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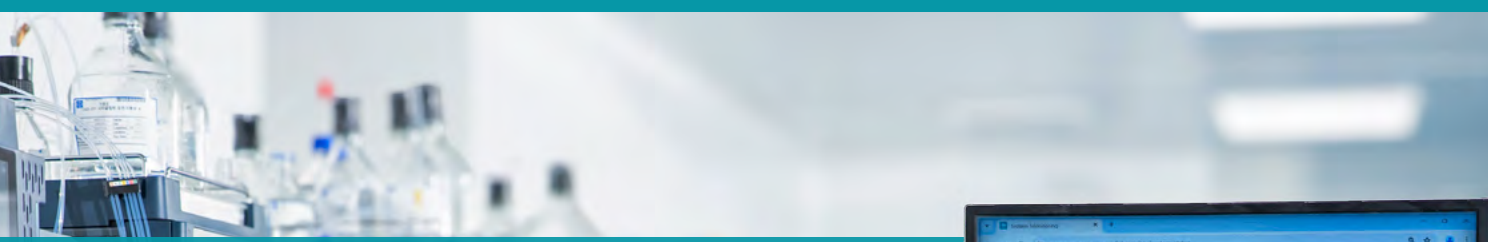

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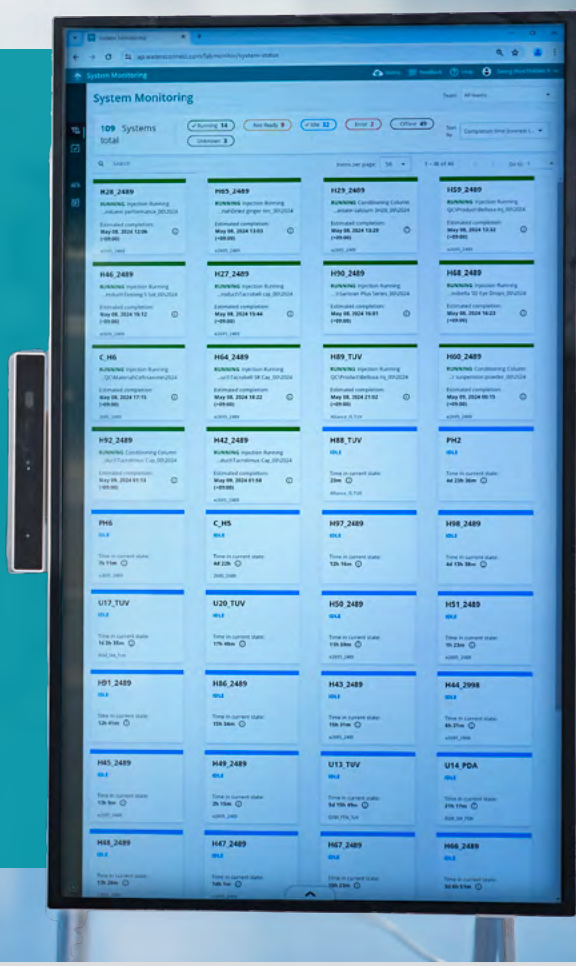

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A close-up shot of golden honey being drizzled from a wooden honey dipper.

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NOTE FROM THE CEO

AS THE YEAR DRAWS TO A CLOSE, we're thrilled to present our November/December 2024 Resource Issue, a comprehensive guide featuring company profiles, product profiles, application notes, and a directory of companies. In addition, this editorial provides informative, useful columns, articles, and interviews, making it a treasure trove of knowledge, spanning life sciences, instrumental advancements, and green analytical methods. Let's explore what awaits you in these pages!

First, Dwight R. Stoll returns with his highly regarded "LC Troubleshooting" column, starting a multi-part series on aqueous buffers in liquid chromatography. Here, Dwight will begin to demistify why buffered aqueous solutions are critical for pH control and detector compatibility, offering foundational insights that pave the way for upcoming troubleshooting scenarios.

"Focus on Biopharmaceutical Analysis," delves into the complexities of proteomics with "LC-MS-Based Proteomics for Biomarker Quantification for Both Prognosis and Diagnosis in the Clinical Setting." This column explores cutting-edge methods like SILAC, iTRAQ, and TMT for uncovering low-abundance biomarkers, emphasizing the transformative impact of LC–MS on clinical diagnostics.

In "Sample Prep Perspectives," Mary Ellen McNally illuminates the versatility of "Sample Preparation with Molecularly Imprinted Polymers (MIPs)." From SPE clean-ups to stir bar sorptive extractions, this piece chronicles three decades of progress and highlights MIPs as cost-effective, selective alternatives to natural receptors.

In "Pharmaceutical Perspectives," Claudio Brunelli and Wayne Callar present "Analytical Method Lifecycle of SFC Methods from Development Use to Routine QC Implementation." This column underscores the importance of selecting robust analytical techniques early on, focusing on the "green" advantages of SFC in supporting chiral and hydrophobic compounds throughout drug development.

On the food analysis front, researchers Silvia Valverde and Ana María Ares discuss their work on "A Green Analytical Method for Simultaneously Determining Plasticizers Residues in Honeys from Different Botanical Origins." Their innovative use of GC-MS exemplifies how sustainability and precision can coexist in contaminant analysis.

Will Wetzel's featured interview with Anne Marie Smith reflects on the HPLC 2024 Conference and "The Future of Digital Method Development." Discover how the ICH Q14 guidelines are reshaping method development strategies for analytical scientists.

Finally, our feature article, "Next Generation Peak Fitting for Separations," authored by M. Farooq Wahab, Troy T. Handlovic, and Daniel W. Armstrong, introduces a game-changing approach to tackling overlapping peaks in complex separations. Their discussion of iterative curve fitting showcases its versatility across analytical and preparative settings.

We hope this issue serves as your go-to resource for inspiration, innovation, and practical solutions.

Please accept our wishes for a joyous holiday season, and we'll see you in 2025!

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

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Mobile Phase Buffers in Liquid Chromatography: A Review of Essential Ideas

Dwight R. Stoll

Buffered solutions that resist changes in pH are very important to various aspects of the practice of liquid chromatography (LC). In this installment, I discuss several essential principles related to when and why buffers are important, as well as practical factors such as commonly used buffering agents that are recommended for use with different types of detectors. This installment is intended as a prelude to subsequent installments that will explore specific troubleshooting scenarios that are impacted by effective use of buffered solutions.

IN MY CONVERSATIONS with people in all corners of the field, I find too often that there is information and understanding missing from users' thinking about, and uses of, buffers. I think it is easy to imagine why this happens—for example, we see liquid chromatography (LC) users transplanted from other fields or disciplines, who may not have ever been exposed to the idea of buffer capacity. It can be really eye-opening, however, to field questions from users wondering why their peak shapes are bad, or their retention times are not repeatable, only to find that the problems originate with poor choices related to buffers. They have the latest LC instrument make and model. They have a shiny new mass spectrometer that gives them accurate mass and high resolution. But the major weakness of the method and analysis is the buffer. This is a situation we should avoid, of course. In response to my questions about choices related to buffers in situations like this, I also find too often responses along the lines of "That's what the person before me did," or "This method came from a different group, and we have to do it that way." I certainly understand there are situations where one must implement a method that has been handed to them for a variety of possible reasons, and there is nothing I can do in this article to change that. However, I think there are many opportunities to prevent these kinds of poor choices from being made in the first place through education and formation of good habits. Upon inheriting a method, please ask, "Do these conditions make sense? Is there a better way? Has the community learned anything in the recent past that might influence what conditions we use here?" Let's not continue propagating the old errors, myths, and bad habits of the past.

Much has been written about buffers in this "LC Troubleshooting" column, this magazine, the *LCGC* Blog more broadly, and in lots of other places in the past. This is an instance where I think it is fair to say that the problem is not so much a lack of information as it is digesting and sifting through the large volume of information out there to figure out what is relevant to your particular situation. This installment of "LC Troubleshooting" tries to help with that. I give an overview of the main aspects to think about, brief summaries of the prevailing wisdom in the field, and then point to examples of relevant, trustworthy sources for more information for those who want to dig deeper. The discussion is colored in a way that is most relevant to reversed-phase (RP) and hydrophilic-interaction (HILIC) separations. Of course, many of the aspects are relevant to other separation modes as well, such as ion-exchange and size-exclusion separations, but we don't have space to address them in detail here. Perhaps we'll follow up with those details in a different month and year.

What, and What For?

Before getting into any other details, we should establish what exactly we are referring to when we say "buffer," and also establish why we need buffers at all. The textbook definition of a *buffer* is a solution that resists changes in pH upon the addition of acid or base to the solution. The extent to which a particular solution acts as a buffer depends on a handful of factors, including the

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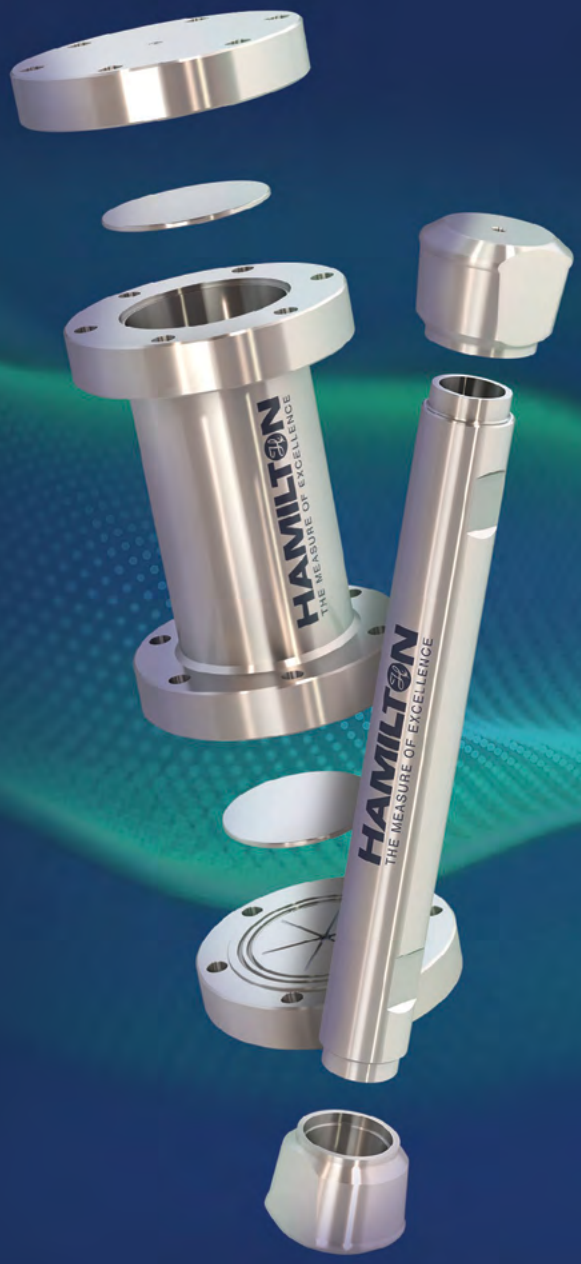

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nature of the buffering agent (which itself must be a Bronsted acid or base), its concentration, and the pH of the solution relative to the pK_a of the buffering agent. This buffering ability can be quantified using the concept of *buffer capacity* (see next section). First, though, let's consider the chemistry that is responsible for this buffering against pH changes using the simple example of a solution of acetic acid and sodium acetate in water. If we add 10 millimoles of acetic acid and 10 millimoles of sodium acetate to enough water to make a liter of solution, we will find that at equilibrium the concentrations of protonated acetic acid (HAc for short) and deprotonated, negatively charged acetate ion (Ac^- for short) are roughly equal, and the pH of the solution will be approximately 4.8. We illustrate the idea that the quantities of these species are related by showing the following chemical reaction:

$$HAc_{(aq)} \rightleftharpoons H^+_{(aq)} + Ac^-_{(aq)} \quad [1]$$

Now, if we consider what happens when we add some strong acid to the system, perhaps through the addition of some hydrochloric acid (HCl), which will introduce some H^+ (because HCl is fully dissociated in water), Le Chatelier's Principle tells us that some of the Ac^- will be consumed by reaction with H^+ to produce HAc. In equation 1, the H^+ we add from HCl is a product in the reaction, and adding a product species to a system at equilibrium will shift that equilibrium such that species on the reactant side of the equation (HAc in this case) is produced from species on the product side (Ac^- and H^+ in this case). In this way, the H^+ ions that are added to the solution from the addition of HCl are "soaked up" by the Ac^-, and the net change to the concentration of H^+, which dictates the pH of the solution, is small. In this case, the solution acts as a good buffer, resisting a major change in pH, even through strong acid has been added to the solution.

But...so what? Why is it important for the mobile phase to have this buffering ability in LC? The short answer, and I think the aspect that probably gets the most attention, is that mobile phase pH can dramatically affect the separation selectivity for ionogenic compounds—that is, compounds that are prone to gain or lose protons as the pH of the solution is changed. In RP and HILIC separations, these gains and losses of protons are also accompanied by changes in the charge state of an analyte (for example, see equation 1 where acetic acid goes from zero charge to a -1 charge upon the loss of a proton), which in turn dramatically affects the water solubility of the compound and retention under RP and HILIC conditions (see the section below on selectivity effects for more information about this aspect). However, there are many other effects of buffers on LC separations and detectors, and some of these can actually be more important from a troubleshooting and method optimization point of view. A short list includes effects on peak shape, retention repeatability and reproducibility, and signal-to-noise (S/N) ratio and detection limits (particularly when using mass spectrometric detection).

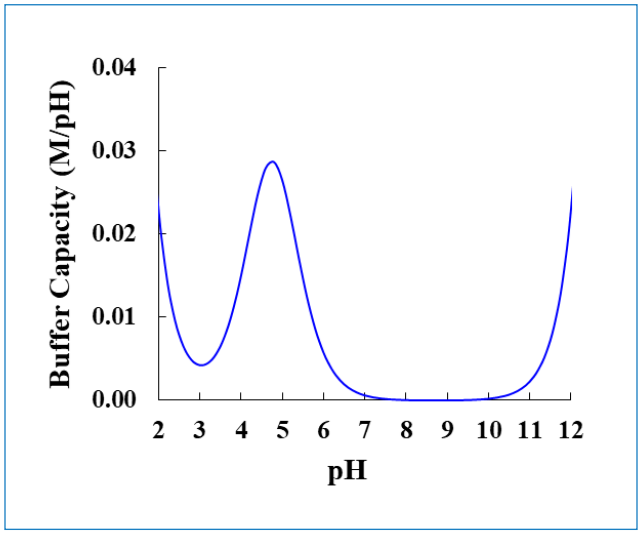

FIGURE 1: Plot of the buffer capacity versus pH for an acetic acid/acetate buffering system. The buffer capacity maximizes when the pH is equal to the p*K*a of the acidic component of the buffering agent.

The Concept of Buffer Capacity

The extent of the ability of the buffer to mitigate changes in pH because of the additions of acids or bases can be quantified using the concept of buffer capacity (β), as expressed in equation 2 (1). In this expression, $[H^+]$ and $[OH^-]$ are the concentrations of hydrogen and hydroxide ions in solution, K_{HA} is the dissociation constant of the acidic form of the buffering agent (acetic acid, for example), and C_{HA} is the "formal" concentration of the buffering agent, which, in the case of the acetic acid/acetate buffering system, would be the sum of the actual concentrations of the protonated acetic acid and deprotonated acetate ion in solution. Figure 1 shows a plot of the buffer capacity versus pH for the acetic acid/acetate system. It turns out that β maximizes when the solution pH is equal to the pK_a of the buffering agent. We see this in Figure 1 with the maximum in the curve around pH 4.8. The buffer capacity also increases at very low and high pH; in these regions the high concentrations of H^+ and OH^- actually act as good buffers themselves.

$$\beta = 2.3 \cdot [H^+] + 2.3 \cdot [OH^-] + \frac{2.3 \cdot K_{HA} \cdot C_{HA} \cdot [H^+]}{\left(K_{HA} + [H^+]\right)^2} \quad [2]$$

The buffer capacity concept is also very useful for illustrating the point that just because a solution contains a buffering agent does not mean that the solution is actually a good buffer at every pH. Indeed, Figure 1 shows that the acetic acid/acetate system has very little buffer capacity (that is, it is a terrible

buffer) in the pH range of about 7 to 10. When I talk with people about their mobile phases, I frequently hear them describe a solution prepared by adding ammonium acetate to water, without further pH adjustment. The pH of this solution will be approximately 7, and Figure 1 shows that this solution actually has little ability to buffer. It is true that the ammonium ion can contribute to buffering, but the useful range of the ammonium/ammonia system only extends down to approximately pH 7.5. We should be careful to manage our expectations about what such a solution can and cannot offer to a LC method in terms of pH control of the mobile phase.

Effects of Buffers on Primary Separation Metrics

I think the two direct effects of buffers on primary separation metrics that receive the most attention are the effects of buffer pH on selectivity, and the effects of buffer type, pH, and concentration on peak shape.

Effect of pH on Selectivity

Although there are several potential effects of mobile phase pH on selectivity, which can come from changes in the ionization state of the analyte, the stationary phase, or both, changes in the ionization of the analyte are those we typically point to first. A simple view of this effect is shown in Figure 2, where I have illustrated two RP separations of a simple mixture of neutral (N), acidic (A), and basic (B) compounds. We see that at pH 2 the neutral is separated, but the acid and base are coeluted. If the acidic compound has a carboxylic acid functional group, it will be protonated and neutral at pH 2. The basic compound will be protonated and positively charged. When we move to pH 7, however, we see the retention time of the acid decreases dramatically, because it has a pK_a between 2 and 7, loses a proton upon the move to pH 7, and becomes negatively charged and much more water soluble. The base, however, remains unchanged in the move from pH 2 to 7, because its pK_a is well above 7, and the ionization is unaffected by the move from pH 2 to 7. This simple illustration emphasizes the importance of considering mobile phase pH as a variable during method development. Readers interested in learning more about this aspect are referred to reference (2).

Effects on Peak Shape

There are also several different mechanisms through which a buffer can affect peak shape in RP and HILIC separations. These include effects on the chemistry of interaction partners influencing retention (for example, whether or a silanol group on the surface of a silica particle is charged or not), and shielding of injected analytes from charged molecules adsorbed to the stationary phase surface, which can affect "loadability" (that is, the amount of analyte mass that can be injected before poor peak shapes are observed). There is extensive literature on these topics. For representative articles that discuss these aspects, readers are referred to references (3) and (4).

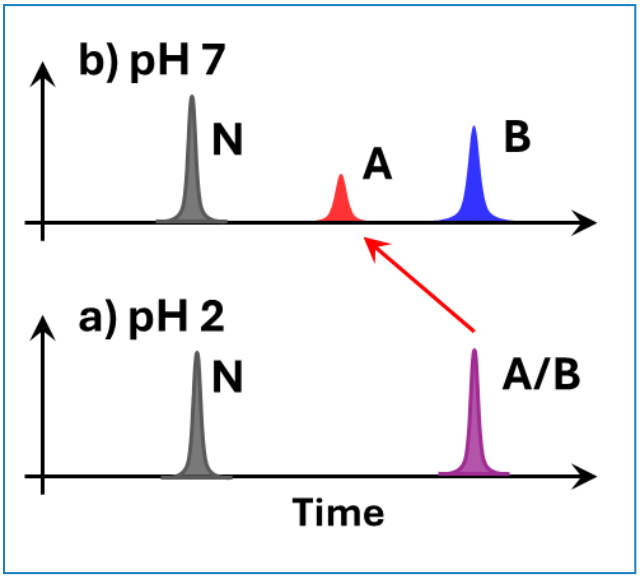

FIGURE 2: Simple illustration of the effect of mobile phase pH on selectivity of an RP separation when the sample contains ionogenic compounds.

Practical Factors

In addition to the conceptual aspects of what a buffer is, its effective pH range, and its primary effects on actual separations, there are many "practical factors" to consider when choosing a buffering agent and how to use it.

Buffers and Bugs

Microbes will grow readily, and sometimes rapidly, in solutions that are buffered at or near physiological conditions (for example, dilute phosphate buffers around pH 7 are microbe-friendly). This means that, if you are trying to buffer mobile phases under these conditions, you will need to do something to prevent microbial growth, otherwise microbes can cause serious damage to LC columns and other instrument components. The most common approaches to prevent microbial growth (aside from working far from pH 7, which is not always possible) are to use additives that will kill the bugs or prevent their growth in the first place, but not affect the chromatography (for example, sodium azide), or add some (10% or so is a good starting point) organic solvent such as methanol or acetonitrile to the buffered solution. Of course, this is not always an option. Another approach is to maintain sterile conditions for the buffer solution at the time of preparation (for example, through filtering) and throughout its use (for example by preventing infiltration of microbes from laboratory air). Readers interested in learning more about these details are referred to references (5) and (6).

Solubility

Different buffering agents vary tremendously in terms of their solubilities in water. This becomes more interesting when we introduce organic solvents into the picture, as is the case with mobile phases used for RP and HILIC separations. For example, my research group has found that ammonium acetate is quite soluble in even neat methanol, whereas ammonium formate is not nearly as soluble. One place where these solubilities are very important, and I think this might be surprising to many chromatographers, is when we mix two or more solvents in LC pumps. For example, it is common to use something like 50 mM sodium phosphate in water in one pump channel, and neat acetonitrile (ACN) in another pump channel. Sodium phosphate is not soluble in all proportions of mixtures of ACN and water, thus we have to be extremely careful that we don't instruct the pump to produce an ACN/buffer mixture that will cause the phosphate to precipitate inside the pump. Doing so can rapidly cause damage to the pump because of the abrasive nature of the precipitates. The most extensive, systematic study I am aware of on the solubility of buffering agents commonly used in LC in organic solvent/water mixtures was published by Schellinger and Carr nearly two decades ago (7), and I highly recommend incorporating the findings of this work into your knowledge base for LC work.

Care for Your Column

When choosing a buffering agent, and especially the pH range of interest, we must keep the well-being of our columns in mind. The conventional advice for columns containing silica-based particles that have been modified by covalently bonding ligands to the silica surface is to work in the pH range of about 2 to 8. At pH levels below 2, the siloxane bonds tethering the ligands to the silica surface can be hydrolyzed, and at pH levels above 8, the silica itself will dissolve. Over the past two decades, there has been a flurry of research and development among manufacturers working to beat this limitation of conventional silica-based phases, and now it is easy to find products that can be used safely below pH 2 and above 8. However, each of these products is a little different, and users should pay close attention to the manufacturer's advice provided with documentation inside the column box (or on their websites). Of course, there are many other exceptions as well. For example, materials based on organic polymers (for example, polystyrene-based phases) or other metal oxides (for example, alumina, titania, and zirconia) generally do not have the same pH limitations as silica, but have other practical limitations. Readers interested in learning more about these aspects are referred to references (8–10).

The Tug-of-War with Detectors

One of the cruel twists of nature that appears in the practice of LC is that the buffers that are optimal for the chromatography itself are often not optimal for the detector that is coupled to the outlet of the column. For example, phosphoric acid and phosphate salts are great buffering agents that are transparent to ultraviolet (UV) detection at least down to 210 nm. However, these same phosphate-containing buffers are effectively forbidden when using mass spectrometric (MS) detection because the phosphate is not at all volatile. On the other hand, MS-friendly buffering agents, such as dilute formic acid and ammonium salts of organic bases such as acetate and trifluoroacetate, absorb UV light pretty strongly around 210 nm, which leads to a number of complications, including baseline drift and noise. Readers interested in a more detailed discussion of these issues are referred to the excellent discussion in reference (11).

