkanes and alkenes, are commonly analyzed in petrochemical and chemical industries (15,16). Figure 2 shows an overlay of 1000 ppm (v/v) of alkanes, alkenes, and carbon dioxide, each in nitrogen, with excellent separation. Due to the non-polar nature of the stationary phase, alkenes are eluted before alkanes. In contrast, with adsorbents employed as stationary phases such as aluminum oxide or sintered silica, alkanes are eluted before alkenes. Increased retention allows to fully separate methane, carbon dioxide, ethylene, and ethane.

Increased retention might compromise our ability to effectively analyze less volatile compounds. Therefore, we investigated the potential of this set up to elute longer chain alkanes by analyzing C_8-C_{24} alkanes standard mixture. Figure 3 shows effective elution could be accomplished up to C_{14} with an approximate boiling point of 250 °C, subsequent peaks are eluted having substantial peak broadening. This result suggests that thick film column can provide an advantage of substantially improved separation of highly volatile compounds, and at the same time cover relatively wide range of analytes. The elution order obtained with complementary stationary phases with dissimilar separation mechanisms can be employed advantageously to

increase the confidence in identifying a hydrocarbon component in complex hydrocarbon process streams.

Arsine and Phosphine

Phosphine (PH_3) and Arsine (AsH_3) are compounds of an inorganic chemical nature, present as impurities in petroleum and its derivatives (17–19). The formation of AsH_3 is associated with natural processes in the geothermal steam, and PH_3, with biogenic processes, or the biological reduction of phosphate. These and other impurities are considered inhibitors of Zieglar-Natta catalysts during the synthesis of polypropylene (20,21). They are of occupational importance in petroleum and petrochemical complexes because of their effects on human health, since the arsine exposure limits established by the United States National Institute for Occupational Safety and Health (NIOSH), and the Occupational Safety and Health Association (OSHA), are 0.002 mg/m^3 for 15 min and 0.2 mg/m^3, respectively (22,23). The simultaneous quantification of AsH_3 and PH_3 at these trace and ultra-trace levels is an analytical challenge of considerable importance in polyolefin-producing plants. Shown in SM_3 is a reconstructed chromatogram of 10 ppm (v/v) PH_3 and AsH_3 in nitrogen, demonstrating the high degree of inertness and the resolution achieved by the column.

Volatile Sulfur Compounds:

Volatile sulfur compounds are of interest for several applications including hydrocarbon feedstock, environmental monitoring, and emission control (24,25). Figure 4 shows an overlay of sulfur compounds by SCD and FID in tandem using a 40 m × 0.32 mm id × 3 µm PDMS and a 60 m × 0.32 mm id × 8 µm PDMS. An *R* value of 1.5 was obtained with the 3 µm column approaching baseline resolution. In contrast, due to the increased retention, an *R* value of 5.9 was obtained with the 8 µm column. The improvement in resolution aids in providing more accurate measurement for hydrogen sulfide especially in complex hydrocarbon matrices such as liquefied petroleum gas or raw natural gas. A mixture of 13 sulfur-containing compounds at a nominal concentration of 10 ppm (v/v) each, including sulfides, disulfides, and mercaptans, could also be efficiently separated, as shown in Figure 5, with highly reproducible peak areas (< 3.8 % RSD, $n = 6$) and retention times (< 0.02 % RSD, $n = 6$). The use of the SCD provides sulfur specific detection, employing an FID as a hyphenated tandem detector, offers additional information related to the boiling distribution of the analytes, as well as a more complete sample composition particularly for organic compounds.

Oxygenated Compounds in Ethylene Oxide

Ethylene oxide (EO) is a chemical of industrial significance (26,27). It is a reactant for the manufacturing of ethylene glycol, as well as one of the strongest sterilizing agents for surgery preparation (28). Common impurities in EO, such as ethylene, ethane, cyclopropane, and acetaldehyde, were well separated with the column described, as illustrated in Figure 6.

Method Performance Enhancements with Carbon Compound Independent Response

Despite the popularity of the FID, two key drawbacks were observed: a) carbon-containing compound specific response and several important volatile carbon-containing compounds has low to no response on FID due to the lack of formation of CHO^+ ions, such as carbon monoxide, carbon dioxide, formaldehyde, carbonyl sulfide, and carbon disulfide. The employment of post-column with catalysis can defeat the constraints encountered. Table II lists the relative response of several probe compounds used. A relative response of 1.0±0.05 was achieved, demonstrating the effectiveness of the technique. This approach of using one single standard to quantify other carbon-containing compound of interest can substantially improve lab safety as analysts can avoid handling and disposing toxic compounds, lower cost-of-ownership without the need to acquire materials that are difficult to synthesize or are reactive and unstable.

Conclusions

We successfully demonstrated the use of low phase ratio column technology in partition gas chromatography to characterize highly volatile or reactive compounds in difficult matrices with respectable chromatographic performance. In addition to increase retention for the volatile analytes, an added benefit of having thicker film is its aiding in enhancing overall chromatographic inertness. The incorporation of a post-column two-stage reactor enabled carbon compound independent response. ■

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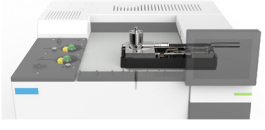

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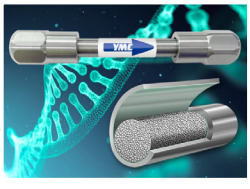

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