Baselines and Purity

Adding anything to a LC mobile phase, whether used for buffering the pH or some other purpose, increases the risk of introducing impurities that can later show up in the detector in the form of drifting baselines, new peaks in chromatograms that having nothing to do with injected samples, or even serious interferences with detection in general. One of the most spectacular examples of this from my own research group was a case where a new bottle of formic acid contained an impurity that suppressed ionization of proteins we were working with to the point that we thought something was seriously wrong with our mass spectrometer. In fact, simply switching back to a previous source of formic made the problem go away entirely. When choosing buffering agents and preparing the buffers, one should be careful to consider the availability of high purity reagents, and the ways that low level impurities might impact that the LC assay at hand. Readers interested in learning more about this aspect are referred to reference (12).

Making the Buffer in the Laboratory

When we go to actually make a buffer in the laboratory, there are multiple approaches we can use. The major distinction I see has to do with deciding whether to use a pH meter-guided approach or a gravimetric approach. Consider the preparation of a sodium phosphate buffer at pH 6. In the pH meter-guided approach, we might start by adding phosphoric acid to water, and then add sodium hydroxide until a pH meter tells us we have arrived at pH 6. This approach is very common in biochemistry laboratories. In the gravimetric approach, we would first calculate the masses of the mono- and dibasic sodium hydrogenphosphate salts needed to reach our target pH of 6. Then, we go to the laboratory, weigh out the salts, and add them to water. No pH meter is needed, other than to verify that a gross error was not made along the way. We find that the gravimetric approach is much more repeatable than the pH meter-guided approach and tends to be much faster as well. Readers interested in learning more about this aspect are referred to references (13) and (14).

Filter, or Not?

When preparing a buffered mobile phase, one must eventually decide whether or not to filter the solution prior to use with an LC instrument. On the surface, the answer seems simple—the solution should of course be filtered to remove any particulates or debris that might have been added to the solvent from the buffering agent. However, the filtration process itself can actually introduce more problems than it solves, if not executed properly. For example, filter materials can leach impurities into the solvent, and a filtration apparatus and environment that are not clean can also be a source of unwanted impurities in the filtered solution. In my laboratory, we use the guideline that we do not filter solutions after adding buffering agents that are liquids themselves (for example, formic acid, phosphoric acid, or ammonia), but we do filter after adding solid reagents such as sodium phosphate and similar salts, and we find this works pretty well on average. Readers interested in learning more about these details are referred to reference (6).

Other Important Aspects

One aspect that is very important when considering buffers for RP and HILIC separations is that the addition of organic solvents to water can have a dramatic effect on the apparent pH of the resulting solution compared to the pH prior to the addition of solvent. This is a result of the effect of the organic solvent on the acid dissociation constants of components in solution, whether they are buffering agents of the mobile phase, or analytes that we inject into the mobile phase for analysis. Readers interested in learning more about this aspect are referred to references (13) and (15).

Summary

In this installment, I have reviewed the essential considerations for effective use of buffers in RP and HILIC separations. Although we often point first to the effects of mobile phase pH and buffers on separation selectivity and peak shape, there are actually a number of other practical factors that are also strongly influenced by buffer choice. The LC community has learned a lot over the past few decades about effective use of buffers, and users are encouraged to implement these learnings at the method development stage. When problems arise with existing methods, this knowledge base also serves as a tremendous resource for fixing buffer-related problems. ■

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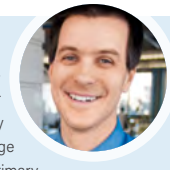

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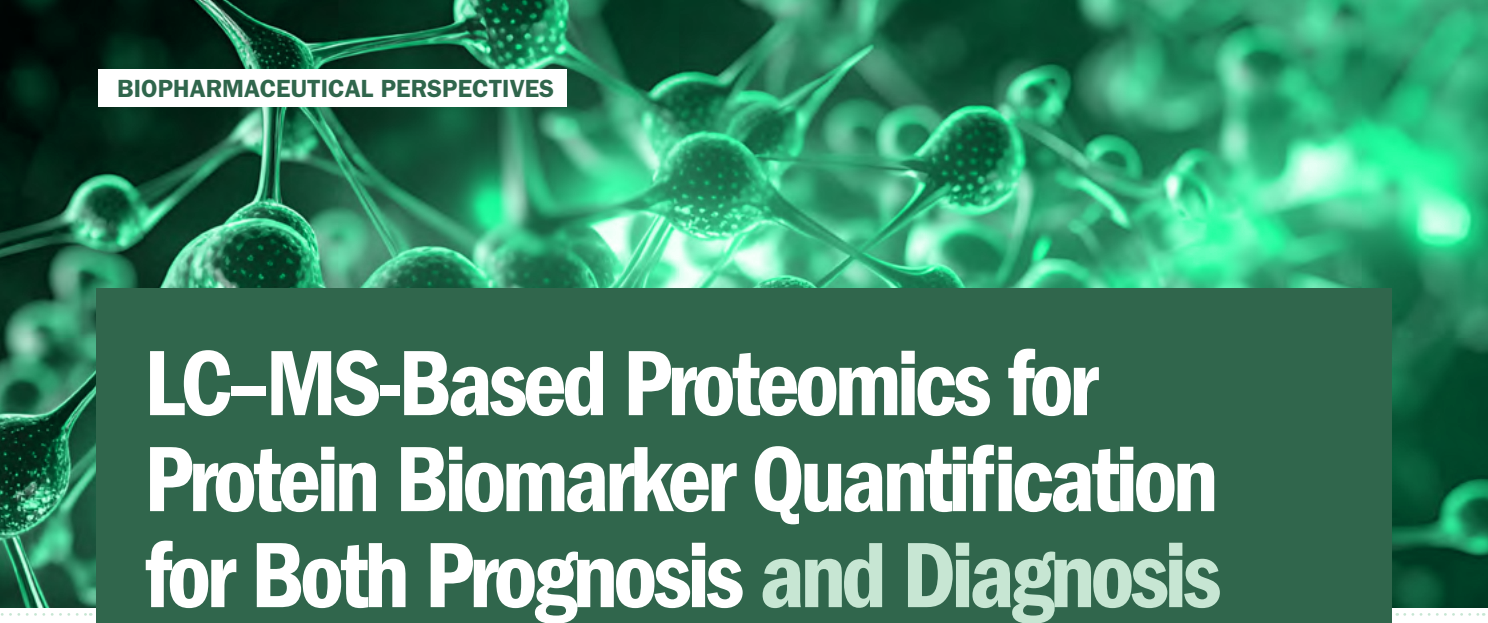

LC–MS-Based Proteomics for Protein Biomarker Quantification for Both Prognosis and Diagnosis in the Clinical Setting

Jared R. Auclair, Anantdeep Kaur, and Anurag S. Rathore

Biomarkers play a significant role in evaluating disease risk and treatment by acting as indicators of biological processes as well as pharmacological reactions to therapy. Candidate protein biomarkers are highly promising, specific biomarkers. These provide more functional information and reflect a more precise physiological cellular state. However, reliable and robust measurement of low-abundance protein biomarkers remains a challenge, primarily because of the presence of an array of post-translational modifications (PTMs). In recent years, advances in protein quantification technologies that provide higher sensitivity and specificity are expected to accelerate protein biomarker discovery and verification. In this column, we discuss the label-free and stable isotope labeling proteomics approaches that help in biomarker discovery. We also discuss the different enrichment techniques, such as stable isotope labeling by amino acid in cell culture (SILAC), isobaric tags for relative and absolute quantitation (iTRAQ), and tandem mass tags (TMT, that help in measuring low-abundance protein biomarkers.

BIOMARKERS PLAY A KEY ROLE in evaluating disease risk and the human body's response to therapeutic interventions. But what are biomarkers? *Biomarkers* are measurable indicators of biological processes or states, such as disease states, and can be specific cells, genes, hormones, or gene products, such as proteins. Protein biomarkers specifically provide insights into cellular functions and physiology, making them valuable in both research and clinical settings. But protein biomarkers do not come without their challenges. In particular, low-abundance proteins are difficult to detect because of post-translational modifications (PTMs) (1–3). PTMs are covalent processing events that occur on amino acids after their biosynthesis, changing the proteins properties and, in some cases, function (4). Thus, robust and sensitive measurement techniques are a necessity in detecting these low-abundance proteins (1–3). To that end, we will discuss mass spectrometry techniques using label-free and stable isotope labeling proteomics approaches. This will include different enrichment techniques, such as stable isotope labeling by amino acid in cell culture (SILAC), isobaric tags for relative and absolute quantitation (iTRAQ) and tandem mass tags (TMT), that help in measuring low-abundance protein biomarkers.

Protein Biomarkers

Why protein biomarkers? Along with protein biomarkers, RNA and DNA biomarkers are perhaps the most commonly used in diagnostics. However, RNA and DNA biomarkers provide information at the genetic level, which represents gene expression or the genetic code. Protein biomarkers, in contrast, give a more functional perspective by representing biological processes and functional states. DNA and RNA biomarkers do not give information about the physiological state of a cell, as they do not account for PTMs (or protein interactions), which often represent the true physiological state of a cell (5). That is to say, protein biomarkers provide more detailed functional, physiological, and pathological information about an organism, which makes them more promising as a diagnostic tool and for monitoring the efficacy of therapeutic interventions (5–7).

PTMs, as we mentioned, can influence protein structure and function in addition to potentially causing challenges in the detection and quantification of

low-abundance proteins. PTMs, such as phosphorylation, glycosylation, and ubiquitination, which change the molecular mass of proteins, can mask the presence of proteins in a sample and introduce variability in measurements. PTMs also create heterogeneity in protein populations, which complicates their measurement, not to mention that the PTM-modified protein itself may be the protein state of interest in detection. Thus, reliable and sensitive measurement techniques, such as mass spectrometry, are needed to advance protein biomarker discovery (5–7).

Challenges in Measuring Low-Abundance Protein Biomarkers

As we have discussed thus far, detecting and quantifying low-abundance proteins is a challenge due to such things as PTMs. In addition to PTMs, low-abundance proteins may be difficult to detect because they are overshadowed by highly abundant proteins. That is, highly abundant proteins dominate the available signals, which obscures or even masks the presence of low-abundance proteins (8–10). In proteomic approaches, it is particularly problematic because the protein concentrations vary greatly. For example, in a proteomic sample digested with trypsin, there are more tryptic peptides from the high-abundance proteins than the low-abundance proteins, causing low-abundance peptides to be masked. To detect these low-abundance peptides in proteomic approaches, more extensive and sophisticated chromatographic separation is needed. This reduces the dynamic range and biases identification towards the abundant proteins (8).

To overcome the challenges of PTMs and high-abundance proteins, improved sensitivity and specificity in protein quantification methods are needed. There are numerous ways to accomplish this, including selective depletion (high-abundance proteins) or enrichment techniques (low-abundance proteins) (8–10). Antibody arrays, ligand libraries, and chromatographic prefractionation can be used to reduce high-abundance proteins, whereas techniques that exploit the abundance-dependent Michaelis-Menten kinetics of tryptic digestion can be used to selectively digest and deplete abundant proteins (DigDeAPr) (8). In addition, the use of mass spectrometry-based proteomics with novel fractionation, enrichment techniques, and advances in data analysis, can be used to detect low-abundance proteins (8–10).

Advances in Protein Quantification Technologies

Over the last several years, there have been significant advances in protein quantification technologies that have positively impacted the detection of low-abundance proteins, and thus allowed for advances in protein biomarker discovery. We will briefly discuss two: *label-free proteomic approaches* and *stable isotope labeling approaches.*

Label-free proteomic approaches, as their name suggests, do not require the use of any labeling, but instead rely on the mass spectrometric signal's intensity or the number of spectra matching each protein (spectral count). Label-free methods allow for the analysis of any number of samples without the limitations of the number of available labels. They also enable large-scale comparisons, which makes them ideal for high-throughput studies, including high-throughput biomarker discovery studies. Because of the lack of labeling, these studies tend to be simpler (due to easier sample prep, as just one example) and more cost-effective. That said, label-free approaches do have their limits. They tend to be less reproducible and less sensitive than other techniques. In addition, variability in instrument performance and sample processing can introduce errors, thus affecting the accuracy of results, including biomarker identification (11–13).

Stable isotope labeling approaches, as you might have guessed by now, require the labeling of samples using stable isotopes. There are several different stable isotope labeling techniques used, including stable isotope labeling by SILAC, iTRAQ, and TMT. These techniques tend to offer more controlled and precise quantification of proteins and peptides by incorporating heavy isotopes into them. Briefly, SILAC works by growing cells in media containing isotopically labeled amino acids, resulting in near complete labeling of proteins. iTRAQ and TMT, on the other hand, utilize in vitro chemical labeling after proteolysis and allow for multiplexing, where many samples can be labeled and analyzed at the same time (higher throughput) (11–13).

Enrichment Techniques for Low-Abundance Proteins

Stable isotope labeling approaches, compared to their label-free counterparts, allow for enhanced sensitivity, specificity, and reproducibility, in particular when analyzing complex biological samples. This, however, comes at an increased cost, and is more time-consuming when compared to label-free methods. That said, the enhanced precision and reliability of these isotope-labeled methods make them ideal for biomarker discovery, specifically biomarker discovery of low-abundant proteins (11–13). Thus, let us take a closer look at each of these isotopic labeling techniques.

SILAC is an in vivo metabolic labeling technique that incorporates isotopically labeled amino acids (13C or 15N) into proteins during cell growth. In this method, cells are grown in media containing either light or heavy forms of amino acids and are spiked together before analysis. SILAC is often used to quantify protein expression levels across different conditions and is effective at studying dynamic biological processes, such as signaling pathways, due to its ability to detect changes in protein abundance in an accurate and reproducible way. In addition, SILAC can quantify low-abundance protein biomarkers because it minimizes variability due to sample processing. SILAC allows for direct comparison of protein expression across different cell states, making it an effective technique to monitor dynamic biological and disease states. As the name implies, labeling happens in cell culture, which can be a limitation. Not all cells are capable of growing in the necessary media, thus limiting its applicability. Also, it is not useful for tissue, organs, or other in vivo studies (14,15).

iTRAQ is an in vitro chemical labeling technique that enables the simultaneous quantification of proteins from up to eight different samples in one experiment. In iTRAQ, isobaric tags are attached to peptides at the N-terminus and lysine residues, which then release reporter ions during mass spectrometric analysis. The major advantage of iTRAQ is its ability to multiplex, allowing for the comparison of multiple samples aimed at assessing biological variability. A disadvantage of iTRAQ, which could have a significant impact on protein biomarkers, is reduced sensitivity due to sample complexity and interference from high-abundance proteins (14,15).

TMT is a similar technique to iTRAQ in that it allows for multiplexing, but instead of only 8 different samples, TMT allows for the analysis of sixteen samples in one experiment. Like iTRAQ, TMT labels peptides at their N-terminus and lysine residues and releases reporter ions during tandem mass spectrometry. As you might expect, the major advantage of TMT is its throughput, or its ability to analyze sixteen samples at once. TMT is also advantageous in characterizing complex biological samples, such as plasma, as it allows for the comparison of different conditions at the same time. This is particularly useful in analysis of clinical studies or biomarker discovery. The major disadvantage to TMT is the potential for ratio compression, where signal intensities from different samples may interfere with each other, thus providing less accurate quantification (14,15).

Future Directions in Protein Biomarker Identification

Several emerging technologies will have an impact on protein biomarker discovery. Next-generation proteomics techniques, such as single-cell proteomics, will allow for the analysis of proteins at an individual cell level. This will allow for a greater understanding of cellular heterogeneity, which is valuable in monitoring tumors or immune responses. Single-cell proteomics allows for the unique protein expression in each cell to be monitored and not lost in the averaging of expression across multiple cells (16–18). In addition to single-cell proteomics, miniaturization and automation will likely impact protein biomarker identification. As both suggest by their names, miniaturization of instrumentation will allow for point-of-care like protein biomarker characterization, and automation will potentially allow for more accurate less complicated assays (19).

In addition to new techniques like single-cell analysis, machine learning likely will have an impact on protein biomarker discovery. Machine learning will allow for the analysis and better management of large experimental datasets. This analysis will enable patterns and correlations to be identified that were not possible before. These new patterns and correlations can improve the sensitivity and specificity of biomarker detection, leading to more accurate diagnostics (16–18).

These emerging technologies will have a significant impact on accelerating the transition from discovery to clinical validation and application. For example, single-cell proteomics provides a more nuanced understanding of disease at the cellular level. This could lead to the identification of novel biomarkers that are more precise in monitoring disease progression or therapeutic response. In addition, improvement in sensitivity in mass spectrometry techniques could lead to the discovery of low-abundance biomarkers, permitting early diagnosis, intervention, and personalized treatments. Machine learning and other advanced computational techniques allow for the rapid analysis of datasets, enabling the identification of biomarkers with greater precision and speed. In both cases, the pipeline from discovery to clinical application could be streamlined, allowing for faster development of diagnostics and therapeutic targets, ultimately improving patient outcomes. Thus, the integration of novel techniques and novel computational tools likely will bridge the gap between research and clinical application (16–18).

When considering emerging technologies, however, it is important to consider potential limitations and challenges. Analyzing the vast amount of data from single-cell proteomics, even with machine learning, is a challenge. The complexity of proteome-wide studies and variability introduced by PTMs require novel computational approaches to ensure accuracy and reproducibility. Improving the sensitivity of mass-spectrometry-based proteomics is a necessity in detecting low-abundance proteins that are often the most informative biomarkers. Machine learning tools also need continued refinement to handle complex datasets and reduce noise without losing critical information. These and other emerging technologies hold great promise for clinical biomarkers; however, further development and refinement are needed to realize their use in a clinical setting (16–18).

Conclusion

Protein biomarkers are instrumental in being able to detect and diagnose diseases as well as to detect and monitor the efficiency of therapeutics. This is in no small part due to their ability to monitor complex biological processes and cellular states. The detection of low-abundance proteins, which are critically important in disease states, is a significant challenge in biomarker discovery due to post-translation modifications and high-abundance proteins. Highly sensitive and specific measurement techniques are needed to overcome these challenges and allow for the detection of low-abundance proteins.

Label-free and stable isotope labeling approaches play a role in addressing these challenges. Label-free methods are simple and cheap, whereas stable isotope labeling techniques are more precise and reproducible. In addition, isotopic labeling allows for multiplexing, which is an advantage when characterizing complex biological samples. In both cases, there are strengths and weaknesses, but the strengths have allowed for improved measurements of low-abundance proteins, improving reliable biomarker discovery.

Biomarker discovery and clinical diagnostics will be positively impacted by innovations in enrichment and quantification. Single-cell proteomics and machine learning will enhance the sensitivity of proteomic studies, allowing for more precise analysis of cellular heterogeneity and complex biological processes. New advances like these will accelerate the translation of biomarkers into clinical applications, which will lead to more personalized and effective medical interventions, improving patient outcomes. ■

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Applications of AI and GenAI to Help Optimize Purification and Yield of Antibodies From Plasma

Krish Ghosh, PhD, MBA
President
TCG Digital

How AI and GenAI can streamline the process of deriving antibodies from plasma

There are several steps in deriving antibodies from plasma, but each is critical in ensuring a safe, effective, and high-quality final product. *LCGC International* sat down with Krish Ghosh, PhD, MBA, President of TCG Digital, to discuss how artificial intelligence (AI) and generative AI (genAI) can be leveraged to optimize the overall process from plasma collection to storage.

LCGC: Can you describe the process used to derive antibodies from plasma?

GHOSH: Many companies are deriving antibodies from plasma to produce drugs, specifically for treating immune deficiencies. Deriving antibodies from plasma products involves several steps, from plasma collection to purification of the desired antibodies. There are essentially seven steps. First, plasma is collected from donors, where blood is drawn, separated, and screened to ensure there are no contaminants. The second step is plasma pooling, where plasma from multiple donors is combined into a single pool to get a broad range of antibodies and increase yield. The third step is fractionation, which has two components. One is precipitation, where plasma proteins are separated using techniques like ethanol fractionation. This can involve adjusting temperature and pH to precipitate different protein fractions. The other component is centrifugation, which separates the precipitates based on density. The fourth step, antibody purification, commonly uses Protein A affinity chromatography. Ion exchange (IEC) or size exclusion chromatography (SEC) are other purification methods. After purification is concentration and formulation. The purified antibody solution is concentrated using techniques like ultrafiltration. The antibodies are then formulated into a final product and tested for antibody activity to ensure sterility. Once quality control is complete, the purified antibodies are packaged under sterile conditions and stored, usually frozen, in warehouses. This process results in a purified antibody product for therapeutic purposes like drug production, diagnostic tests, or research.

LCGC: How do AI and GenAI models help drive these steps, and what differentiates them from traditional analytics methods?

GHOSH: We have publications describing how we've implemented advanced analytics solutions through an enterprise data lake for clients. The enterprise platform, tcgmcube, focuses on plasma pooling efficiency, donor plasma inventory management, yield prediction, manufacturing key performance indicator (KPI) monitoring, and the application of genAI. The outcome highlights significant advancements in production efficiency, data accessibility, and real-time decision-making. I'll review two examples, starting with data pooling. One company we work with sources its plasma in multiple centers, company-owned and third-party. Blood donors come, each batch is collected, and batches from different donors are assembled in a case. These cases are shipped to a warehouse, frozen, and stored. After plasma has been stored for three, four, or five years, it may lose effectiveness. This client wanted to prioritize older shipments and optimize RSV plasma use in certain pools. Creating multiple plasma pools simultaneously was important, with flexible criteria to choose which shipments, cases, and centers to include or exclude. This process required automation and digitization. There are also hundreds of customizable parameters in these models. Simple parameters include age, expiry date, and minimum unique donor constraints, and more complex parameters include plasma volume, weighted average titer, and center-specific volume contributions. To handle these parameters, we developed methodologies and algorithms, which ultimately led to the application of AI. We used a heuristic approach based on volume to determine center contributions and ensured adherence to specific percentage ranges, handling multiple centers with varying contributions. My second example is yield optimization. The production and manufacturing process takes, in this case, about 10 days and involves several steps, starting with plasma pooling and ending with the purified protein. You can't produce 160 kg one day, 80 kg the next, and 250 kg on the third day—that's not ideal. There's a range in which the yields must be consistent after 10 days, typically within 20 kg. Forecasting this accurately is difficult, so we used algorithms. We looked at key variables like blood collection centers and donor age. For predictive models, we examined target variables like bulk weight or chiroplasma and explored model types, basic linear regression and others, like XGBoost. The goal was to use machine learning (ML) to predict yield, and these methods, while complex, led us to AI.

LCGC: Can you provide an example where AI models demonstrated time reduction and cost savings?

GHOSH: This process enabled the optimization of plasma unit selection based on multiple criteria including age, adherence to the Code of Federal Regulations (CFR), expiry, and volume to ensure optimal plasma pools. The solution significantly reduced manual effort for pool creation, enhancing productivity and accuracy. It allowed for real-time adjustments to pooling strategies based on dynamic inventory levels, ensuring that production can quickly adapt to demand and supply condition changes. For example, if there's a demand for a drug that requires purified proteins for manufacturing, those proteins must be created consistently. But if demand exceeds supply, you need a strategy to increase production, prepare more manufacturing plants, and achieve visibility over the next 9 to 12 months. Implementing the plasma pooling process, which has historically been manual, immediately streamlined the supply chain function. These efficiencies included reducing FTE hours required for pool creation, ensuring the most efficient mix of plasma for each product, and achieving a standardization that manual pool creation cannot.

LCGC: Can you provide an example where AI models demonstrated an increase in profitability?

GHOSH: We developed different solutions for a company for over a year, such as plasma pooling, yield optimization, etc. This company has been profitable in recent quarters. Analysts expect this to continue, which has given tremendous confidence to investors. This is reflected in its market capitalization, a function of profitability and investor confidence; theirs grew fivefold over the past year and a half.

LCGC: How does this help in the quality control process?

GHOSH: One element we focused on was KPI monitoring and outlier detection. The tcgmcube has dynamic KPI dashboards that provide real-time visibility into KPIs across the manufacturing process. This system employs advanced statistical algorithms for outlier detection, flagging deviations from expected performance ranges. This solution streamlines the operational process and helps maintain compliance, ensuring that every batch meets the highest quality standards. Implementing the business intelligence features offered by tcgmcube has allowed the company's management to use a single dashboard to monitor a range of KPIs across the manufacturing process, which were previously housed in multiple applications. The data pipeline has also reduced the manual effort previously required to create reports and charts used to identify manufacturing trends.

LCGC: Can these models help management in better understanding the output in the short to mid-term range for planning of raw material and resources accordingly?

GHOSH: I'll come back to batch yield prediction. The platform integrates advanced ML models to predict production batch yields within that 20-kg range. This data must be handled accurately, as deviations can cause serious issues, sometimes requiring reports to regulatory authorities. The system leverages historical data and real-time process parameters. This predictive capability allows adjustments for future production batches, optimizing yields and reducing waste. By using advanced ML models to forecast yield, the company can now better plan raw material procurement, schedule production runs, and manage logistics, leading to improved operational efficiency and cost savings. This approach enables informed decision-making and optimization strategies, driving improved batch margins. It provides visibility into a 9- to 12-month window of the manufacturing process—critical for the continued growth of a product portfolio.

LCGC: Do you have any final thoughts to share?

GHOSH: Large language models are widely used today, with many open-source companies providing these services. Through LabVantage Analytics, powered by tcgmcube, we have developed private or local large language models that leverage the capabilities of larger models but are tailored to a company's specific documents. For example, one company had thousands of documents to mine for key information. Previously, they would have hired people for several months to complete this task. Our model, however, scanned those documents, gathered the necessary information, and brought in references. These references give the end user confidence, as they can verify information. While no model is 100% accurate, we've achieved a very low error rate. Instead of hiring 10 people for six months, this local large language model provides answers in minutes. This capability extends beyond manufacturing; it can be applied across the entire drug development continuum from discovery and animal testing to clinical trials, manufacturing, and commercial operations. Enterprise search using genAI is becoming more common, but it also helps companies apply AI to their own data, preserving intellectual property and ensuring sensitive information is protected. The genAI chatbot we developed for this company replaced manual search processes, which weren't sustainable. This client's adoption of LabVantage Analytics, powered by tcgmcube, is an important milestone in biopharmaceutical manufacturing, setting new standards for efficiency, productivity, and innovation. Our solution successfully addresses key challenges, such as integrating disparate data sources, breaking down organizational silos, and scaling data architecture for future growth. The value delivered through this digitization process exemplifies the potential of AI and advanced analytics to drive the next wave of innovation in biopharmaceutical manufacturing.

Sample Preparation with Molecularly Imprinted Polymers (MIPs)

Mary Ellen McNally

The first report of using molecularly imprinted polymers (MIPs) as a selective sorbent in sample preparation was in 1994 for the solid-phase extraction (SPE) cleanup of urine to analyze pentamidine. Now, 30 years later, the applications for MIPs are widespread. The molecular recognition abilities of these stable polymers offer high selectivity for a sample preparation method. MIP synthesis is inexpensive, easy, and a clear alternative to the use of natural receptors. Sample preparation techniques using MIPs are broad. Beyond SPE, MIPs are also used in microextraction, matrix solid-phase dispersion, coated fibers, monoliths, and stir bar sorptive extraction, to name a few. This article will describe the current highlights of using MIPs in sample preparation.

WHAT IS A MOLECULARLY IMPRINTED POLYMER (MIP)? Simply, it is a tailor-made polymer which recognizes an analyte of interest, following the same principles of the lock and key enzyme mechanism. Many recent advances have brought MIP technology to a wider audience, specifically for sample preparation, and applications now abound. If the inception of MIPs is taken as Sellegrin's landmark paper in *Analytical Chemistry* on the analysis of pentamidine (1), this is a 30-year-old technology, and even with the breadth of publications on preparation and applications, it has been slow to develop on a commercial scale. Likely, this is because producing a tailor-made polymer is a tedious and time-consuming process. Has the technology reached the precipice of commercial viability? MIPs' time may finally be here.

How MIPs Are Made

The most common process to make a MIP through polymerization is called the *noncovalent process*. Procedurally, the template molecule (analyte of interest) and functional monomers are put in a solution (the solvent is called the *porogen,* so named because it infiltrates the pores) and complexed via noncovalent bonds. A cross linker and an initiator are then added to initiate polymerization of the monomers around the template molecule. The whole system is washed extensively to remove the template molecule, and cavities or binding sites are then created. The binding sites are similar in size, shape, and position of functional moieties to the target molecule. The necessity of a large number of organic solvents under acidic or basic conditions is a limitation to the noncovalent MIP polymerization process. Relatively speaking, the experimental approach is simple; monomers of many types are readily available for a variety of desired template analytes. The template-monomer interactions are equilibrium-driven in noncovalent processes, and typically, a monomer content is added in excess; this can create non-selective bonding sites in the MIPs (2).

Two other processes are used to make MIPs: *covalent* and *semi-covalent*. The covalent approach, which can be limited, was introduced by Wulff and Sarchan (3) in the early 1970s, but not used for sample preparation methods until much later. It differs from the noncovalent approach in that the bonds between the template molecule and the monomers are reversible covalent bonds in the first step of the polymerization process. To remove the template molecule, the covalent bonds that were formed need to be broken, and mild conditions are required. These conditions are often difficult to determine, which is the most prevalent limitation to the covalent approach.

In the semi-covalent approach, before polymerization, the target analyte is covalently bound to the chosen functional monomer, or the imprinting step. The rebinding step relies only on classic noncovalent interactions, such as hydrogen bonding and hydrophobic, as well as ionic interactions.

The polymerization technique most frequently used for all three of these polymerization processes is bulk polymerization; however, if regularly shaped particles are required, suspension or precipitation polymerization processes are better choices (4). Bulk polymerization includes a milling step,

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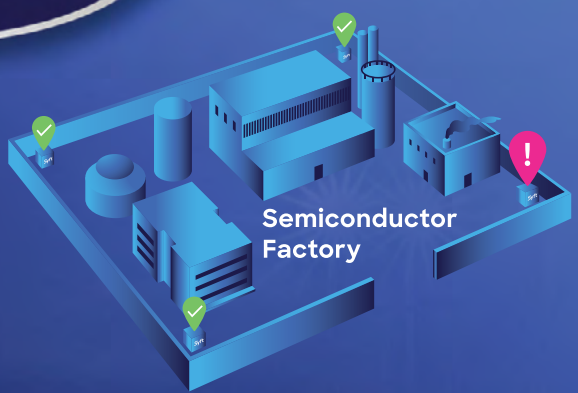
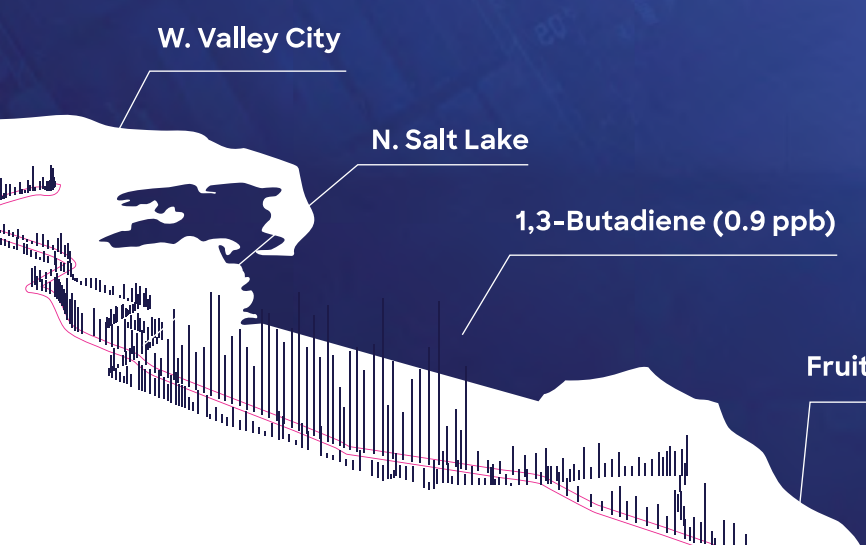


Mobile Monitoring



which can produce irregularly-shaped particles and destroy binding sites. Suspension and precipitation polymerization, by nature of the processes themselves, generate spherical MIP particles. Spherical particles provide optimal surface contact for the target analytes; irregular surfaces are less ideal.

It is important to note that not all MIPs are made with a single target molecule. There is a double template approach, where two analytes of interest are used and complex sample extraction is made easier. Reports have shown higher adsorption capacity and a reduction in sample preparation steps when double templates are used (5). Dummy molecules can also serve as the targeted molecule. Generally chosen by their resemblance to the specific desired target in terms of shape, size, and functional groups, the advantage of using a dummy is the elimination of high recovery values caused by residual targeted molecules not removed with the wash step. This potential interference is more important when low-level quantitative analysis is the end analysis goal.

From an environmentally conscious perspective, synthesis procedures used to develop MIPs have only recently considered the 12 principles of Green Chemistry, and many improvements can be made. MIPs frequently use chemicals such as acrylic acid, vinyl pyridine, and styrene as the functional monomer, and ethylene glycol dimethacrylate or divinylbenzene as the cross linker. The reduction of organic solvent use and the choice of greener polymerization strategies, as well as greener poragens, are just starting to be mentioned in the literature (4,6). The use of ionic liquids and deep eutectic solvents are on the rise, as is the use of biopolymers and natural monomers.

MIP in SPE

Batch SPE, conventional offline SPE, and online SPE have all used MIP technology (2). Offline SPE with MIPs simply substitutes the classical C-18, C-8, and other conventional phases in the cartridge with the MIP phase. The column is then loaded with the sample after conditioning, washed with the selected solvent, and the target analyte is eluted. The extract is then analyzed chromatographically, as in conventional SPE. Online SPE places a cartridge or column loaded with the prepared MIP in a direct line between the injection port and the chromatographic column when a switching valve is turned. In this way, the sample can be loaded onto the MIP cartridge, and then rinsed with the mobile phase. Depending on the valving configuration, the MIP cartridge can be rinsed with a different eluent to eliminate interferences or a sample matrix before being introduced to the chromatographic column. Batch SPE has been replaced by the offline and inline procedures described above, except for the case of magnetic molecularly imprinted particles (MMIP).

Magnetic Molecularly Imprinted (MMI) SPE

The main advantage of magnetic molecularly imprinted (MMI) polymers is the enhanced selectivity for a target molecule available through MIP technology coupled with the ability to be quickly isolated from sample matrices with an external magnetic field (6). The procedure to make these MMI materials consists of a sol-gel reaction with different types of magnetic sorbents; Fe_3O_4 is a frequently used magnetic sorbent. Particles, nanotubes, and nanosheets have all been tried and reported as potential magnetic sources. The goal of these systems is to maintain enough magnetic properties that a magnet can be used to extract molecules of interest after the separation occurs on a material of high specific surface area which still contains adequate molecularly imprinted binding sites or cavities for the target analyte.

MIP in Dispersive SPE (dSPE)

Dispersive SPE (dSPE) is conducting by adding the adsorbent particles directly to the sample matrix, allowing an extraction to occur over a selected extraction time, after which the adsorbent is recovered either by centrifugation and filtering, or, if a magnetic core particle was used, magnetic field. A desorption step follows with a suitable solvent. Choice of desorption solvent, extraction time, and stirring rate, all control the extraction efficiency, and therefore must be determined. This is a very common approach used in MIP sample cleanup. For MIPs prepared by bulk polymerization techniques, a specific amount of the MIP is added to the sample matrix, and the process ensues as for standard dSPE. Pichon and associates reported that over 50% of MIP sample extraction was conducted by dispersive SPE. Since single lab-made MIP materials are the most common, dSPE is the easiest approach to use for sample cleanup (6).

MIP Thin Films and Fibers

High-throughput analysis and lower consumption of organic solvents than conventional SPE methods have been obtained using in situ synthesis of MIPs on the surface of microfiltration glass fiber membranes in multi-well filter plates or onto polyethylene frits (4,7). Similar surface imprinting methods have been utilized for MIP-coated fibers and stir bars for SPME and stir bar sorptive extraction (SBSE). Activation by a silylation reaction, followed by immersion in the prepolymer solution, enables the polymerization reaction to take place on the fibers or glass magnets. The coating thickness and porosity of the final imprinted fiber are controlled by the polymerization time and the choice of porogen. There are polymerization reactions that allow for better control of the thickness of the polymer; for example, surface reversible addition–fragmentation chain transfer (RAFT). Additional work on these techniques is needed to advance MIPs on thin films.

MIP Monoliths

Fibers, disks, and columns have been prepared as monolithic imprinted polymers for sample extraction (8). Monoliths can be both organic polymer-based or inorganic-based materials. Both types offer the absence of frits to retain the column material in the column. The monoliths are a single piece of material in the column, fiber, or disk, and show good permeability, low pressure drop, and allow for preparation within the structures. The selection of the porogen and the template need to be chosen carefully during preparation, since both affect the final morphology of the monolith, as well as the extraction effi-

ciency. Additional flexibility, high stability, and enhanced mechanical properties have been seen when hybrid organic-inorganic MIPs were created. MIP monoliths have potential for both proteins and peptides in bioanalysis.

MIP in SPME

As early as 2001, solid-phase microextraction (SPME) tubes have been coated with MIPs (6), but more applications have been seen from 2016 to the present. Typical SPME tubes are coated with polydimethylsiloxane (PDMS), polyacrylate (PA), or divinylbenzene (DVB). Both stainless steel and silica glass have been used to create MIP-based SPME tubes, the capillaries (200 to 300 µm diameter) are coated by immersing them in a larger tube with a diameter of at least 1 mm (6). This larger tube is filled with the polymerization solution and serves as an outer mold to contain the MIP layer. As for all extraction techniques, the measure of effectiveness is based on temperature, extraction time, the sorbent itself, and the analyte. For MIPs that are not stable at elevated temperatures (the temperature of a GC inlet), desorption can take place in a liquid solvent, which is then transferred to a sample vial, and a standard injection is made. For trace level analysis, this is less desirable, as dilution can result using a solvent for the desorption step. Frequently, a non-imprinted polymer (NIP) is used as the control. With an NIP, no target analyte is added in the polymerization process, but the process is identical to the one used when the MIP is created. Precision for fibers produced using this coating polymerization process have been reported at less than 5% for one fiber used repeatedly, and less than 6% for five fibers used for the same target analyte. Reusability of the fibers showed they remained effective up to 100 cycles.

MIP SPME fibers prepared in this way showed higher extraction efficiencies than fibers based on the chemistries of PDMS, PA and DVB, but the extraction phase amount on the MIP SPME phases was higher based on the larger amount of extraction phase present—this trend is, therefore, as should be expected (7).

Commercial Availability

Figure 1 from (6) shows the groups of molecules for which MIPs have been developed during the two-year period from 2018 to 2020; development has touched every area of sample preparation from simple and complex matrices. However, most of the MIPs that have been created were generated for use in individual labs by select researchers. The commercially available MIPs, although growing, remain more limited. Supelco, with their SupelMIP SPE cartridges, currently offer the following target analytes: clenbuterol, beta agonists, beta blockers, beta receptors (which are a combination of beta agonists and beta blockers), and tobacco metabolines, such as 4-(methynitrosamino)-1-(3-pyridyl)-1-butanol, (MMAL), N-nitrosonornicotine (NNN), 4-(methynitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N '-nitrosonanabasine (NAB), and N'-nitrosoanatabine (NAT). They also have MIP SPE cartridges for chloramphenicol, triazine, riboflavin, and amphetamines. Likewise, Affinisep have developed their own separate line, AFFINIMIP SPE, particularly for food and pharma analysis. Thus far, their target molecules include mycotoxins (patulin, zearalenone, ochratoxin A, fumonisins), drugs (amphetamines), and endocrine-disrupting compounds (bisphenol A and estrogens [estradiol]).

Based on all the developed MIPs in the literature, the number available for commercial purchase is relatively small. There is good news, however—both Affinisep and MIP Technologies, a subsidiary of Biotage, will custom make MIP phases for desired target analytes. This service opens the field to those in commercial laboratories that do not have the time or resources required to develop MIP separation phases for SPE.

Summary

Without a doubt, MIP technology offers an exclusive selectivity for analytes of interest; simply, the MIP is designed specifically for the target analyte. This selectivity comes at the cost of a labor-intensive process to produce the MIP. Extensive research and a wide variety of sample preparation options are available, and current efforts are focused on making the established synthesis and polymerization pathways simpler and more compliant with the principles of Green Chemistry. In addition, commercial offerings for high-value target analytes and MIP synthesis for hire should broaden the viability of MIPs in the routine laboratory. After 30 years, this technology appears to be on the precipice of making a significant contribution to the routine analytical laboratory. ■

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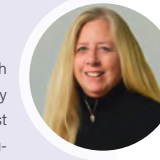

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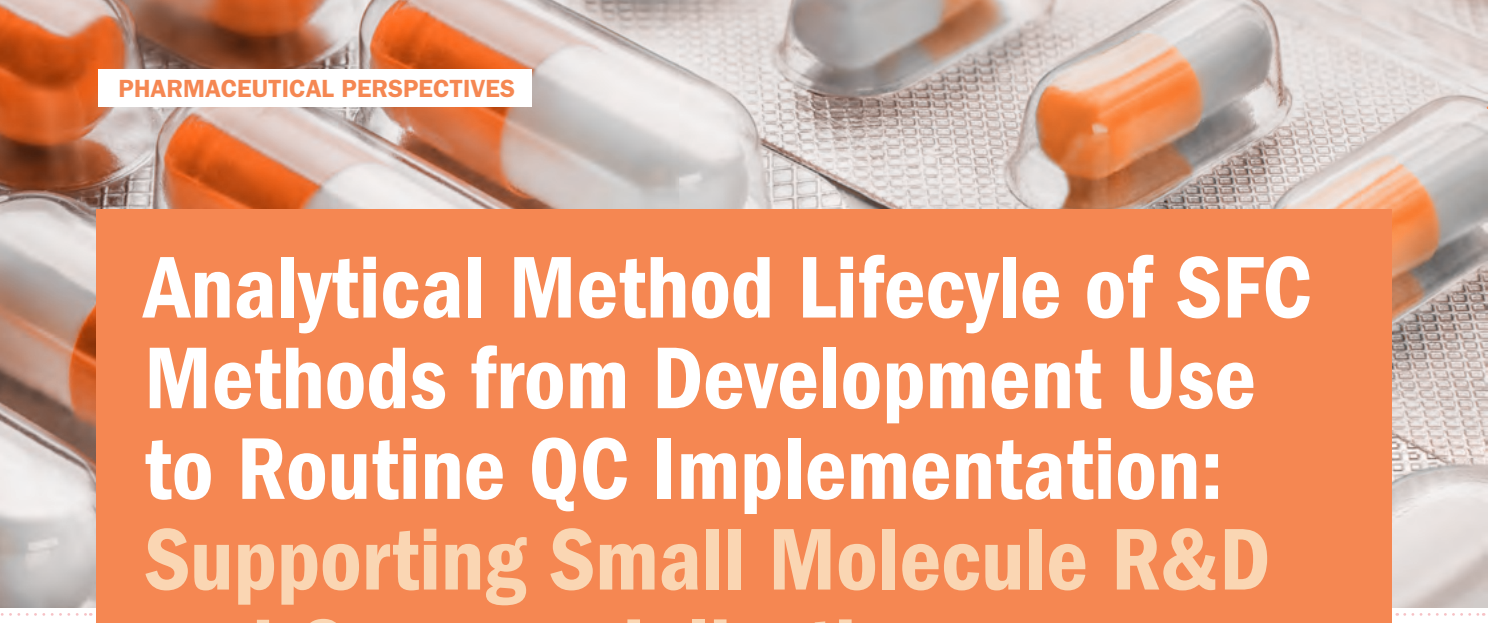

Analytical Method Lifecyle of SFC Methods from Development Use to Routine QC Implementation: Supporting Small Molecule R&D and Commercialization

Claudio Brunelli and Wayne Callar

Selection of the correct analytical technique from the outset of method development is key in securing the optimal and most robust method to be used throughout a product's development journey from R&D to commercialization. In this article, we review some key considerations for chromatographic technique selection and method development across the full drug process–from early-stage active pharmaceutical ingredient (API) synthesis to routine commercial release activities. We describe the implementation of supercritical fluid chromatography (SFC) in the pharmaceutical industry, especially for chiral, water sensitive analytes, and low to high LogP and LogD hydrophobic compounds, while demonstrating high levels of method robustness and support of "green" analytics.

THE PRODUCT DEVELOPMENT lifecycle in the pharmaceutical industry can be divided into three distinct phases: discovery, development, and registration and manufacturing.

In the discovery phase, the analytical impetus is normally on speed and throughput. However, once a candidate has passed the proof-of-concept stage selected for development and used in clinical trials, the analytical methodology that accompanies the development should take into consideration the overall method lifecycle, which may extend through to routine product manufacturing support. Such analytical methods need to meet requirements of United States Pharmacopeia (USP) resolution (*USP <621>*) (1–2) and sensitivity (limit of quantification [LOQ]and limit of detection [LOD]). Eventually, the full development to registration and manufacturing has to guarantee robustness and ruggedness, ensuring good transferability of the method. Meeting sustainability goals is also a key driver that should be considered, as these analytical methods could be used for a considerable period of time (in the order of years).

The required performance of the analytical methodology, defined in the product analytical target profile (ATP) serves as the starting point for method technique selection and method development (3). The correct marrying of "method requirement," "analyte properties," and "technique capability" is what ultimately ensures robustness of the method. Specificity, sensitivity, accuracy, and reproducibility are typical parameters that measure the performance of the analytical method. The technique should be able to meet these performance criteria versus the analyte, instrumentation and transferability across laboratories, departments and business units.

Prior knowledge or experimental verification relating to the performance of available analytical technologies enables the analyst to select the most appropriate technology to meet the requirements of the ATP. Where more than one technology has been demonstrated to meet the ATP, a review of business requirements (such as throughput, automation, downstream availability) should be performed to aid selection. Procedure specific performance indicators should be defined. These can include critical resolution of defined impurities (also known as the key predictive sample set [KPSS]), as well as specific sensitivity requirements, and this becomes the starting point of method development.

Discussion

The choice of analytical technique plays a critical role in the success of the analytical method. The key requirements from the method are defined in the ATP, and the analytical technology needs to be able to satisfy all these requirements—not only during the development, but also throughout the project lifecycle from development to commercialization. If a non-robust or poorly optimized method is developed in the first instance, subsequent method redevelopment will be needed downstream, generating a cumulative resource burden.

In the pharmaceutical industry, reversed-phase liquid chromatography (RPLC) is by far the most widely employed analytical technique, and it carries several advantages over alternative techniques, including analyst familiarity and versatility for many different molecular classes. Although alternative separation modes are often proposed during development, technique selection is often driven by business considerations, such as instrument availability across downstream or partner laboratories. In agreement with ICH Q14 (4), other techniques should be prioritized for method development if they provide higher method robustness, instrument ruggedness, validation success, and transferability.

Although the analyst can rely on the availability of a wide toolbox of separation techniques (including, but not limited to, RPLC, supercritical fluid chromatography [SFC], gas chromatography [GC], ion exchange [IEX] chromatography, capillary electrophoresis [CE], hydrophilic-interaction chromatography [HILIC], normal-phase liquid chromatography [NPLC], and size-exclusion chromatography [SEC]), each of them can perform optimally only within defined constraints of physicochemical properties, such as pK_a, logD, logP, or solubility. Each chromatographic mode has an operating "sweet spot," and, within this sweet spot, the optimal performance and robustness for the analytical method is unlocked. In this article, we discuss the application of SFC identified as the optimal approach to meet ATP requirements in various measurement challenges.

The Road of Enabling SFC from Development to QC

As discussed earlier, SFC may be the first choice for analytes that have compatible physicochemical "sweet spots," for example:

Retention:

- Analysis of polar compounds, (LogD <-1): While SFC provides adequate retention, RPLC risks eluting such compounds with the solvent front unless appropriate fully aqueous-compatible stationary phases are selected or ion pairing reagents are utilized. This leads to additional method complexity and often require additional method controls.

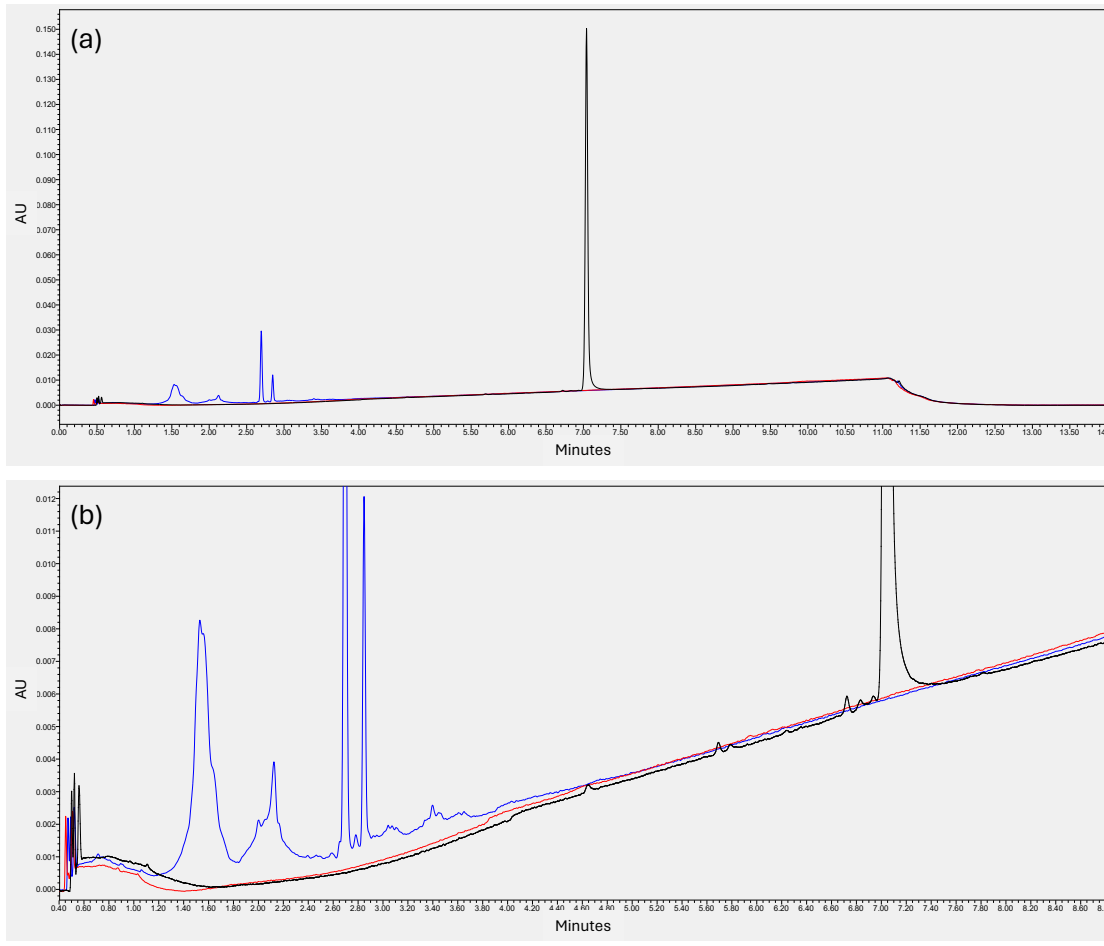

FIGURE 1: Overlay of the SFC chromatograms of an API (black trace), its formulation excipients (blue trace), and the sample diluent (DCM) – blank (red trace). (a) full scale, and (b) zoom (Torus DIOL (100 x 3.0 mm, 1.7 µm), methanol 10 mM ammonium formate (2%, hold 0.5 min, to 42% in 10 min), 40 °C, 120 bar, 1.7 mL/min, 2 µL, 292 nm).

- Hydrophobic compounds (LogD >4): With RPLC, such analytes are typically eluted later in the gradient... or not at all.

Solubility:

- SFC is a good technique for analyzing lipophilic analytes with high solubility in organic solvents (and poor solubility in water). Selecting RPLC for such an application elicits a risk of precipitation (column blockage), diluent or mobile phase incompatibility (potentially leading to poor peak shapes), insufficient column loading, or carry over.

Stability:

- The "water-free" mobile phases employed with SFC offer the advantage of being the ideal choice for the analysis of water labile compounds.

Selectivity:

- SFC has useful orthogonality (chromatographic selectivity) to RPLC. This alternative orthogonality has been shown to offer superior positional isomer selectivity, which can be useful in resolving difficult critical pairs (4).
- SFC is Pfizer's (and many other companies) default choice for chiral separations.

The Use of SFC–MS in an Open Access Environment

In our laboratories, an open access (OA) SFC–MS instrument has been in use since 2021 by both chemists and analysts. The availability of easy-to-use generic method screens (chiral and achiral) facilitate high throughput screening of a large number of process chemistry samples.

In 2022, over 3000 samples were analyzed on this open access SFC. The OA SFC screen has significantly decreased method development time, and, by incorporating SFC earlier on in the development workflow (at the initial process chemistry stage), has led to the organic growth

of the technique in the department. The inclusion of an open access SFC has therefore lowered the energy-barrier for wider SFC application in development at Pfizer.

SFC for Drug Product Development

Because of its applicability to analytes with low solubility in aqueous environments, SFC also is used in drug product (DP) analysis. The application detailed here is for an injectable DP that required an oil-based formulation. Castor oil, soybean oil, sesame oil, and oleic acid were explored by the formulation team as potential excipients for an injectable solution of an Active Pharmaceutical Ingredient (API). A risk assessment of a potential RPLC method highlighted several risks, such as the accumulation of the oil matrix or risk of precipitation of the API on column, the risk of inaccurate analyte recovery, intensive sample preparation, tighter system control requirements, and higher maintenance burden. With SFC being a "water-free" chromatographic technique, and with the API and formulation oils being soluble in organic solvent and CO_2 mobile phases, most of these risks were mitigated. On a polar stationary phase, the API peak eluted in the middle of a 2% to 42% organic modifier gradient. As the formulation oils were unretained, good separation of the oil matrix and the API was achieved, with no impurity co-elution issues and with simpler sample preparation than the RPLC method.

Figure 1a and 1b shows the overlay of the chromatograms of API (black trace) dissolved in dichloromethane (DCM, red trace) versus the formulation matrix oils (blue trace). Acetone was later selected as the method diluent to replace DCM as a "greener" option (5).

The drug product application of SFC detailed above started with a risk assessment of the sample characteristics versus the ATP. It is an example of a science-based approach to successful method development. The method development took no more than one day, compared to several weeks dedicated to RPLC method development and troubleshooting. This highlights the alternative utility of SFC in pharmaceutical analytical laboratories with challenging matrices.

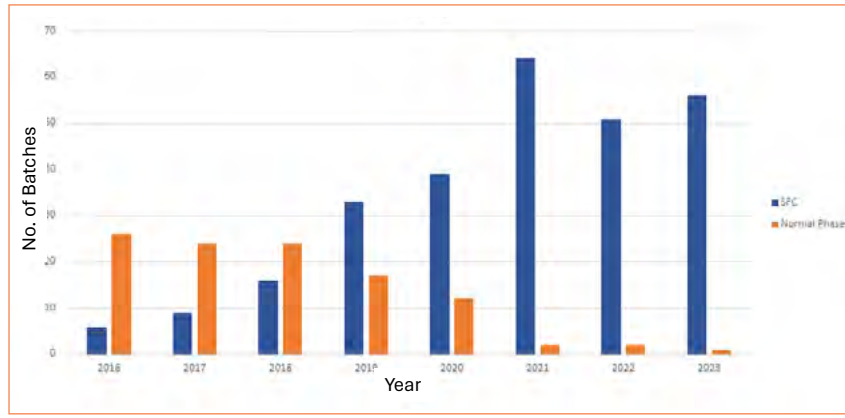

FIGURE 2: SFC vs. NPLC in Pfizer, GMP Analytics (Sandwich) for release of Clinical API, Intermediates and IPCs. Bar graph shows the number of batches tested at Pfizer's API clinical manufacturing (Sandwich, UK R&D) site from 2016 to 2023 by SFC (blue bars) and by NPLC (orange bars).

SFC in the QC Laboratory: API Manufacture and Release Testing

The superiority of SFC for chiral separations is well known and widely described in the literature (6–9). SFC has been the technique of choice for decades in compound discovery departments and for preparative and purification separations. It is only recently that SFC technology became reliable enough that its use could satisfy the strict requirements for validation, method transfer, and ultimately implementation in QC laboratories. Several reports have been presented in the literature highlighting GMP applications in recent years (10–13).

Implementing modern SFC instrumentation in QC laboratories has added a new platform for the chiral control strategy of APIs which were predominantly being analyzed using normal-phase LC (NPLC). Although the use of NPLC for chiral measurements can be satisfactory, because of long analysis times and toxic or non-green mobile phases, incorporating organic solvents such as hexane or heptane, it is rarely the optimum separation approach.

Figure 2 displays the increase in number of batches tested with SFC as part of Pfizer's API clinical manufacturing control strategy (Sandwich, UK R&D site) from 2016 to 2023. As can be seen, SFC has replaced NPLC for clinical API manufacturing support almost entirely over this timespan.

The confidence in the robustness of SFC methods created during development, allowed Pfizer to transfer SFC methods to commercial operations. Between 2016 and 2020, SFC chiral methods were redeveloped to NPLC equivalents at point of registration or commercial transfer because of a lack of SFC systems in manufacturing units. Since then, three marketed products have been registered with SFC methods as part of their control strategy:

- **Abrocitinib (cibinqo):** Determination of process impurity content in water-sensitive intermediate by SFC where NPLC gave poor and unreproducible chromatography.
- **Ritlecitinib (litfulo):** API identity and chiral purity evaluation by SFC.
- **Nirmatrelvir (paxlovid):** Determination of API stereoisomer content by SFC.

The transfer of abrocitinib in 2020 from clinical to commercial manufacture opened the door for SFC to Pfizer's commercial manufacturing partners.

Two subsequent commercial chiral

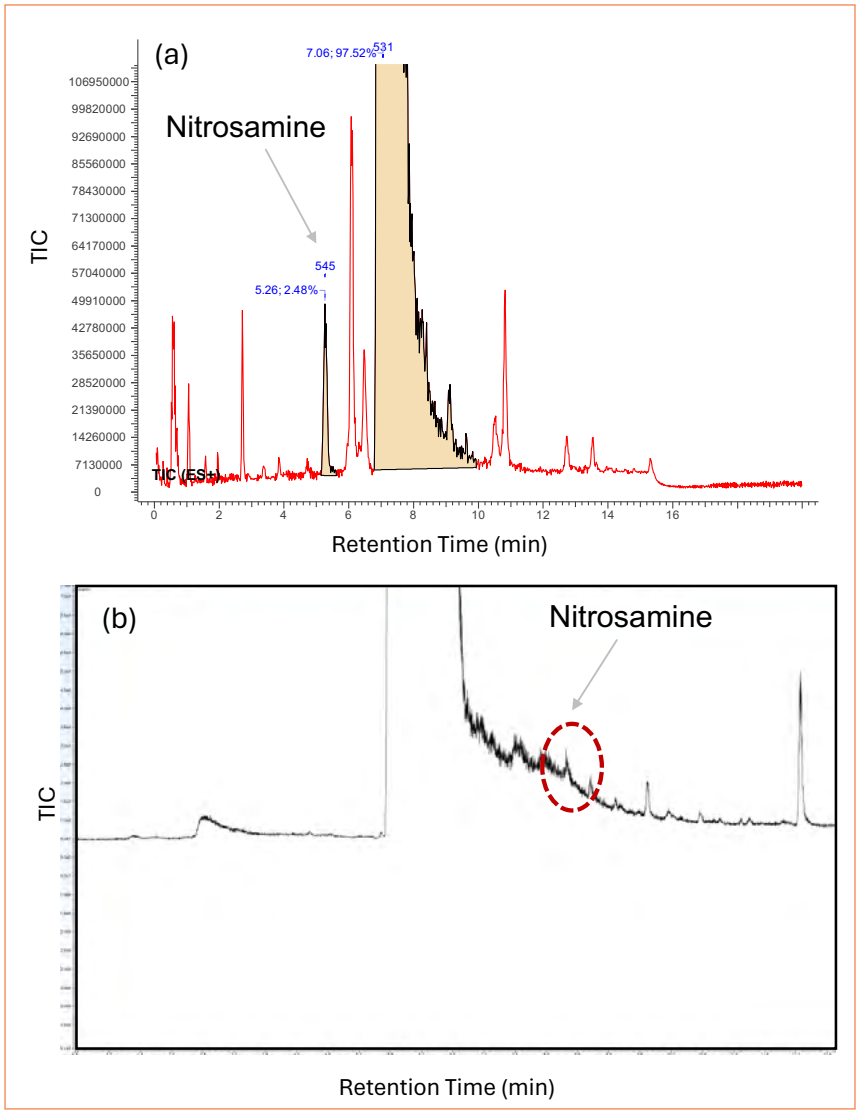

FIGURE 3: Total ion chromatograms (TIC) – API (30 mg/mL) spiked with NO-Am (0.05 mg/mL) for (a) an SFC–MS (Torus DIOL (100 x 3.0 mm, 1.7 µm), methanol 10 mM ammonium formate [5% to 20% in 20 min], 40 °C, 120 bar, 2.0 mL/min, 10 µL); and (b) LC–MS, RPLC (Zorbax Eclipse Plus [100 x 2.1 mm, 1.8 µm], MPA: 10 mM ammonium acetate, pH 4.5, MPB: acetonitrile, gradient 20% hold 2 min to 80 % in 13 min, 0.3 mL/min, 40 °C, 10 µL injection vol.) of an API spiked with its related nitrosamine.

API methods were successfully transferred to Pfizer commercial API sites, without method transfer issues. Furthermore, the SFC methods were well received by the QC teams.

Since its implementation in the Ringaskiddy (Ireland) API manufacturing site, over 110 individual release runs were completed using SFC since 2020, over half of which were for Nirmatrelvir. Modern SFC instrumentation has proven to be very reliable in this environment.

Nitrosamine Determination

In 2020, the FDA published guidance (14) discussing the risk of nitrosamine drug substance related impurities (NDSRIs) and mitigation strategies. In 2023, the FDA published recommended acceptable intake limits for these mutagenic impurities (15). Although a risk assessment and mitigation approach can be taken for the control of NDSRIs, identification and quantitation by analytical methods are still required to appropriately support the risk assessment. Here, we consider SFC as a technique to aid in the control strategy of NDSRIs (16).

Sensitivity to low- or sub-ppm levels are required to reliably determine levels of NDSRIs, often requiring mass spectrometric (MS) and tandem mass spectrometric (MS/MS) detection. While nitrosamines are often analyzed by GC or RPLC, SFC can also be considered for the following reasons:

1. **Orthogonal selectivity to RPLC:** Nitrosamine derivative of the API often are eluted after the API with RPLC, whereas they often are eluted prior to the API using SFC which can, in turn, aid quantification.
2. **Compatibility of SFC with MS detection**
3. **Often enhanced MS sensitivity because of simpler mobile phase desolvation**
4. **High on column drug loading:** High concentration samples are possible through dissolution in organic solvents, such as acetone. These strong organic strength solutions are highly compatible with SFC.

Figure 3 shows the total ion chromatogram (TIC) plots of an (a) SFC–MS chromatogram, and (b) RPLC-MS chromatogram. The nitrosamine derivative is eluted before the main component in SFC–MS providing a reduced risk of matrix effects in the ion source and improved quantification.

Conclusion

Systematic science-based method development is essential to ensuring analytical methods can successfully support drug development through the entire lifecycle. We have shared a series of applications demonstrating successful method development and validation with SFC. We have demonstrated the application of SFC in QC and commercial manufacturing support and highlight how the technique can be used beyond discovery applications. Additionally, through these examples, we have shown SFC can provide a higher degree of speed, robustness, and simplicity over alternative or more established techniques.

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A Green Analytical Method for Simultaneously Determining Plasticizers Residues in Honeys from Different Botanical Origins

Interview by John Chasse, *LCGC International*

Recent research by the Analytical Chemistry Group of the University of Valladolid (Spain) focused on determining nine plasticizers in honey samples by gas chromatography–mass spectrometry (GC–MS). While the team points out that there have been studies conducted previously focusing on the assessment of other contaminants in honey (such as metals, pesticides, antibiotics, and veterinary drugs), there are comparatively few that have been done specifically regarding plastics. *LCGC International* spoke to Silvia Valverde and Ana María Ares of this group about their research and the resulting article.

You mention in your paper (1) that there have been few studies focused on assessing the presence of plastic contaminants in honey in the past. Why do you think this is? Are there any that were especially inspirational in your research?

Despite plastics having been used for decades, our awareness of their pollution and widespread presence is relatively recent, as is our understanding of their adverse health effects. The studies conducted by Peñalver and associates (2), Kartalovic and co-authors (3), and Di Fiore and associates (4), served as inspiration for this research. These studies have played a crucial role in advancing research on the presence of contaminants, particularly plastics, in diverse environmental and food contexts, such as honey.

While it goes without saying that we shouldn't want plasticizers in our honey, do their presence or other environmental chemicals affect the bee, the hive, or the honey in any way?

The presence of plasticizers and other pollutants in honey can negatively impact bees and the hive by weakening their immune systems and interfering with their behavior and reproduction patterns. This can lead to a decline in the beehive population. Additionally, these contaminants can compromise the quality and safety of honey for human consumption by altering its taste and potentially making it toxic. Therefore, it is essential to monitor and minimize honey contamination, as well as the factors exposed to bees.

Which plasticizers were the most prevalent, and what is their source in the environment?

The most prevalent plasticizers in our study, BBP (benzyl butyl phthalate), DEP (diethyl phthalate), and DEHA (di-2-ethylhexyl adipate), can reach honey through various environmental pathways:

i) *Soil and water contamination*. Plasticizers can seep into soil and water near sources of contamination, such as factories or landfills. Honeybees may collect contaminated water or nectar from plants growing in polluted soil.

ii) *Contaminated air*. Plasticizers can disperse in the air, and honeybees can encounter these contaminants during their flight and bring them back to the beehive.

iii) *Deposition on plants*. Plasticizers from air and water can settle on plants. Honeybees gather nectar and pollen from these plants, transferring contaminants into the honey.

iv) *Use of contaminated products*. Products containing plasticizers, like plastics in hive structures or agricultural practices nearby, can directly transfer these chemicals to honey through contact.

Why were gas chromatography (GC) and high performance liquid chromatography (HPLC) your techniques of choice in your analysis?

GC is particularly suitable for analyzing phthalates due to its ability to separate volatile organic compounds based on their vapor pressures. Phthalates, being volatile, can be effectively detected by GC at very low concentrations, which is critical for assessing contaminants in food products like honey, where even trace amounts matter. GC offers rapid analysis, facilitating high sample throughput, and ensures excellent quantitative accuracy, which is essential for precisely measuring phthalate levels in honey samples.

How are the analytical methods you selected and developed environmentally friendly or green in their approach?

An attempt has been made to develop an analytical methodology that is as environ-

Image Credit: Tierney - stock.adobe.com

mentally friendly as possible by using ethyl acetate as an extractant, known as a green solvent, and employing minimal volumes (< 5 mL) of this solvent. The sustainability of this approach has been evaluated using several green analytical metrics, with the most significant drawback identified as the energy consumption of the gas chromatography-mass spectrometry (GC–MS) instrumentation, which is essential for these analyses and unavoidable.

Briefly state your findings in this study.

The study developed a novel analytical method using GC–MS to analyze nine plasticizers in honey samples. Efficient sample treatment involving dual solvent extraction with ethyl acetate and cleanup with florisil resulted in satisfactory analyte recoveries (77–118%). Chromatographic analysis on an Agilent HP-5MS column demonstrated quick analysis times (< 21 minutes) under optimized temperature conditions. The method was validated for selectivity, low limits of detection (0.1–3.1 µg/kg), quantification (0.2–10.3 µg/kg), linearity, matrix effect, trueness, and precision (relative standard deviation < 9%). Analysis of thirty samples from various sources revealed residues of five plasticizers in most samples. Importantly, health risk assessment based on detected levels indicated no significant risks to consumers.

You analyzed honeys from three different botanical origins (multifloral, rosemary, and heather). Were there any types of honey more susceptible to plasticizers or other environmental contaminants? Were there any other variables, like hive location, that influenced their presence?

This study, based on the analysis of 30 honey samples, provides initial insights that necessitate a more comprehensive investigation for conclusive results. Initial indications suggest that multifloral honeys might have higher concentrations of contaminants, while honeys sourced from local markets show potentially higher levels compared to those from experimental fields. However, no consistent pattern based on hive location has been discerned. As emphasized, a larger and more extensive study is required to draw definitive conclusions regarding the susceptibility of different honey types to plasticizer contaminants.

Do your findings correlate with what you had hypothesized?

The findings generally align with our hypotheses. We anticipated detecting plasticizer residues in honey samples due to potential environmental exposures. The efficient sample treatment method we developed allowed us to detect five specific plasticizers across various honey sources, confirming our expectations. However, the precise distribution and levels of these contaminants exceeded our initial predictions, highlighting the need for robust analytical methods to assess environmental and health-related impacts accurately.

Was there anything particularly unexpected that stands out from your perspective?

During the optimization of sample treatment, a strong matrix effect was observed

across all three botanical origins evaluated, which could be effectively minimized with appropriate clean up steps. It was surprising to find that all origins exhibited a similar response to this matrix effect, a phenomenon not commonly observed with other contaminants studied. Regarding the results, it was unexpected to detect the presence of phthalates in all samples, specifically three of them, regardless of the type and origin of the honey.

Were there any limitations or challenges you encountered in your work?

In terms of challenges, analyzing phthalates presents a significant analytical hurdle due to their widespread presence in common laboratory materials. It requires meticulous cleaning of all equipment to ensure they are free from these contaminants, thus preventing false positives. Moreover, as previously mentioned, the limited number of samples analyzed underscores the need for a larger sample size to enhance the reliability of the conclusions drawn.

What best practices can you recommend in this type of analysis for both instrument parameters and data analysis?

Cleanliness in sample preparation, including rigorous cleaning of equipment and selecting efficient sample treatment methods, helps minimize contamination and ensure accurate measurements.

It is recommended to use statistical methods, such as calculating mean concentrations, standard deviations, and performing ANOVA, to effectively compare sample groups and identify significant differences. Additionally, employing visual tools like box plots is advised to illustrate data distribution and outliers, enhancing the interpretation of complex datasets, such as those from contaminant assessments in various types of honey.

Are there any other natural food crops that you think might benefit from this sort of analysis?

This analytical approach could be beneficial for assessing a wide range of natural food crops. It could be particularly valuable for crops exposed to environmental contaminants or those requiring strict quality control measures. Examples include fruits, vegetables, grains, and herbs often subjected to pesticides and heavy metals.

Can you please summarize the feedback that you have received from others regarding this work?

The project has sparked significant interest across various sectors of society, both within the scientific community and among the general public. This has led to its presentation at several international conferences and its coverage as a notable news story in Spanish newspapers.

Do you think more of this type of analysis should be performed on other natural foods?

Additionally, products like milk, meat, and seafood could also benefit from rigorous contaminant analysis to uphold food safety standards. Such comprehensive analyses are essential for ensuring consumer health and maintaining the integrity of food products worldwide.

What are the next steps in this research, and are you planning to be involved in improving this technology?

The next steps would involve expanding the study to clarify conclusions regarding the accumulation of plasticizers in honeys, exploring differences based on botanical and geographical origins, as well as packaging of the product. ■

This article has additional supplemental information only available online.
Scan code for link.

ABOUT THE AUTHORS

Silvia Valverde Bastardo

is an Assistant Professor in Analytical Chemistry at the University of Valladolid specializing in the development and enhancement of analytical methodologies for bioactive, pesticide, and pollutant compounds determination in agri-food matrices. She has a strong focus on chromatographic techniques (LC and GC) coupled with several detectors, specifically mass spectrometry and tandem mass spectrometry, as well as on sample treatment and extraction techniques. She completed her Ph.D. in 2018 at the University of Valladolid. Subsequently, she worked in the private sector and undertook a postdoctoral stage at the Autonomous University of Madrid, where she was closely associated with the fertilizer sector, collaborating with prominent companies such as Fertinagro Biotech SL, Tradecorp, and Tessenderlo Kerley. In 2022, she joined the Department of Analytical Chemistry at the University of Valladolid as an Assistant Professor, where she currently works. She has authored 30 scientific publications, 4 book chapters, and received over 500 citations. In addition to participating in nine research projects, both publicly funded and financed by recognized companies in the wine and fertilizer sectors. She serves as an external reviewer for several journals and continues to contribute significantly to the field of analytical chemistry.

Ana María Ares Sacristán

is an Associate Professor in Analytical Chemistry, researcher, and secretary of TESEA group at the University of Valladolid-UVa. She has contributed toward expanding the knowledge about some relevant issues, such as food safety and quality, including the analysis of contaminants and bioactive compounds, and in development of new analytical strategies, especially those that involved green chemistry. She studied chemistry at UVa and received her PhD degree in 2015 at the same institution. After five years working as a postdoctoral researcher in Analytical Development and Validation in a pharmaceutical industry and continued to be linked as a professor at UVa, she obtained a full position in the same institution. In the last few years, she has mainly worked in the apiculture area, especially in developing new strategies for determining pesticides and bioactive compounds in bee products. During her scientific career, she has worked with several analytical separation techniques as HPLC, GC, CE, and SFC coupled to several different detectors. She has also contributed as author/co-author to more than 60 scientific publications, 30 congress presentations, 5 book chapters, and 1 patent. In addition, she has also participated as Principal Researcher/Researcher in 4 research projects or contracts with various industries and government agencies. She is also a member of the Editorial Board of several journals.

Addressing Sample Prep Challenges in Bioanalysis

Troy Voelker, PhD
Senior Director of Laboratory Operations
Aliri Bioanalysis

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Biological samples exhibit a high level of complexity rendering the sample preparation in Bioanalysis a formidable challenge. Effective extraction of analytes and removal of matrix components is a crucial step for the success of a bioanalytical workflow. If not addressed strategically and with the right tools, sample preparation challenges can become a bottleneck that cost labs time and precious resources.

Biotage sat down with Dr. Troy Voelker, Senior Director of Laboratory Operations at Aliri Bioanalysis, to discuss overcoming sample prep challenges in Bioanalysis.

Dr. Voelker leads all laboratory divisions including method development, method validation, instrument operations, and production groups. Aliri Bioanalysis provides a range of bioanalytical services to the pharmaceutical and biotech industry in support of early-stage discovery through NDA submission. Aliri is recognized as an industry leader for its bioanalysis services utilizing platforms such as High-Resolution Mass Spectrometry and traditional LC-MS/MS to identify and quantify drug analytes in sample matrices such as plasma, serum, and various tissues.

LCGC: What are some of the challenges of Sample Prep in Bioanalysis?
VOELKER: "The challenge is getting to as clean of a sample in as few steps as possible. Everyone needs high throughput, and everyone is trying to cut both time and expense out of the process. We typically look to the Biotage® ISOLUTE® SLE+ for fast throughput sample clean up. He added: In my experience, Biotage's Sample Prep products have proven to be reliable and accessible.

LCGC: How do you determine the right Sample Prep product to use in your lab?
VOELKER: "When I assess SPE solutions, there are some factors I consider upfront and one is how reproducible is the product batch-to-batch and I have never seen an issue with Biotage's plates, whether that's SLE or SPE, always been very reliable. A second factor is accessibility, if I have a worry that I can't keep your product on my shelf to get samples through, then that's a concern that I would have to relay over to a sponsor."

LCGC: What is the rate-limiting step of Sample Prep for Bioanalysis and how do you address them?
VOELKER: "We do a lot of oligonucleotides work here, and there is a dry-down step associated with those methods. Typically, we were looking at evaporation times taking anywhere from 1-1.5 hours being fast, to 2.5-3 hours being somewhat typical. With the TurboVap® 96 Dual, our main focus was to expedite that part of the process. We set up the system and programmed it. I challenged one of our MD Scientists to see how fast he can dry down a plate of sample extracts from an oligo SPE workflow. It was dry in 45 minutes which was twice as fast as our fastest previous time.

We looked at programming the system as a walk-away device that would free up our analysts from having to systematically go back and raise the plate or increase the flow. It was a time-saver that allowed our analysts to concentrate more at the bench and worry less about getting samples ready for injection."

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Q&A

The Future of Digital Method Development

Interview by Will Wetzel, *LCGC International*

Following the HPLC 2024 Conference in Denver, Colorado, *LCGC International* spoke with Anne Marie Smith of ACD/Labs about the new ICH Q14 guidelines and how they impact analytical scientists and their work.

DIGITAL METHOD DEVELOPMENT is the process of using digital tools to collect and analyze data sets and is the norm in most laboratories. But the introduction of new tools like artificial intelligence (AI) and machine learning (ML), could shake up the space.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is a collaborative effort between regulatory bodies and pharmaceutical companies to discuss and provide guidelines for pharmaceutical product development (1). Recently, the ICH put forth new guidelines, titled "Analytical Procedure Development Q14," which focused on analytical procedures for drug substances (1).

Anne Marie Smith, a Product Manager at ACD/Labs, presented a poster at the 2024 HPLC conference in Denver, Colorado titled "Practical Implementation of ICH Q14 Guidelines in Digital Method Development," where she discussed these new guidelines in depth and what it means for digital method development (2).

Smith sat down with *LCGC International* to discuss the future of digital method development and data analysis.

Q Please briefly describe the new ICH Q14 guidelines are and how it impacts method development.

The guideline came into effect in June 2024, and it provides approaches for developing and maintaining analytical procedures for drug substances and products. It focuses on the use of science and risk-based approaches. It is complimentary to other guidelines like ICH Q2 (validation), and it uses some approaches and tools referenced in ICH Q8–12. The guidelines work to create robust methods with reduced effort by leveraging prior knowledge and using a more strategic approach. Two methodologies are outlined that can be used to meet the guidelines—minimal and enhanced approaches.

Q What are the most important takeaways that analytical scientists should be aware of from these new guidelines?

It's important to recognize that these are guidelines and not requirements, so some interpretation will be required. Both minimal and enhanced approaches are acceptable for method development. However, the enhanced approach can give better certainty of procedure performance, serve as a foundation for the *analytical procedure control strategy*, and the additional development data/knowledge can improve efficiency in regulatory post approval changes. One of the best ways to get started is to build your knowledge base now. Getting data into a database can help as we learn more about how to utilize artificial intelligence (AI) and machine learning (ML) and give you a better starting point for your method development.

Q What role does software play in developing robust analytical procedures?

Software can make it easier to develop a robust analytical procedure, reducing the number of runs a user needs to obtain and reducing solvents, making the process greener. It can give you a better

Getting data into a database can help as we learn more about how to utilize artificial intelligence (AI) and machine learning (ML) and give you a better starting point for your method development.

starting point by calculating PhysChem properties like pK_a, log*P*, and log*D*, which helps determine a suitable pH for method development based on your compounds and column and help determine the most suitable or varied columns. It can also be used to help transfer methods between laboratories or types of instruments (high-performance liquid chromatography [HPLC] to ultrahigh-pressure liquid chromatography [UHPLC]) potentially leading to shorter, greener methods. Software can help you make data-driven decisions as you design your experiments for screening, optimization, and robustness leading to better separations with fewer experiments. By understanding the design space, you can understand the impact of slight changes and ensure the robustness of your method. Furthermore, software makes it easier to keep track of everything you have done, leading to accurate and complete documentation with the ability to leverage that knowledge in the future.

Q What are the biggest challenges or roadblocks you see laboratories facing today when it comes to software for research and development? How can they address these challenges?

There has been a move to software-as-a-service (SaaS) and cloud-based services. Applications can be delivered through a browser, but that does not address where the data is stored or how you get it to the cloud. Addressing this involves various infrastructure strategies like determining what data is required on demand and edge computing.

Q What are the biggest trends that you think we'll see in analytical chemistry informatics over the next few years?

There is a strong push and interest in AI and ML, though everyone is still learning how to do this.

Q What role will artificial intelligence and machine learning play in this space moving forward?

Companies gather a plethora of data daily. Being able to learn from this data can lead to quicker development of more robust methods. It removes the low-hanging fruit, enabling the scientist to have a better starting point and focus on the science. It can also reduce the number of true experiments needed, leading to many environmental benefits.

Q Is there anything else our readers should know about this topic?

In what ways does use of prior knowledge help with method development? How does one get started with setting up a knowledge base? Using prior knowledge helps users reduce work by finding an existing method that would work for their compound, or something similar enough that they only need to make minor tweaks to it. This reduces time for experiments to be completed and turns the method development process into a greener process. Setting up the knowledge base is easy and can often be automated. Structures, data and metadata are linked together so that the information can easily be extracted and found when needed. ■

This interview was lightly edited for clarity.

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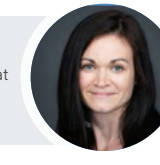

Next Generation Peak Fitting for Separations

M. Farooq Wahab, Troy T. Handlovic, and Daniel W. Armstrong

Separation scientists frequently encounter critical pairs that are difficult to separate in a complex mixture. To save time and expensive solvents, an effective alternative to conventional screening protocols or mathematical peak width reduction is called *iterative curve fitting*. This method does not sharpen the peaks to enhance the chromatographic resolution, but extracts the original shape from overlapping peaks in a complex separation, as if an isolated compound were injected. The generalized family of Haarhoff-van der Linde of peak functions accounts for most chromatographic peak shapes under analytical, isocratic, or gradient elution, and mass-overloaded conditions. Four illustrative examples are discussed: (i) sub-second separation of five compounds; (ii) area extraction from 30 partially resolved peaks separated in under a minute; (iii) iterative curve fitting and baseline correction for a nicotine containing E-liquid; and (iv) advantages of fitting an overloaded peak shape for preparative separations. The large *F*-statistic, and R^2 near to 1.0 in all cases, shows excellent modeling of the data's variance.

MANY REAL-WORLD chromatographic separations have difficult critical pairs that fail to resolve under most experimental conditions (1,2). Standard advice in such instances is to employ a different column chemistry, design a better gradient, or select a different mobile phase system. Screening conditions to produce a chromatogram without peak overlap consumes costly time and solvents in a busy laboratory. More sophisticated and expensive options include two-dimensional liquid chromatography (2D-LC), two-dimensional gas chromatography (2D-GC), and information-rich detectors such as mass spectrometers, or photodiode array detectors. Also, the optimal choices of mobile and stationary phases are not always sustainable, and greener solvents often produce skewed and low-efficiency peaks (3,4). With a current emphasis on "greening" separation methods and improving workflow in terms of saving time and solvents (3,5,6), it is the right time for separation scientists to resort to digital signal processing (DSP) approaches. DSP techniques are essential in medical imaging, astronomy, and spectroscopy. If physicians can trust digitally enhanced medical images for life-altering health decisions, separation scientists should also fully utilize the benefits that DSP could bring to their research work. Currently, and largely unbeknownst to most users, all mass spectrometers and nuclear magnetic resonance (NMR) signals are highly processed mathematically. Although not the focus of this work, multiple DSP techniques in the literature reduce the peak widths and increase chromatographic resolution (7). Herein, we discuss one compelling alternative DSP technique that offers digital resolution without altering peak widths or shapes called iterative curve fitting. This approach, also referred to as *peak fitting*, allows for the mathematical modeling of a given peak shape to subsequently calculate peak areas, heights, statistical moments, and other chromatographic figures of merit in a noise-free environment. The term *iterative* implies that the curve fitting process is repeatedly improved until the *residuals* (the difference between the data and the model) are minimized, usually through least squares formulation and non-linear optimization algorithms. With this protocol, the profile of each peak, overlapping or non-overlapping, is obtained as if a single component were injected instead of a mixture.

Almost all chromatographic peaks have a degree of asymmetry (8,9), yet the most common methods to calculate figures of merit (such as resolution, efficiency, and retention factor) are based on Gaussian distribution approximations. The interest in resolving partially overlapping chromatographic signals with mathematical models dates back to the early 1970s, when it was realized that the simple Gaussian model for fitting peaks could not capture the variance of the data effectively (10). Despite this understanding and the development of over 200 different versions of chromatographic peak functions (11), two models have remained consistently popular: the Gaussian and the exponentially

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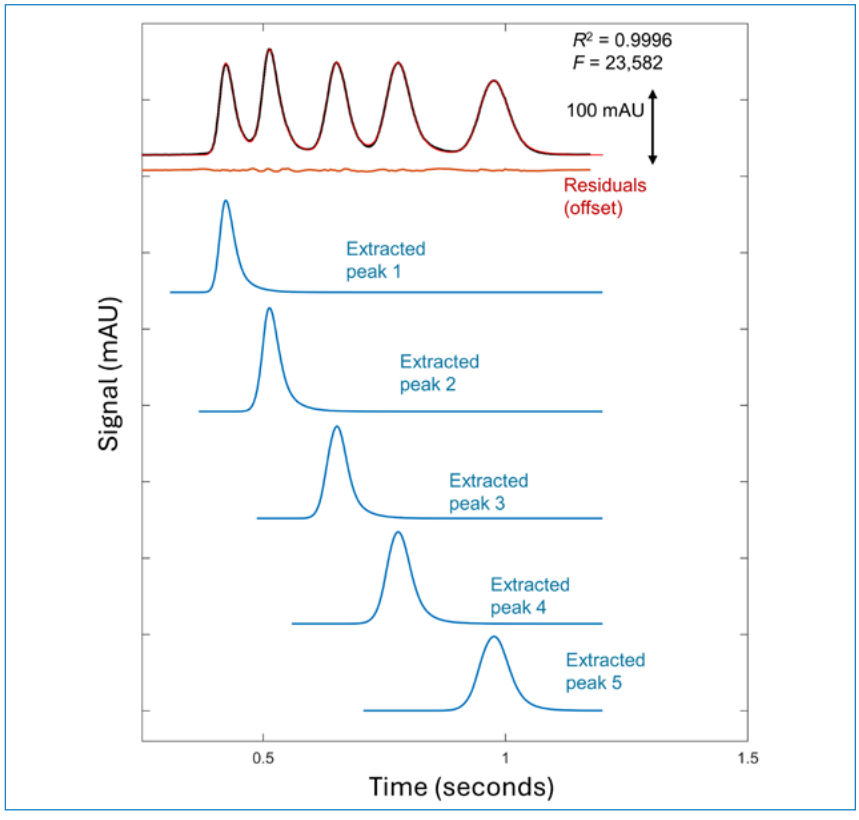


FIGURE 1: Sub-second HILIC separation of five nucleosides (6-aza-2-thiothymine, thymine, 5(hydroxymethyl)uracil, 6-aminouracil, and isocytosine) fit with five Gen-2HVL<ge> functions. (Conditions: Vanquish UHPLC, 10 mm x 3.0 mm (i.d.), 1.9 µm bare SPP silica, 250 Hz, 8.0 mL/min, UV detection at 254 nm). Raw data (black, modeled peaks (red). Data reproduced from Patel et al. (16).

modified Gaussian (EMG) distributions. The current need to address the demands posed by complex separations in today's laboratories is to develop new powerful models that can fit or consider most experimental isocratic or gradient peak shapes under analytical or overload conditions. Secondly, the peak fitting procedure should automatically attain the global minimum of the least square objective function through iterative non-linear optimization without manual intervention or requiring the user's initial estimates. The key advantage of curve fitting in chromatography is that the extracted peak data is drift and noise free. This information can be obtained with high statistical confidence even when signals from single channel detectors such as ultraviolet-visible (UV-vis) spectroscopy evaporative light scattering, charged aerosol, and refractive index detectors coincide to a significant degree. The same concepts apply to gas chromatography, with single-channel data from flame ionization, barrier discharge, or thermal conductivity detectors.

Popular chromatography data systems (CDS) currently do not extract peak information from modeling, and can only do the rudimentary perpendicular drop or tangents method to obtain crude area information (9,12). The proposed peak functions here are new theoretical models derived from the Haarhoff-van der Linde peak function (HVL)(13), extended by generalizing the underlying statistical Gaussian distribution and incorporating instrument response functions (*I*) (14). These statistical innovations add third and fourth-moment adjustments to the core peak shape (the *probability density function* [PDF]), and the signal processing innovations add the *I* as a convolution to the core peak PDF (15). These models are supersets of the HVL that manage the non-idealities of tailed and fronted shapes that increase with non-linear chromatographic concentration effects. Consequently, the results computed from these fitted models are accurate and precise for single and overlapping peaks. The estimated and derived parameters include accurate peak areas, retention times, resolutions, means (t_r), variances (σ^2), skews, and kurtosis values while mapping nearly all the data's variance. Here, a new peak model called the twice-generalized HVL model will be abbreviated as Gen2HVL, and when convolved with a half-Gaussian and exponential decay, *I* term, as Gen2HVL<ge>. The mathematical documentation of these functions is available elsewhere (14), and can be applied using built-in curve fitters combined with custom-made Python or MATLAB scripts. Eventually, we believe that advanced signal processing will be embedded into all commercial chromatography data systems, just as it is for NMR, mass spectrometry (MS), Raman, and Fourier transform infrared spectroscopy (FT-IR).

Experimental

Columns

C18 columns were purchased from Supelco (Ascentis) in the dimensions of 150 mm x 3.0 mm (i.d.) and from Agilent (EC-C18) in the dimensions of 50 mm x 3.0 mm (i.d.), both packed with 2.7 µm superficially porous particles (SPPs). The HILIC separation shown was conducted using a custom prepared bare silica column in the dimensions of 10 mm x 3.0 mm (i.d.) with 1.9 µm SPPs as fully described elsewhere (16). The analytical overloaded separation shown was conducted using a NicoShell column in the dimensions of 100 mm x 4.6 mm (i.d.) with 2.7 µm SPPs acquired from AZYP, LLC.

Instruments

Example chromatograms displayed here were collected on two systems: Thermo Vanquish UHPLC and Agilent 1220 HPLC. The Vanquish contained a quaternary pump (VF-P20A), a split sampler (VF-A10-A), and a variable wavelength detector (VF-D40-A). The Agilent 1220 HPLC contained a G4290B UV detector. Separations were conducted at ambient temperature unless otherwise noted to avoid the additional volume of tubing required to use a column oven. Mobile phases are reported as volume-to-volume ratios.

Software

All chromatograms were analyzed using PeakLab (v. 1.05.02) from AIST Software. Baseline correction (when noted) was done in PeakLab using the non-parametric linear model or constrained cubic splines (Figure 3; note that all figures in this article can be accessed through the QR code on the bottom of page 79) with manual piecewise sectioning. Peak fitting was achieved with the "local maxima peaks," "hidden peaks/second derivative," or "hidden peaks residuals" options. All figures were made by exporting the fits from PeakLab and plotting them in MATLAB (2023a, The MathWorks, Inc.).

Results and Discussion

To demonstrate the power of advanced peak fitting, we have curated four vastly different case studies discussed below that we believe will be of interest to a practicing chromatographer. These examples include three modes of chromatography (reversed-phase liquid chromatography [RPLC], hydrophilic-interaction chromatography [HILIC], and normal phase liquid chromatography [NPLC]) in both chiral/achiral and analytical/overload settings. It should be noted that iterative curve fitting is *not* a peak deconvolution process, where the goal is to reduce peak width and increase analytical resolution by reversing the mathematical process of convolution. In a loose sense, scientists use deconvolution when they mean the *decomposition of a signal*, which refers to the breakdown of a complex signal into its constituent signals. For example, in liquid chromatography-mass spectrometry (LC–MS), the total ion chromatogram (TIC) can be decomposed into extracted ion chromatograms for each monitored *m/z*, and then all extracted ion chromatograms can be summed to regain the TIC. Curve fitting mathematically allows for a similar decomposition, but only uses one data channel. Here, each peak is transformed into its own fully resolved signal as if it were injected into the chromatograph alone and in its pure form, even if the original chromatogram contains overlap.

Sub-Second Separation of Five Nucleosides Using HILIC

Current instrumentation technologies now allow for the separation of components on the sub-second scale in both gas (17) and liquid (16) chromatography. To achieve this level of speed in LC, small columns with high flow rates must be used with a pump capable of delivering flow rates as high as 8 mL/min at ≥ 800 bar. Figure 1 shows an example of HILIC separation where five nucleosides (6-aza-2-thiothymine, thymine, 5-(hydroxymethyl)uracil, 6-aminouracil, and isocytosine) were separated in just ~ 1 s using a short 10 mm × 3.0 mm custom made bare silica superficially porous particle (SPP) column (16). Ultrafast separations are excellent case studies for peak fitting, as the high number of analytes present in a short time window and low column efficiencies frequently produce peak overlap in the chromatogram. These peaks are also subject to further significant shape deformation from extra-column effects due to their low retention factors and the relatively large system volumes compared to the small volumes of the narrow and short columns (15,18).

The coefficient of determination (R^2) is commonly used within chemistry and known to be equal to one minus the ratio of the residual sum of squares (SS_{rs} or SSE) to the total sum of squares (SS_{tot} or SSM). The F-statistic is another metric widely used by statisticians but less so by chemists. When statistically generalizing models, one must use caution in adding parameters to the function since the model with more parameters will fit the given data at least as well and often better with a higher R^2, than the one with fewer parameters. However, this increase is not always statistically significant. The F-statistic can be calculated to see if the improvement in the fit is significant using the same SS_{rs} and SS_{tot} data, accounting for the number of parameters used in each model (14). Therefore, we consider the function that produces the highest F-value to be the best model to estimate the experimental data. Note that no R^2 or F value can indicate satisfactory goodness-of-fit (GOF) alone, but they help compare functions when modeling the same data set (19,20). Visual inspection of the residuals is of the highest importance in assessing GOF, and unfortunately, no GOF determination within classical statistics is better than human interpretation. Ideally, the residuals produce a flat line with random fluctuations from the noise in the experimental data and are not modeled by the peak functions. Any deviation from this flat randomly oscillating line indicates misfit between the data and the model.

Figure 1 shows Gen2HVL convolved with the half Gaussian and exponential instrument response, Gen2HVL<ge>, fit to the ultrafast chromatogram (red) discussed above. The modeled data (black) describes the experimental peak profiles (red) with an excellent GOF. As expected, when using this effective model, the R^2 and F values were 0.9996 and 23,582, respectively. The residuals here are ideal and reflect the variance from the noise of the experimental data not being represented by the model. This result represents a greater than ten-fold increase in GOF compared to the classical Gaussian function (F = 2,983). With the data modeled through the iterative curve fitting process in 1D, each analyte signal can now be treated as if it were a separate chromatogram. Figure 1 shows the individual extracted peaks (blue), which can be integrated, quantified, and statistically described accurately in the absence of noise. The sum of all extracted peaks recreates the original chromatogram with the added advantages of no drift, noise, or overlap.

Separation of 30 Peaks in just 1 Minute

Giddings applied statistical overlap theory to chromatography, predicting that a column has a finite peak capacity roughly equal to $1/2 \times \sqrt{N_{avg}}$ (valid only with isocratic elution considering a resolution of one and moderate retention) (2). Furthermore, it was predicted that only 18% of a column's peaks would contain a single component in a random chromatogram (2). Fast separations, with samples containing many components, will often result in peak overlap due to the limited number of plates provided by small columns used at high linear velocities. Peak capacity can be physically improved by gradient elution as the column's apparent efficiency increases or by 2D separations, which increase time and expense. Alternatively, peak fitting can be used when operating above peak capacity without changing the experimental conditions. Figure 2a presents a reversed-phase linear gradient separation of 30 components in just 1 min on a 5 cm by 3 mm (i.d.) C18 column. The large amount of peak overlap in this separation makes it an appropriate application to evaluate the limits of peak fitting science. In Figure 2a, the chromatogram is fitted with the Gen2HVL function. The resultant fit is highly accurate with an R^2 of 0.9997 and a F-statistic of 249,725. More importantly, the residuals observed in this separation are ideal, showing a nearly flat line with random fluctuations due to the noise in the experimental data. This example shows an ideal outcome from peak fitting and provides an application for fast separations with high peak density. As demonstrated in Figure 2b, each fitted peak can now be individually treated as its own fully resolved signals without noise. Integrating the resolved fitted peaks (Figure 2b) to gain their individual areas is highly accurate and reproducible, whereas it is difficult or impossible with the raw data (Figure 2a).

Analysis of a Commercial E-Liquid with Gradient Elution RPLC

E-liquids are fluids used in electronic cigarettes, which are popular amongst today's youth (21). A recent study using LC paired with high-resolution mass spectrometry (LC–HRMS) found over 2,000 components in some commercial E-liquids (22). Of these components, most of their identities are unknown, meaning their human activity and toxicology are also unknown. Methods to separate, identify, and quantify components in these liquids are extremely important for public health and policy making. When short wavelengths (~190–220 nm) are used to analyze these samples, molecules that do not contain or have weak chromophores can be detected. This wavelength region is problematic when the chromatographer uses alcohols (such as methanol) in the mobile phase as they absorb deep UV (3). In isocratic elution, the mobile phase absorbing deep UV light results in a raised baseline and reduced sensitivity. This absorption becomes more problematic when using gradient elution as the baseline drifts from the low absorbing water to the stronger absorbing methanol. Chemically, the drifting baseline can be reduced by using acetonitrile as the organic solvent, but this is more expensive and far less environmentally benign (3).

Figure 3a (purple) shows a cogent example of the analysis of a commercial E-liquid using gradient elution RPLC with two key issues: a heavily drifting baseline and severe signal overlap. Combining UV detection at 210 nm

with methanol as the organic solvent in the gradient creates an undesirable drift in the baseline (Figure 3a, purple). Even with the reduced sensitivity from the high background signal, this chromatogram provided significantly more detectable components than when the sample was identically analyzed at 254 nm. To solve this problem with post-analysis calculations, the baseline was first extracted (Figure 3a, blue) using a constrained cubic spline with user-controlled piecewise sectioning of the baseline in PeakLab. The baseline correction can also include undesirable peaks and features. The result of the baseline subtraction is presented in Figure 3a (black), showing a zeroed baseline with preservation of the peaks, their location, and their intensity. The area of these peaks and their retention time can be accurately determined by fitting the baseline corrected chromatogram. Figure 3b shows a "zoomed in" portion of this chromatogram fit with 20 Gen2HVL functions. The R^2 from this fit is 0.9997, the *F*-statistic is 324,597, and the residuals (Figure 3b, orange) are random with low relative intensity. These merits indicate a robust and accurate fit; peak area and location information are now readily available from this problematic chromatogram.

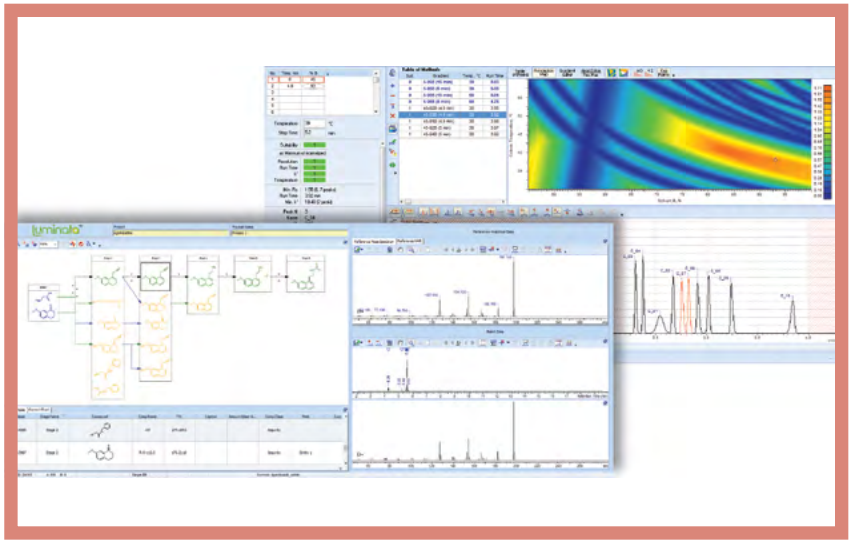
Mass Overload of a Drug's Enantiomers in NPLC

Preparative liquid chromatography revolves around overloading the column to collect the largest amount of desired components per mass of stationary phase per unit of time (productivity) (23). The band profiles and retention times are challenging to understand and predict when overloading columns (24). To obtain this information experimentally, a loading study is often completed on smaller columns with the same packing and then directly scaled to the larger, more industrial sized column (5,25). Overloading the analytical sized column can also be helpful for "semi-preparative" chromatography when only a small amount (µg to mg scale) of a highly pure compound is needed in environments where a larger column is unavailable. Semi-preparative chromatography is often used to isolate enantiomers of a compound when small masses are needed for stereospecific activity testing. This process involves using a chiral stationary phase, overloading the phase, and then collecting the fractions for each enantiomer.

Figure 4 contains an example of part of a mass loading study for *R*/*S*-alprenolol using a semi-synthetic macrocyclic glycopeptide chiral stationary phase (NicoShell) in the normal phase. Even though the column is of analytical size (100 mm x 4.6 mm i.d.), up to ~0.5 mg of the drug can be loaded on the column per injection. When doing loading studies, it is beneficial to overload the column to the point where

(CONTINUED ON PAGE 78)

Advanced Chemistry Development, Inc. (ACD/Labs)



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YEAR FOUNDED
1994

Company Description

ACD/Labs is a leading provider of scientific software solutions that facilitate effective decision-making and digital transformation in chemical and pharmaceutical R&D.

We enable organizations to leverage harmonized analytical, structural, and molecular information—for use by chemists and data scientists alike—in a FAIR-compliant environment. Our time-tested, science-centric solutions, supported by cloud-enabled technologies, automate, manage, and proliferate chemically intelligent knowledge.

For 30 years, ACD/Labs has been privileged to assist leading R&D organizations in many industries worldwide. Through digitalization and engineering of data for AI initiatives, we are helping create efficiencies in workflows that improve productivity and accelerate scientific innovation.

Chief Chromatographic Techniques Supported

- HPLC
- UHPLC
- LC/UV
- LC/MS
- LC/MS/MS
- GC/MS

Markets Served

ACD/Labs serves companies in pharma, biotech, fine chemicals, consumer packaged goods, agrochemicals, and petrochemicals; academic institutions; and government laboratories.

Major Products/Services

- **Method Selection Suite—**Software to help develop rational methods by predicting physicochemical properties, simulating LC and GC separations, and running 3D optimizations.
- **AutoChrom®—**Assists expert chromatographers in developing methods using a QbD approach. Design and develop workflows, execute experiments, analyze data, perform DoE optimization, and create reports from one place.
- **MS Workbook Suite**—Work with LC/MS and GC/MS data by importing datasets from nearly all major file formats. Deconvolute chromatographic traces, identify components by spectral search and predicted fragmentation, and archive data in a searchable database.
- **Luminata**—Make informed decisions in chemistry, manufacturing, and control (CMC) by monitoring your entire CMC process from one piece of software. Input reaction schemes and analytical data to develop process control, track impurities, manage stability studies and your supply chain, and efficiently answer regulatory enquiries.

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YEAR FOUNDED
1990, since 2010 in USA

Company Description

For over 34 years, Antec Scientific has been the world's leading supplier of analytical instrumentation based on electrochemistry (EC). Antec's line of instruments include electrochemical detectors (ECD), analyzers built on HPLC-ECD, and electrochemical reactors for use with MS (EC–MS) and synthesis.

Electrochemical Detectors for HPLC
The DECADE™ line of ECDs (Elite™ and Lite™) have become the benchmark in detection, and can be used with any third party (U)HPLC system. Different flow cells are available to cover a broad range of applications from the analysis of antibiotics to sugars or pharmaceuticals.

Dedicated Analyzers
The ALEXYS™ Analyzers based on HPLC with ECD are turnkey instruments built for highest performance and to ensure ease-in-use. Guaranteed applications have been developed with the ALEXYS Analyzer for the analysis of antibiotics, neurotransmitters, and pharmaceuticals. For sensitive and reliable analysis of all classes of carbohydrates the ALEXYS Carbohydrates Analyzer based on HPAEC-PAD is available.

Electrochemical Reactors for MS and Synthesis
With the introduction of the ROXY™ Exceed EC system, REDOX reactions can be monitored directly by mass spectrometry (MS). The on-line coupling of electrochemical reactors with MS (EC–MS) has found many application areas from proteomics, (reduction of disulfide bonds), drug metabolism, and drug stability testing, to environmental degradation. Another emerging application of Antec's flow and batch reactors is in Flow Chemistry for rapid electrochemical synthesis of products.

SenCell™ for Detection
Proprietary Adjustable Spacer Technology (AST) for highest sensitivity (US Patent 9,310,330).

µ-PrepCell™ for Reactions
Redox reactions upfront MS: disulfide bond reduction in proteins, mimicking drug metabolism, drug stability testing/degradation.

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The new benchmark for superior separation of all classes of carbohydrates using HPAEC with PAD or MS detection.

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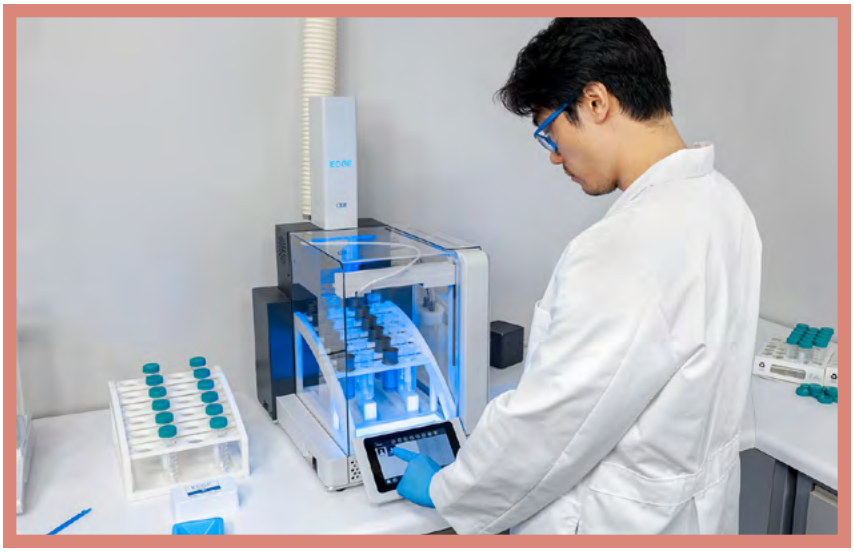

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NUMBER OF EMPLOYEES
USA: 260
Elsewhere: 90

YEAR FOUNDED
1978

Company Description

CEM Corporation is a leading global company specializing in scientific solutions for critical laboratory applications. We design and manufacture systems for analytical laboratories, bioscience applications, life sciences, and processing plants worldwide. Our product portfolio includes innovative instrumentation for sample preparation for elemental or chromatographic analysis, chemical synthesis, peptide synthesis, and other applications.

Chief Chromatographic Techniques Supported

- LC
- LC–MS
- GC
- GC–MS

Markets Served

CEM instrumentation is used by chemists, scientists, and technicians in private industry, as well as leading universities, analytical laboratories, and research facilities around the world. We support numerous markets, including the pharmaceutical, chemical, environmental, applied materials, food, and petroleum industries, among others.

Major Products/Services

Good analysis starts with sample preparation. The CEM suite of products is designed to bring speed, simplicity, automation, and accuracy to sample prep for chromatographic analysis.

EDGE PFAS™ and MARS 6 PFAS™ are designed specifically for PFAS extraction. With no background contamination, simple operation, and the flexibility of either sequential automated extraction, reducing the need for SPE, or high throughput extractions, PFAS preparation has never been easier.

The Discover Prep™ is an automated microwave sample prep workhorse for applications such as hydrolysis and extraction of samples like plastics/polymers, pharmaceutical/nutraceuticals, consumer products, and more.

Extractions that typically take hours are complete in minutes using a closed system that is safer for laboratory staff.

The MARS 6™ Extraction system can be used for the same applications as the Discover Prep but in a high-throughput platform that allows for processing of up to 40 samples per batch, still in a fraction of the time of traditional extraction methods.

The EDGE® is a fully automated extraction system that performs solvent addition, extraction, filtration, and system wash in a fraction of the time of other techniques. The EDGE supports extraction of foods, cannabis, soils, and more.

Facility

CEM's global headquarters, research laboratories, and manufacturing facilities are located in Matthews, North Carolina. The company has subsidiaries in the United Kingdom, Ireland, Germany, Italy, France, Japan, and Singapore, as well as more than 50 distributors worldwide.

Hamilton Company

Chromatography Products for HPLC and GC

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YEAR FOUNDED
1953

Company Description

Hamilton Company is an industry leader in the design and manufacture of liquid handling, process analytics, robotics, and automated storage solutions. For 70 years, Hamilton has been satisfying customer needs by combining quality materials with skilled workmanship to ensure the highest level of performance. Hamilton's lifelong commitment to precision and quality has earned a global ISO 9001 Certification.

Founded on the technology of analytical Microliter™ and Gastight® syringes, Hamilton has a broad offering of laboratory products, including manual and semi-automated precision fluid measuring instruments, chromatography products, process sensors, laboratory electrodes, benchtop devices, and more.

Chief Chromatographic Techniques Supported

- HPLC
- Polymeric HPLC columns: reversed phase, anion exchange, cation exchange, and ion exclusion
- Silica HPLC columns
- GC septa
- Chromatography syringes (HPLC and GC)

Markets Served

- Analytical
- Pharmaceutical
- Biotechnology
- Industrial
- Clinical
- Environmental
- Food Chemistry
- Regulatory
- Oligonucleotides
- Cosmetics
- Toxicology
- Oil & Gas

Major Products/Services

HPLC Columns: We manufacture 18 types of polymeric HPLC columns for reversed phase, anion exchange, cation exchange, and ion exclusion separations, providing a wide range of retention characteristics and performance benefits. Our polymer-based HPLC columns provide maximum inertness and pH stability (0–14) with the pressure stability of silica-based columns. With our Polymeric Reversed Phase (PRP™) HPLC columns and resins, the sample dictates the necessary separation conditions, not the limitations of the column. We can also customize HPLC columns to meet a desired application need. Each product is manufactured to achieve the highest level of accuracy.

Chromatography Syringes: We offer the most complete selection of syringes on the market for use in various applications including GC and HPLC (autosamplers and manual injection), thin layer chromatography (TLC), liquid handling, and life sciences. Crafting exceptional syringes is an evolving science, which is why we are dedicated to the continuous research and development of this product line. We constantly enrich our entire syringe offering by either improving existing models or introducing new ones. Using customers' needs and feedback as a guide, we innovate in ways that maximize flexibility, performance, and value.

Microlab® 600 Diluter/Dispenser:
The Microlab 600 is a highly precise syringe pump with a touchscreen interface designed to quickly and easily dilute and dispense fluids, while increasing throughput and reducing cost and wasted buffer. This positive displacement system provides better than 99% accuracy, independent of a liquid's viscosity, vapor pressure, and temperature. The inert fluid path minimizes sample carryover and is compatible with harsh chemicals.

Facility

We are a global enterprise with headquarters in Reno, NV; Hopkinton, MA; Giarmata, Romania; and Bonaduz, Switzerland, and offices worldwide.

HAMILTON®

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NUMBER OF EMPLOYEES
Under 10

YEAR FOUNDED
2014

Company Description

HILICON AB is a leading supplier of hydrophilic interaction liquid chromatography (HILIC) products for the separation of polar and hydrophilic compounds. Four column chemistries in UHPLC and HPLC formats, iHILIC®-Fusion, iHILIC®-Fusion(+), iHILIC®-Fusion(P), and iHILIC®-(P) Classic, provide customized and complementary selectivity, excellent durability, and ultra-low column bleeding. The columns are universal for LC–MS based analysis of polar compounds across various fields, including "Omics" research, food and beverage analysis, pharmaceutical discovery, environmental studies, and clinical diagnostics. In addition, HILICON offers iSPE®-HILIC cartridges and 96-well plates for HILIC sample preparation in solid phase extraction (SPE). It is highly beneficial for studying glycans, glycopeptides, and other polar compounds.

Chief Chromatographic Techniques Supported

- HILIC
- UHPLC
- HPLC
- LC-MS
- HILIC SPE

Markets Served

We provide high-performance HILIC products to the customers worldwide. These products are utilised in diverse applications for analysing polar compounds:

- Omics research, including metabolomics, proteomics, lipidomics, glycomics, and more.
- Pharmaceutical
- Clinical diagnostics
- Life science
- Food and beverage
- Environmental
- Forensic

Major Products/Services

- iHILIC®-Fusion and iHILIC®-Fusion(+) silica-based columns; 1.8, 3.5, and 5 µm; pH 2-8
- iHILIC®-Fusion(P) and iHILIC®-(P) Classic polymer-based columns; 5 µm; pH 1-10
- iSPE®-HILIC in the format of single cartridge and 96-well plate
- Method development for HILIC separation of polar compounds

Facility

HILICON AB began its business at Umeå Biotech Incubator in Uminova Science Park in 2014. From 2018, we relocated to a new facility within Uminova Science Park, Umeå, Sweden.

KIN-TEK Analytical, Inc.

Gas Calibration Standards and Equipment

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YEAR FOUNDED
1970

Company Description

KIN-TEK Analytical, Inc. (KIN-TEK) is a preferred provider of devices and instrumentation for creating trace concentration calibration gas standards and complex gas mixtures. KIN-TEK revolutionized permeation tube technology and further developed it to produce Trace Source™ Disposable and Refillable Permeation Tubes. KIN-TEK's Trace Source™ tube technology is employed in KIN-TEK's Gas Standard Generators to provide accurate, NIST-traceable calibration standards. KIN-TEK's products include a range of stand-alone gas standard generators and generator systems, gas mixers, and permeation devices to fit almost any application that relies on accuracy and traceability.

Chief Chromatographic Techniques Supported

- Calibration gases
- Gas standards
- GC calibration
- GC/MS calibration
- Trace gas standards
- Permeation tubes
- Permeation tube supplies

Markets Served

KIN-TEK delivers trace gas calibration product solutions and services worldwide to solve customer problems in the laboratory, field (portable), and process industries. Industries served include Analyzer Manufacturers, Aerospace and Aviation, Environmental, Food & Beverage, Petrochemical, Pharmaceutical, Refineries, Semiconductor manufacturers, R&D, Universities, and many more!

Major Products/Services

Gas standard generator modules can be operated as stand-alone calibrators or combined into a modular system.

FlexMixer™ Gas Blender (New!)
Multi-Gas Blending/Diluting System for mixing gas cylinder sources.

FlexStream™ Gas Standard Generator
Automated expandable modular permeation system.

491Flex™ Gas Standard Generator
Manually operated expandable modular permeation system.

EcoFlex™ Gas Standard Generator
Manually operated stand-alone simplified permeation module.

Span Pac™ H_2O Trace Moisture Generator
Manually operated trace moisture permeation system.

Span Pac™ I Industrial Gas Standard Generator
Industrial permeation system for on-line calibration of process GCs.

CO395 Flex™ Certification Oven
Permeation tube oven for equilibrating tubes.

Trace Source™ Disposable and Refillable Permeation Tubes
>550 certified gas standards.

Facility

Our corporate headquarters is in La Marque, Texas. All manufacturing, certifications, and operations take place at this facility.

KIN-TEK
The Calibration Specialists

LabVantage Solutions, Inc.

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NUMBER OF EMPLOYEES
1200

YEAR FOUNDED
1983

Company Description

LabVantage Solutions is the recognized leader in enterprise laboratory software and services, as well as advanced analytics. More than 1500 global customer sites across industries rely on LabVantage's highly configurable, 100% web browser-based Laboratory Information Management System (LIMS) platform to innovate faster in the R&D cycle, improve manufactured product quality, comply with regulations, increase cybersecurity, and leverage AI. As a technology and digital transformation leader, LabVantage offers configurable Software-as-a-Service (SaaS), and subscription services. Investments in analytics bring the benefits of AI to customers, while global services provide consistent, knowledgeable deployments, validation, migration, and professional and managed services.

Chief Chromatographic Techniques Supported

LabVantage supports all analytical techniques, including all chromatographic techniques, by interfacing to any commercial chromatography data system (CDS).

Markets Served

- Life Sciences
- Pharmaceutical/ Biotechnology
- Medical Device
- Biobanking
- Food & Beverage
- Consumer Goods
- Oil, Gas, and Energy
- Genetics & Diagnostics
- Public Health
- Healthcare
- Forensics

Major Products/Services

LabVantage's integrated laboratory informatics platform includes the most technologically and architecturally modern Laboratory Information Management System (LIMS) available, with embedded Electronic Lab Notebook (ELN), Laboratory Execution System (LES), and Scientific Data Management System (SDMS). LabVantage Analytics, an advanced analytics solution seamlessly integrated to offer AI, including generative AI, ML, and more through the platform, enhances analysis and insights from a greater range of data: LIMS, business, and external sources. The complete LabVantage platform is entirely configurable without the need for any coding. It features modern technology and architecture that enable remote, compliant user-access from any device; its zero-footprint means no client programs or plugins to install or validate. Purpose-built industry accelerators of its LIMS are pre-configured for ease of use and faster deployment in pharma, biobanking, food & beverage, oil & gas, and more.

Facilities

LabVantage customers are supported locally from nearly two dozen global offices with support and service contracts, as well as training, and over 20 global partners.

LabVantage®

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YEAR FOUNDED
1936

Company Description

For over 85 years, industries around the world have trusted LECO to deliver technologically advanced products and solutions. Today's technologies for separation science reflect LECO's commitment to understanding your laboratory's challenges and providing solutions that can investigate highly complex samples while streamlining your analysis. LECO leverages premier multidimensional-GC separations, time-of-flight mass spectrometry, and advanced software packages to boost your lab's productivity and deepen your chemical insights. For more information, please visit them on the web at www.leco.com.

Markets Served

LECO Separation Science instruments serve a wide variety of Markets, particularly in application areas demanding complex sample characterization, wide matrix compatibility, and unknown identification capabilities. Our core markets include:

- Environmental
- Petroleum, Fuels, and Energy
- Food and Consumer Product Safety
- Aroma and Fragrance
- Metabolomics and Life Sciences
- Pharmaceutical

Major Products/Services

LECO provides end-to-end GC-MS solutions. Our diverse range of autosamplers can be configured with the appropriate chromatography system and mass spectrometer to resolve any level of chemical complexity – ChromaTOF® Software handles every step of method development and acquisition, and our suite of advanced data processing tools reveal the insights you need to make impactful decisions.

Products include:

- **Pegasus® BTX GC–TOFMS:** The newest Pegasus® Time-of-Flight mass spectrometer, with extreme full-mass-range sensitivity and exceptional reliability in a compact benchtop footprint
- **Pegasus BTX4D GCxGC–TOFMS:** Four dimensions of analytical resolution offer a more complete analysis in a benchtop instrument
- **Pegasus HRT+ GC-TOFMS:** with industry-leading resolution (up to 50,000) and mass accuracy for high-information content analysis. High-resolution, accurate-mass TOFMS delivering up to 50,000 resolution with a single ion source for EI, CI, and NCI acquisition modes.
- **Pegasus HRT+ 4D GC-TOFMS:** GCxGC with Paradigm™ or QuadJet™ Modulators: Let flow or thermal GCxGC modulators improve your group/type analyses. Standard with FID, and compatible with other GC Detectors
- **GCxGC with Paradigm™ or QuadJet™ Modulators:** Let flow or thermal GCxGC modulators improve your group/type analyses. Standard with FID, and compatible with other GC Detectors

Facility

LECO's global headquarters in St. Joseph, Michigan include the Elizabeth S. Warren Technical Centre, a 28,000 square-foot facility exclusively dedicated to the research and development of innovative instrumentation and equipment for LECO's separation science line. LECO serves over 75 countries via our global subsidiaries and network of international distributors.

Pegasus® BTX4D with QuadJet™ Thermal Modulator

Markes International

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Offenbach am Main, Germany

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NUMBER OF EMPLOYEES
200

YEAR FOUNDED
1997

Company Description

Markes International—an industry leader in extraction and enrichment (sample preparation) technology for trace organic analysis—manufactures a range of instrumentation and software that enhances the analytical capability and productivity of GC–MS systems.

Markes' technologies enable analysts to discover more about their samples, and to deliver higher throughput for both research and routine applications.

Chief Chromatographic Techniques Supported

- Thermal desorption (TD) as a sample concentration and introduction technique for GC
- Automated sample preparation and concentration (headspace, headspace-trap, SPME, SPME-trap, and high capacity sorbent extraction)
- Time-of-flight mass spectrometry (TOF-MS) for GC and GC×GC (through Markes' sister company, SepSolve Analytical)
- GC–MS data reprocessing software (through Markes' sister company, SepSolve Analytical)

Markets Served

- Defense/Homeland security
- Environmental
- Food and drink
- Forensic and toxicology
- Breath analysis
- Petrochemical

Markes' global customer base includes major industry, government agencies, academia, and the contract service laboratory sector.

Major Products/Services

Markes is globally recognized for its innovation, high-quality products, unrivaled technical expertise, and high level of customer service within the field of separation science. As a global technology leader of thermal desorption and other sample preparation for GC, Markes has introduced many highly successful products and technologies to the laboratory, enabling analysts to discover more and deliver more.

- A range of analytical thermal desorption systems for tube, online, and canister sampling (products include: UNITY-xr, TD100-xr, TT24-7 and CIA *Advantage*-xr)
- Centri® sample automation and concentration platforms for GC-MS
- Micro-Chamber/Thermal Extractor for fast sampling of emissions from products and materials
- TC-20 sorbent tube conditioner
- Wide range of supplies and consumables for sample preparation (extraction and enrichment)

Facility

Markes International's factory, technical center, and headquarters are located near Cardiff, UK. The company also has technical centers in Germany, USA, and China. It also supports a global distributor network. Markes and its sister company, SepSolve Analytical Ltd, are companies of the Schauenburg Analytics Ltd group.

MARKES international

Porvair Sciences

Porvair Sciences
Clywedog Road South,
Wrexham, LL13 9XS

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01978 661144

E-MAIL
int.sales@porvairsciences.com

WEBSITE
www.microplates.com

Company Description

Porvair Sciences are global leaders in the manufacturing and development of cutting-edge porous plastic materials and microplate technologies for the biotechnology and life science industries. From microplates and assay kits to automated laboratory equipment, our company is committed to the creation of workflow solutions with high quality products for improved analysis. Offering customers a diverse portfolio dedicated to high quality sample preparation, our innovative products are designed to increase productivity and accelerate scientific discovery with integrity.

Primary Sample Preparation Techniques

- Solid Phase Extraction (HLB, SCX, SAX)
- Phospholipid Removal
- Supported Liquid Extraction (SLE)
- Protein Precipitation
- Sample Concentration
- Sample Sealing for Protection

Markets Served

- Chromatography
- Biotechnology
- Pharmaceutical
- Clinical
- Drug Discovery
- Environmental
- Forensics

Major Products/Services

Porvair Sciences' comprehensive range of products for sample preparation includes a wide selection of consumables, such as the Microlute® range, sample collection plates, and vials. Additionally, we provide instruments such as the Ultravap® evaporators, vacuum manifold and UltraPPM LITE positive pressure manifold to ensure optimal sample preparation.

- **Microlute® CSi** for silica solid phase extraction (SPE)
- **Microlute® PLR** for phospholipid removal
- **Microlute® PPP** for protein precipitation
- **Microlute® SLE** for supported liquid extraction
- **Ultraseal™** range of adhesive and heat sealers for sample protection and storage
- **Ultravap®** range of blowdown evaporators for sample concentration
- **UltraPPM LITE** for positive pressure processing
- Deep well microplates for sample collection, handling and storage

Facility

Our UK based operation combines manufacturing expertise with state-of-the-art Research and Development facilities. Porvair Sciences fully equipped site features industry leading production equipment and class 8 clean rooms.

Princeton Chromatography Inc.

Princeton Chromatography Inc.
259 Prospect Plains Road,
Building L,
Cranbury, NJ 08512

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(609) 860-1803

FAX
(609) 860-1805

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info@pci-hplc.com

WEBSITE
www.pci-hplc.com

NUMBER OF EMPLOYEES
5

YEAR FOUNDED
1994

Company Description

With over 30 years of experience, Princeton Chromatography Inc. delivers premium chromatography products backed by unmatched technical support. From SFC to HPLC, analytical to preparative, all columns are subjected to rigid quality standards. We are one of the earliest developers of novel commercial SFC phases, and continue to lead in this area. We also provide bulk chromatographic media, column packing services, and consulting.

Chief Chromatographic Techniques Supported

- UHPLC
- HPLC
- SFC
- Analytical
- Preparative

Markets Served

Globally providing chromatographic columns and media to the following markets: pharmaceutical, bioscience, cannabinoids, education, and drug discovery.

Major Products/Services

Princeton Chromatography Inc. offers a wide range of stationary phases to support analytical, prep, and ultrahigh-pressure liquid chromatography workflows. With new, innovative phases being added periodically, we continue to expand our catalog to meet your evolving HPLC and SFC needs. All phases are available in a range of particle sizes, column diameters and lengths, from 2.0 mm i.d. to 50.0 mm i.d. Popular phases include C18, 2-Ethylpyridine, Diol, and Cyano. In addition to our standard phases, we also specialize in custom phases as well as column packing services, and bulk chromatographic material.

Facility

Headquarters, production, and testing laboratories are located in Cranbury, NJ.

PRINCETON CHROMATOGRAPHY INC

Restek Corporation

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E-MAIL
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WEBSITE
www.restek.com

NUMBER OF EMPLOYEES
500+

YEAR FOUNDED
1985

Company Description

For over 30 years, Restek has been a leader in developing technologies and manufacturing products for gas and liquid chromatography (GC and LC), including columns, reference standards, sample preparation materials, accessories, and more. We have decades of hands-on, practical experience in chemistry, chromatography, and engineering, and our reputation for going the extra mile with Plus 1 customer service and top-performing products is well known throughout the chromatography community. Restek is proud to assist analysts around the world with monitoring the quality and safety of air, water, soil, food, pharmaceuticals, and petroleum. We proactively offer integrated solutions—products, applications, and assistance—perfectly matched to your needs, regardless of your industry. www.restek.com

Chief Chromatographic Techniques Supported

- UHPLC
- HPLC
- LC–MS
- GC
- GC–MS
- GC×GC

Markets Served

- Air monitoring
- Chemical
- Clinical
- Environmental
- Food safety
- Forensic
- Industrial hygiene
- Petrochemical
- Pharmaceutical

Major Products/Services

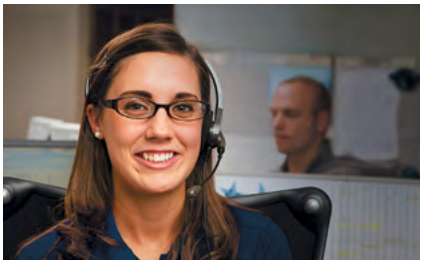
Plus 1 Service in everything we do. Living this corporate core value every day ensures we will surpass your expectations every time you contact us! Our customer service team will suggest time- and money-saving options, and is dedicated to getting your products to you fast. Our technical service chemists can help you from set-up to method development. Visit our website, where you can interact with our chemists' blogs and explore an extensive library of technical publications, chromatograms, product documentation, step-by-step guides, interactive calculators, animations, and educational material.

Restek's commitment to continuous innovation in chromatography sets us apart from our competitors. We introduce and stock hundreds of new products every year, designed by chromatographers for chromatographers.

- Exceptional columns for UHPLC, HPLC, LC–MS, GC, GC–MS, and GC×GC
- Innovative accessories, instrument replacement parts, and consumables
- Air monitoring canisters and sampling supplies
- Sample preparation products
- Reference standards: stock and custom-prepared formulations
- Thousands of innovative products, hundreds of chromatograms

Facilities

Restek opened for business in 1985 in a small business incubator in central Pennsylvania. Today, more than 500 employee-owners work, play, and celebrate milestones in a state-of-the-art 140,000-square-foot facility in Pennsylvania, and in our regional offices in China, England, France, Germany, Italy, Spain, and Japan.



SCION Instruments

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WEBSITE
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Company Description

Built on the history of Varian in GC and GC–MS, SCION Instruments was acquired by the Techcomp group in 2014. SCION Instruments is committed to continuing the 50+ year legacy of products, service, and innovation. We design, develop, supply, and support GC, GC–MS, LC, Headspace, and CompassCDS (chromatography data system) product lines. SCION Instruments maintains a global infrastructure to support sales and service not only for SCION Instruments customers but also for users of legacy Varian and Bruker systems. Our gas and liquid chromatography solutions help you boost productivity and generate data confidently.

Chief Chromatographic Techniques Supported

- Gas Chromatography (GC)
- GC Mass Spectrometry (GCMS)
- High-Performance Liquid Chromatography (HPLC)
- Static and Dynamic Headspace
- Autosamplers for GC, GC–MS, and HPLC
- Chromatography Data System (CDS)
- Preventative Maintenance and Instrument Repair
- Laboratory Accessories
  - GC Columns
  - HPLC Columns
  - GC Gas Filters
  - GC Inlet Liners and Septa
  - Syringes
  - Vials
  - Balances
  - Temperature Control Units

Markets Served

- Petrochemical and Refineries
- Pharmaceutical
- Food & Beverage
- Flavors & Fragrance
- Chemical
- Contract Laboratories
- Environmental
- Forensic
- Academic
- Cannabis

Major Products/Services

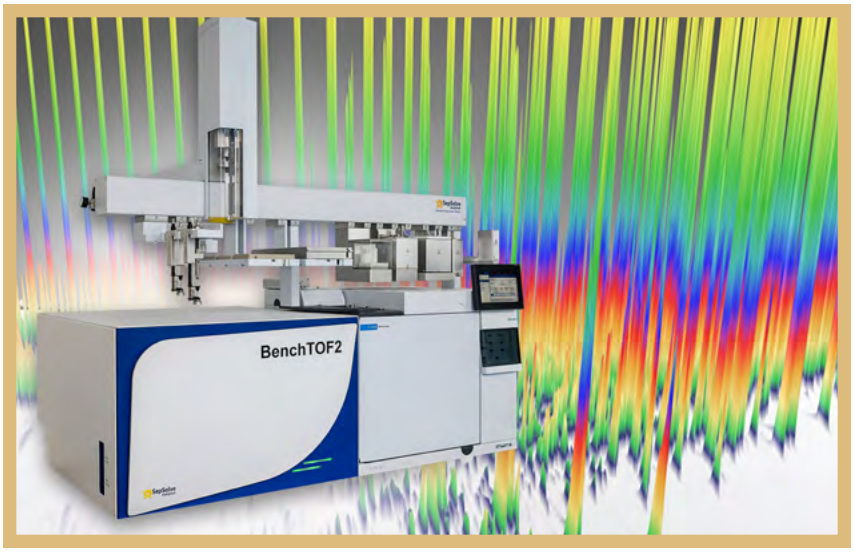
- **SCION 8500 GC** and **8300 GC** offer versatility and superior performance for any application. Detectors: FID, TCD, ECD, PFPD, NPD, PDHID, MS
- **SCION 8700 SQ** and **8900 TQ** are designed for today's fast-paced labs. Both have a small footprint without compromising on quality.
- **SCION LC6000** aims for confidence in results through outstanding lifetime performance and superior gradient precision.
- SCION's **Versa** and **HT3** provide static and dynamic headspace solutions for any laboratory.
- **SCION 8400Pro** and **8410Pro** offer dual injector access with one autosampler.
- **CompassCDS** is our industry-proven, powerful, and operator-friendly chromatography data system software solution.
- Preventative Maintenance and Instrument Repair

Facility

SCION Instruments prides itself on global sales and service infrastructure with the US office located in Fulton, MD. Our instruments are designed at our R&D facility in Livingston, Scotland and manufactured at our headquarters in Goes, The Netherlands.

SCION INSTRUMENTS
A Techcomp Company

SepSolve Analytical Ltd.



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WEBSITE
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YEAR FOUNDED
2016

Company Description

SepSolve Analytical provides analytical platforms for separation scientists, including equipment for automated sample introduction, advanced GC separation, state-of-the-art mass spectrometry, and powerful data analysis.

With many years of experience in the field and access to a range of leading equipment suppliers, SepSolve is very well placed to advise on the most difficult challenges in analytical science, helping analysts to discover more and deliver more—in everything from environmental monitoring and biomarker discovery to petrochemical analysis, food aroma profiling, and more.

SepSolve Analytical Ltd, and its sister company Markes International, are part of the Schauenburg Analytics Ltd group of companies.

Chief Chromatographic Techniques Supported

- GC and GC×GC
- TOF-MS
- Software for GC/GCxGC-MS
- Sample preparation (extraction and enrichment): Thermal desorption, SPME and SPME–trap, High-capacity sorptive extraction, Headspace and Headspace–trap

Markets Served

- Biomarker discovery
- Food and drink
- Petrochemical
- Fragrance
- Environmental

Major Products/Services

The wide range of products and techniques offered by SepSolve includes the company's own INSIGHT-Flow and INSIGHT-Thermal GC×GC modulators, ChromSpace® and ChromCompare+ software for GC and GC×GC, and BenchTOF range of time-of-flight mass spectrometers with groundbreaking simultaneous hard- and soft-ionization technology—Tandem Ionisation®. SepSolve also offers sample preparation equipment, robotic autosamplers and thermal desorbers from leading global suppliers.

Facility

SepSolve has offices and demonstration laboratories in Peterborough, UK, and Waterloo, Canada, and works closely with partners to support customers worldwide, with facilities in countries including the United States, Germany, and China.

SepSolve Analytical

Shimadzu Scientific Instruments

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WEBSITE
www.ssi.shimadzu.com

NUMBER OF EMPLOYEES
US: 625
Worldwide: 14,200

YEAR FOUNDED
Shimadzu Scientific Instruments: 1975
Shimadzu Corporation: 1875

Company Description

Shimadzu Scientific Instruments (SSI) is the North American subsidiary of Shimadzu Corporation's Analytical and Measuring Division. Headquartered in Columbia, Maryland, SSI offers a comprehensive portfolio of robust, precision-engineered platforms. From teaching environments and QA/QC to innovative R&D projects, customers can count on the stability, experience, and support only Shimadzu offers.

Chief Chromatographic Techniques Supported

- Analytical HPLC, UHPLC
- Prep HPLC, SFC
- Inert UHPLC
- SFE–SFC
- Ion Chromatography
- LC–MS/MS
- Q-TOF LC–MS
- Multiplex LC–MS
- GC
- GC–MS/MS

Markets Served

Shimadzu's product line flexibility enables chromatographers in any environment to select the instrument best suited to their application. Shimadzu instruments are found in a wide range of laboratories, including pharma/biopharma, environmental, food and beverages, petrochemical, life sciences, and clinical. Shimadzu provides free technical support for the life of the instruments, and encourages customer alliances to further product development.

Major Products/Services

Shimadzu offers a wide range of advanced UHPLC/HPLC, GC, and mass spectrometry systems and components. Key systems include:

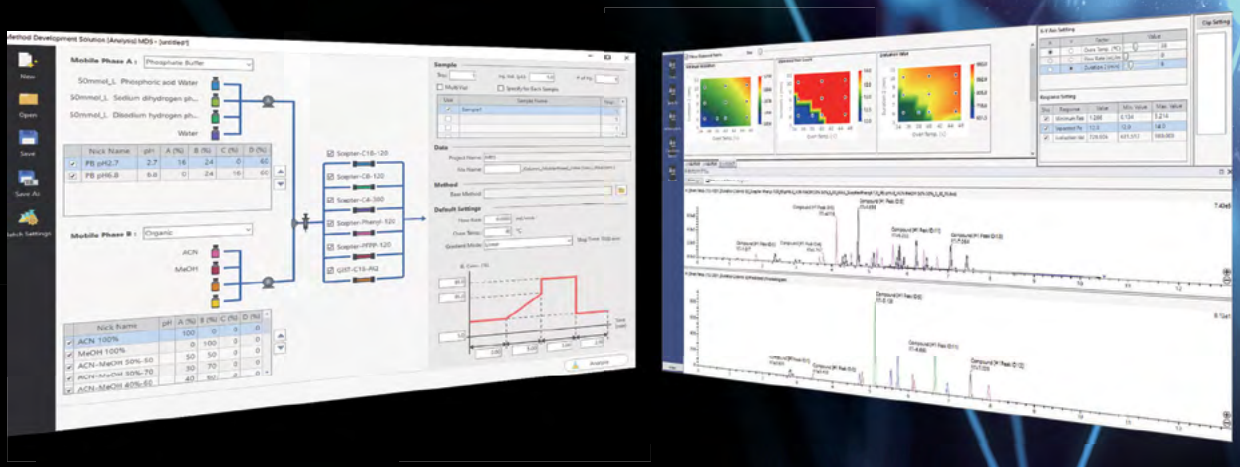
- Nexera 40 series UHPLC—Offering the most advanced performance features available, the 40 series enables smart, efficient workflows and delivers excellent data quality.
- Nexera SFC/Prep SFC—User-focused automation and streamlined software simplify processes, enhance accuracy and increase productivity
- Single-quad LCMS-2050—Compact, robust system provides a complete package of easy-to-use high-level performance
- LC-MS/MS RX Series—Outstanding speed, sensitivity, and robust operation for maximum uptime and the utmost in data quality
- Nexis GC-2030—Offers a modern approach to a classic chromatographic technique, delivering exceptional performance and faster ROI.
- Brevis GC-2050—Best-in-class analytical performance and scalability in an ultra-compact design.
- GC–MS/GC–MS/MS—With Smart Technologies, single and triple-quad systems enable new possibilities in sensitivity, durability, stability, and reliability.

Facilities

Shimadzu's US headquarters includes a customer service and training center, a solution center to showcase technologies, and an R&D center for promoting collaborative projects with customers. Shimadzu's regional facilities, strategically located around the US, provide customers with local sales, service, and technical support.

SHIMADZU
Excellence in Science

SHIMADZU
Excellence in Science



LabSolutions MD: Integrate Everything

DoE, CDS, Reporting, & Compliance

LabSolutions MD is an effective and automated software solution for U/HPLC method development that uses analytical quality by design (AQbD) principles in its workflow.

Key benefits:

- ▶ Greatly reduces the manual time spent on creating methods and batches.
- ▶ Utilizes the power of Experimental Design (DoE) to test the widest range of chromatographic conditions.
- ▶ Uses chromatographic and statistical parameters to arrive at the most optimum method within the design space

An AI driven Automated Gradient Optimization Function also tests and predicts the best gradients to achieve the user's set parameters, such as resolution, USP tailing factor, retention times and many more.

LabSolutions MD works with LC, SFC, and most detectors, including UV, PDA, Fluorescence, Refractive Index, and ELSD.

Shimadzu Scientific Instruments
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SilcoTek Corporation

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WEBSITE
www.SilcoTek.com

NUMBER OF EMPLOYEES
65

YEAR FOUNDED
2009

Company Description

SilcoTek® Corporation is the world's leading provider of flow path deactivations and coatings. Our patented CVD coating technologies improve analytical reliability, increase system uptime, and boost your bottom line. SilcoTek's coatings are used in a large, diverse variety of industries and applications where analytical accuracy and superior performance are critical. Whether for industry-leading chemical compatibility, corrosion resistance, bio-inertness, or hydrophobicity, SilcoTek coatings will expand the material limits of your instruments and products.

Chief Chromatographic Techniques Supported

- Sulfur analysis
- VOC analysis
- Environmental
- Thermal desorption
- Fenceline monitoring
- CEMS
- Flare subpart JA
- Stack monitoring
- Protein analysis
- NOx, SOx testing
- GC
- LC

Markets Served

SilcoTek coating technologies are utilized in diverse applications and industry segments worldwide. Since 1987, customers have relied on SilcoTek coatings to improve the performance of their products and increase revenue in the following markets and applications:

- Process
- Analytical
- Bio/Pharma
- Oil & Gas
- Refining
- Petrochemical
- Semiconductor
- Corrosion
- Coking/Fouling
- Automotive
- Aerospace

Major Products/Services

Major Products/Services: SilcoTek offers custom silicon coatings to OEMs and labs worldwide. Send in your stainless steel, alloy, glass, or ceramic part and we'll apply our signature CVD coatings. Our coating options include:

- SilcoNert®: The most inert coating available.
- Dursan®: An inert, high durability coating. Ideal for lab and field analysis.
- Silcolloy®: A high purity corrosion resistant coating.
- SilcoKlean®: Prevent carbon coking and fouling in automotive and aerospace.
- SilcoGuard®: A low outgassing, high purity coating, ideal for research and semiconductor applications.
- Dursox®: A high purity corrosion-resistant coating.
- Siltride®: SilcoTek's most protective and versatile silicon nitride CVD coating technology.

Facility

SilcoTek applies coatings to customer-supplied products from their newly renovated 70,000 square foot facility state of the art facility in Bellefonte, Pennsylvania. Want to speak to an expert about your application?
Email TechService@SilcoTek.com
or call +1 (814) 353-1778.
To find a sales representative nearest to you, please visit
www.silcotek.com/international-reps

Syft Technologies

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Christchurch
New Zealand

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WEB SITE
https://syft.com/

NUMBER OF EMPLOYEES
>150 worldwide

YEAR FOUNDED
2002

Company Description

Syft Technologies, the pioneer of SIFT MS, is the leading supplier of high-throughput, real-time trace gas analysis instrumentation. Volatile organic compounds (VOCs) and inorganics that cannot be easily targeted by traditional chromatographic methods can be easily detected using SIFT-MS. No sample preparation is required for the direct detection of difficult analytes including those in complex matrices and high humidity environments. SIFT-MS can be used by both MS experts and non-technical users. This direct injection mass spec technology has been developed and proven over 20 years in commercial environments. You can be assured of operational robustness, speed and 24/7 worldwide support.

Chief Spectroscopic Techniques Supported

- Mass spectrometry
- Direct injection mass spectrometry
- Online gas analysis

Markets Served

Pharmaceutical, CDMO / CRO, Semiconductor, Environmental, Petrochemical, Automotive, Consumer Products, Food Packaging, Flavors & Fragrances, Indoor Air Safety, Shipping Container Safety

Major Products/Services

- Syft Tracer
- Syft Tracer Pharm11
- Syft Explorer
- Syft SafetySure

Facility

New Zealand
68 St Asaph Street
Christchurch

Taiwan
4th Floor, No. 455,
Wenhua 3rd Road
Section 2,
New Taipei City

Singapore
211 Woodlands Ave 9
Woodlands Spectrum 2
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Germany
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United States
675 N. Euclid St. #627, Anaheim, CA

South Korea
46, Angol-ro
Bundang-gu
Seongnam-si

Syft™

Tecan Group Ltd.

Tecan Group Ltd.
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Männedorf, Switzerland

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WEB SITE
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NUMBER OF EMPLOYEES
3500

Company Description

Tecan is a global provider of laboratory instruments, reagents, consumables, and solutions in biopharmaceuticals, forensics, diagnostics, and academia. We enable our customers to achieve groundbreaking work in life sciences and medical research through innovative automation, digitalization, and sample preparation tools. Our platforms are designed for flexibility, ensuring precision and efficiency in various lab workflows, from genomics and proteomics to drug discovery, while contributing to a healthier and more sustainable world.

Chief Spectroscopic Techniques Supported

- Liquid Chromatography–Mass Spectrometry (LC-MS) sample preparation
- High-Performance Liquid Chromatography (HPLC) sample preparation
- Gas Chromatography (GC) sample preparation
- Ion Chromatography (IC) sample preparation
- Preparative Chromatography sample preparation

Markets Served

Tecan serves the biotechnology, pharmaceutical, clinical diagnostics, academic research, and industrial markets. Our solutions are vital for genomics, proteomics, drug discovery, diagnostics, and food safety labs, providing end-to-end automation and support in laboratory workflows.

Major Products/Services

- Fluent® Automation Workstations for automated liquid handling
- Resolvex® i300 and Resolvex A200 for automated solid phase extraction
- Resolvex Prep automated high complexity low-to-medium throughput sample preparation
- Solid Phase Extraction (SPE) consumables for optimized workflows
- AffinEx™ Protein A for efficient protein purification
- Reagents and consumables to support a wide range of laboratory applications
- LabNavigator™ and Introspect™ for digital lab management

Facility

Tecan operates state-of-the-art facilities across the USA, Europe, and Asia, ensuring robust global manufacturing and service support for customers worldwide.

TECAN.

Tosoh Bioscience

Tosoh Bioscience

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WEBSITE
www.tosohbioscience.com

NUMBER OF EMPLOYEES
140

YEAR FOUNDED
1987

Company Description

Tosoh Bioscience – Separation and Purification is an acknowledged global leader in liquid chromatography products with a focus on bioseparations. Our team of chromatography experts enables our biopharma partners to provide safe and efficient therapies against life threatening diseases.

Chief Chromatographic Techniques Supported

- Liquid chromatography
- Size exclusion
- Reversed phase, normal phase (hydrophilic interaction)
- Ion exchange
- Hydrophobic interaction
- Affinity
- Mixed-mode

Markets Served

Tosoh Bioscience provides bioseparation products and services primarily to the life sciences and the biopharmaceutical industry. They are used in R&D, manufacturing, and quality control. Our GPC instruments and columns for polymer analysis are utilized in the chemical and petrochemical industry.

Major Products/Services

We offer a comprehensive line of products the analysis and purification of biomolecules: analytical TSKgel® U/HPLC columns; and downstream processing solutions, such as screening tools, SkillPak™ pre-packed columns, TOYOPEARL®, TSKgel, and Ca^{++}Pure-HA™ media, and Octave™ multi-column chromatography (MCC) systems. Our LenS™3 MALS detectors are compatible with GPC, HPLC, and UHPLC systems. For the characterization of natural and synthetic polymers, we offer a line of EcoSEC GPC/SEC instruments and GPC columns.

TOYOPEARL and TSKgel products are renowned for their quality and reliability. Tosoh has a proud history of innovation in size-exclusion chromatography (SEC). TSKgel SW columns are the industry's standard for QC of antibodies by SEC.

Tosoh Bioscience offers extensive technical support such as application development and workshops, and service plans for all instrumentation.

Facilities

In the United States, Tosoh Bioscience has offices in King of Prussia, PA, and Madison, WI; manufacturing operations in Madison, WI, and Grove City, OH; and supply chain operations in Grove City, OH.

In Europe, the Tosoh Bioscience office is in Griesheim, Germany, and supply chain operations are in Tessenderlo, Belgium.

Asia is served by Tosoh Corporation in Tokyo, Japan, Shanghai, China, and Singapore. TSKgel, TOYOPEARL and EcoSEC products are manufactured by Tosoh Corporation, Japan.

Tosoh Bioscience is part of the Tosoh Group, a Japanese chemical and specialty products group with over 100 companies worldwide and a multiethnic workforce of over 14,000 people.

TOSOH BIOSCIENCE

UCT, Inc.

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WEBSITE
www.unitedchem.com

NUMBER OF EMPLOYEES
>100

YEAR FOUNDED
1986

Company Description

UCT is at the forefront of sample preparation technology and a leader in chromatography consumables. The company's wide range of highly reproducible silica and polymeric SPE sorbents provides scientists a consistent extraction technique. UCT is in a unique position within the sample prep and chromatographic sorbent industry; we are one of the few manufacturers of chromatographic silane materials. This expertise provides us with greater control and extensive knowledge of the chemical processes involved in producing high quality bonded phases. UCT's commitment to ensuring customer satisfaction is accomplished by delivering on our promises: top quality, reproducible chromatographic and sample prep products, and unmatched technical support. The company's sample prep product lines include SPE cartridges and well plates, bulk sorbents, QuEChERS tubes, derivatizing reagents, hydrolyzing enzymes, premeasured buffer salts, and extraction manifolds. Chromatography products include HPLC columns and GC liners.

Chief Techniques Supported

- Solid-phase extraction
- QuEChERS
- HPLC

Markets Served

- Clinical
- Environmental
- Food safety
- Forensic
- Pharmaceutical
- Veterinary

Major Products/Services

- CLEAN SCREEN® SPE columns
- Refine® Ultra Filtration Columns & Well Plates
- CLEAN UP® SPE columns
- STYRE SCREEN® SPE columns
- XtrackT® SPE columns
- ENVIRO-CLEAN® SPE cartridges
- ENVIRO-CLEAN® universal cartridges
- Fusion® Ag+
- Chlorofiltr® Sorbents
- QuEChERS
- Quick QuEChERS
- SpinFiltr™
- LipiFiltr®
- Selectra® U/HPLC columns
- SelectraCore core-shell columns
- SELECTRASORB™ bulk sorbents
- SELECTRA-SIL® derivatizing reagents
- SELECTRAZYME® & ABALONASE™ β-glucuronidase enzyme
- SELECT pH buffer pouches
- Positive pressure manifolds
- SPeVAP® Multi-Function Solvent Evaporator
- Vacuum manifolds
- MicroPrep™ 96-Well Microelution Plate
- PFAS Glass Block Manifold

Facility

UCT is headquartered in Bristol, Pennsylvania. Manufacturing and distribution sites are in Lewistown, Pennsylvania, and Wexford, Ireland. UCT is represented worldwide by more than 50 partners and distributors.

UCT

Customer Support
methods@unitedchem.com

FOLLOW UCT ON LinkedIn

PFAS GLASS BLOCK

MANIFOLD SYSTEM

- Corian® lid with Luer Fittings
- Multiple Position Options
- Clear Glass Block
- Vacuum Control and Monitoring
- Chemically Resistant Polypropylene Racks
- PFAS-Safe Components
- Cost-Effective and Flexible

2024 PFAS Solutions Guide

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NUMBER OF EMPLOYEES
US: 365
Europe: 135

YEAR FOUNDED
1968

Company Description

Founded in 1968, VICI is an international group of companies. Our products can be found in nearly every national lab, military facility, and university research department around the globe. For 50 years VICI has driven the field of chromatography with valves, fittings, detectors, and instruments for precision analytical, biomedical, and biocompatible applications. We also customize components and complete systems to improve chromatographic analyses.

Chief Chromatographic Techniques Supported

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- Liquid Chromatography
- Gas Chromatography
- Flow Injection Analysis
- Supercritical Fluid Chromatography
- Gas Permeation Chromatography
- Process Gas Chromatography

Major Products/Services

VICI produces a complete line of valves for analytical chemistry. We specialize in zero dead volume fittings and filters for the separation sciences and are the original creators of the pulse discharge detector (PDD). Our mini-PDD consumes less than one-fifth the amount of helium required by the PDDs used on complete GC systems. It has similar sensitivity to the original PDD with a slightly lower dynamic range.

Our VICI Metronics division produces PEEK, FEP, PFA, and ETFE tubing, ambient temperature gas purifiers, ValcoBond GC capillary columns, and Dynacal gas calibration standards. VICI Precision Sampling manufactures analytical quality syringes, sampling probes, as well as standard and custom formed stainless and special alloy tubing. VICI AG International produces some Valco products as well as the Jour line of HPLC fittings and lab safety products. VICI DBS builds our new line of laboratory hydrogen, nitrogen, and zero air gas generators.

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Facility

With over 150 state-of-the-art CNC machines, our instruments and components are manufactured to exacting standards at our facilities in Houston, USA, and Schenkon, Switzerland. Our other manufacturing facilities include VICI Metronics in Washington, VICI Precision Sampling in Louisiana, and VICI DBS located in Italy. VICI Canada serves our neighbor to the north from its offices in Brockville, Ontario.

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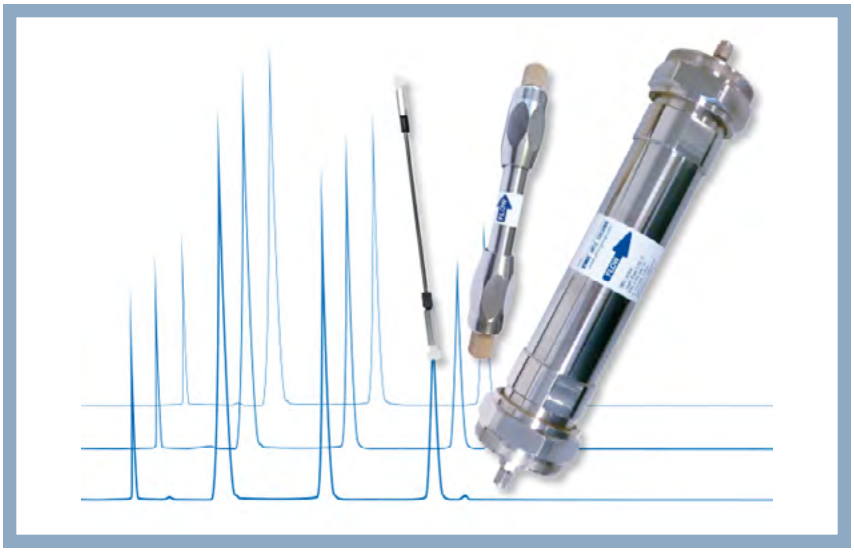

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NUMBER OF EMPLOYEES
50 (Europe)
485 (worldwide)

YEAR FOUNDED
1993

Company Description

YMC is a leading global supplier of high-performance products for liquid chromatography. The broad and innovative product range includes (U)HPLC columns (especially YMC-Triart), dedicated bioinert BioLC columns (for SEC, IEX, RP, HIC) and chiral columns (immobilised or coated). The portfolio further includes bulk media for preparative processes, glass columns (ECO/ECOPLUS) for MPLC and pilot columns (YMC PilotPLUS). YMC provides comprehensive application support, column packing, phase screening and method development services and training programmes.

YMC products are used in R&D, process development, manufacturing, and quality control. YMC's extensive distribution network guarantees availability of YMC products in countries all over the world.

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- HPLC
- UHPLC
- microLC
- nanoLC
- preprative LC
- MPLC
- RP
- NP
- HILIC
- SEC
- IEX
- HIC
- Chiral LC
- SFC

Markets Served

- Pharma
- Biopharma
- Life Science
- Environmental
- Clinical
- Food and Beverage
- Chemical
- Toxicology

Major Products/Services

- scalable to bulk packing materials for lab-scale and production-scale purifications.
- UHPLC/HPLC Columns
- MicroLC/NanoLC Columns
- (Semi) Preparative Columns
- Preparative Bulk Media
- Lab Scale Glass Columns
- Pilot Scale Columns
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- Method Development
- Purification Service
- Seminars & Trainings
- HPLC Tool Kits

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Comprehensive Polar Metabolite Profiling with HILIC-LC-MS

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METABOLIC PROFILING STUDIES using LC-MS technology have enabled the sensitive and reproducible detection of a wide range of metabolites in various biological samples, including biofluids, cells, tissues, and organisms. However, the analysis of polar/hydrophilic metabolites, such as small organic acids, amino acids, nucleotides, and sugars, meets challenges due to their poor retention in traditional reverse-phase LC-MS methods without using ion-pairing reagents in mobile phase or sample derivatization (1).

Hydrophilic interaction liquid chromatography (HILIC) offers a different retention mechanism that is advanced for straightforward separations of hydrophilic compounds (2). Although earlier works showed HILIC technology is effective for a small set of metabolites, it currently has achieved remarkable progress on improving chromatography separation and measurement repeatability, paving the way for comprehensive polar metabolite profiling.

In this application note, we describe a HILIC-LC-MS method using a polymeric iHILIC-(P) Classic column to accomplish a comprehensive polar metabolite profiling of several hundred metabolites in a single run. The results indicate remarkable chromatographic separation and repeatability of retention time. The importance of incorporating medronic acid in HILIC separations (3) and a straightforward sample extraction procedure are also touched.

Experimental

Sample preparation:

1) Metabolite standard samples were prepared in acetonitrile-methanol-water mixture (40:40:20, v/v%) and stored at -80 °C. Their final concentration was 10 µM.
2) 100 µL plasma or DMEM was mixed with 400 µL ice-cold methanol-acetonitrile solution (50:50, v/v%) by vortexing for 3-5s. The mixture was then incubated overnight at -80°C. Whereafter, samples were centrifuged at 15,000 rpm for 15 min at 4°C. Supernatant was transferred to a sample vial for LC-MS analysis.
3) The 293T cells after medium removal were extracted with 500 µL ice-cold acetonitrile-methanol-water solution (40:40:20, v/v %) for about 10 min. The cell extracts were transferred to 1.5 mL tubes and stored overnight at -80 °C. They were further cleaned by centrifuging to remove proteins at 15,000 rpm for 15 min at 4 °C. The supernatant was used for LC-MS analysis.

LC-MS/MS system:

A Shimadzu ExionLC AC HPLC system was connected to a TripleTOF 6600+ mass spectrometer from AB SCIEX. Electrospray ionization (ESI) in both positive and negative mode were used for detection. The ESI source parameters: source temperature at 500 °C, ion source gas 1 and 2 at 60 psi, curtain gas (CUR) at 35 psi, ion spray voltage floating (ISVF) at 5.5 kV or -4.5 kV for positive or negative modes. Mass spectrometer was set at TOF masses of 70–1200 Da.

HILIC separation:

Columns:

150 × 2.1 mm, 5 µm/200Å, iHILIC®-(P) Classic (P/N 160.152.0520, Sorbent Lot: 160-0520-10117 HILICON); Flow rate: 0.2 mL/min

Column temperature: 30 °C

Eluents:

A) 95:5 20 mM ammonium acetate and 0.1% ammonium hydroxide (v/v%) in water/ACN with 2.5 µM medronic acid.
B) Acetonitrile

TABLE 1: Gradient programs for separation with iHILIC-(P) Classic

time [min]	% B
0	85
2	85
7	60
12	35
12.1	20
15.9	20
16	85
23	85

Results and Discussion

Polymeric iHILIC-(P) Classic columns signify an outstanding advancement in HILIC separation under basic pH conditions, which enables successful separation of several hundred hydrophilic metabolites in a single run. This makes them a valuable tool for untargeted metabolomics studies in biological samples, especially when combined with a simplified extraction technique and ESI-MS detection.

Figure 1 demonstrates the untargeted metabolomics measurements for real biological samples. After excluding metabolites belonging to "lipid or lipid-like" classes, a dataset containing 699 unique hydrophilic metabolites was obtained. Among them, 286 were found exclusively in the 293T cells, 300 in plasma, and 113 in both. We were able to use our dataset of 154 metabolite standards to identify 61 metabolites exclusively in the 293T cells, 34 in plasma, and 59 in both. Our method is highly effective for a broad spectrum of metabolites, particularly for those belonging to the class of organic acids and their derivatives and nucleotides.

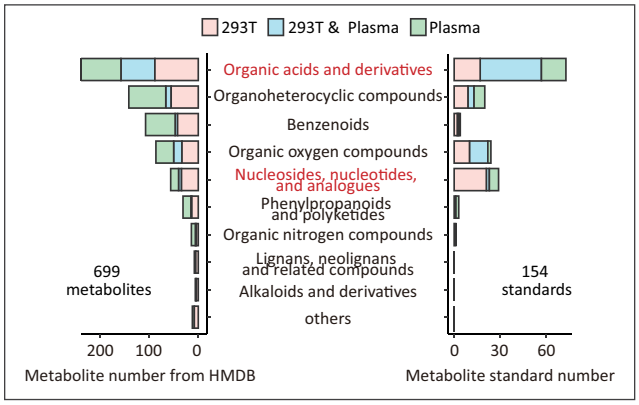

FIGURE 1 Untargeted profiling of hydrophilic metabolites in the 293T cells and plasma with HILIC-MS method in ESI- mode.

The described HILIC-LC-MS method is effective for several biologically important polar metabolites that were difficult to measure. As shown in Figure 2A, highly polar compounds with multiple phosphate groups, such as fructose 1, 6-bisphosphate and inositol hexaphosphate, are easily identified. Additionally, four biologically important metabolites and their isomeric forms were efficiently separated as well, shown in Figure 2B. Moreover, it's worth emphasizing that our sample extraction methods, which avoid drying and reconstitution, ensure the preservation of fragile metabolites, like nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP), as illustrated in Figure 2C.

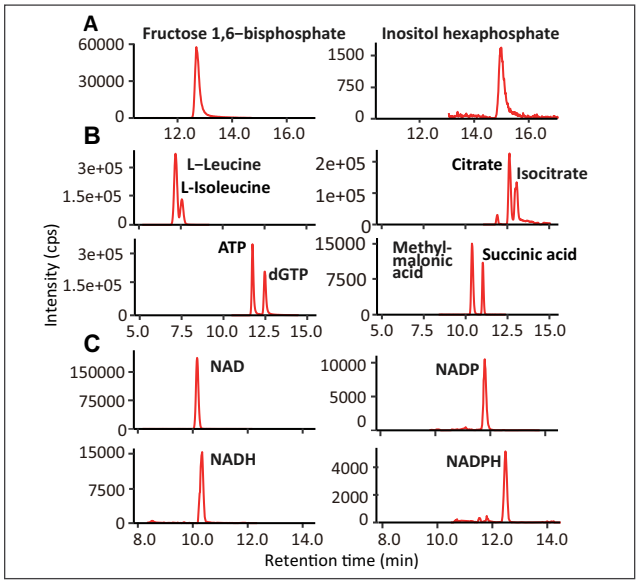

FIGURE 2 Extracted ion chromatograms of polar metabolites from standards or samples.

Figure 3 demonstrates the chromatographic repeatability of the polymeric iHILIC-(P) Classic columns. When analyzing identical amino acid mixture samples, we observed excellent peak shapes and retention time (RT) reproducibility across three different columns (serial number: #20308, #20504, and #40174), marked in green, blue, and red, respectively. In addition, the column (#40174) showed consistent RTs with a median delta RT of less than 0.03 min for >1000 injections of real samples and standards over a four-month period between July and October 2024.

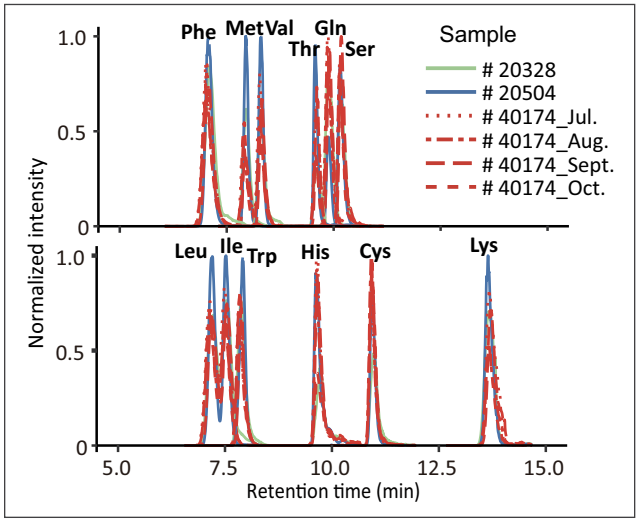

FIGURE 3 Extracted ion chromatograms of amino acids from Dulbecco's Modified Eagle Medium (DMEM) samples.

Conclusion

In summary, the current HILIC-LC-MS method profiles more than 500 polar metabolite standards across various categories in metabolomics studies. It revealed excellent chromatographic separation and retention time stability over several months. Along with straightforward sample preparation, the method enables comprehensive polar metabolite profiling of biological samples.

References

(1) Hu, Q., Tang, H., Wng,Y., Challenges in Analysis of Hydrophilic Metabolites Using Chromatography Coupled with Mass Spectrometry. J. Anal. Test. **2020**, 4, 140–162. https://doi.org/10.1007/s41664-020-00126-z

(2) Alpert A.J., Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds. J. Chromatogr. **1990**, 499, 177-96. https://doi.org/10.1016/S0021-9673(00)96972-3

(3) Hsiao, J.J., Potter , O.G., Chu, TW, Yin H., Improved LC/MS Methods for the Analysis of Metal-Sensitive Analytes Using Medronic Acid as a Mobile Phase Additive. Anal Chem. **2018,** 90, 9457-9464. https://doi.org/10.1021/acs.analchem.8b02100

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LC-MS/MS determination of mycotoxins in cannabis and cannabis derived products using different sample preparations

Juliane Kramer, Giorgia Greco, KNAUER Wissenschaftliche Geräte GmbH

THE U.S. FOOD & DRUG ADMINISTRATION (FDA) and Commission Regulation (EC) No 2023/915 set limit values for mycotoxins in human food and animal feed. AOAC SMPR®2021.010 defines aflatoxins B1/B2, aflatoxins G1/G2 and ochratoxin A as analytes of interest for quantification and qualification in cannabis biomass and cannabis derived products. Here, we describe sample preparation methods and an analytical chromatography LC-MS/MS method to detect mycotoxins in different hemp matrixes at ppb level.

Sample preparation

Four different samples, cannabis/hemp pellets, cannabis/hemp seeds, commercially available hemp flour, and hemp oil were investigated, and four different sample preparation procedures were processed. The following procedures were applied: P1 – solid-liquid extraction/liquid-liquid extraction (SLE/LLE), P2 - a standard QuEChERS extraction with dispersive cleaning, P3 - extraction with following CrossTOX cleanup and P4 - extraction with following solid phase extraction using immunoaffinity columns (IAC SPE). For a detailed description of all procedures please refer to KNAUER application note VDF0193.

FIGURE 1: Simplified overview of sample preparation procedures

Results

Mycotoxins were separated under reversed phase liquid chromatography (LC) conditions. They were detected with mass spectrometry (MS). Multiple Reaction monitoring (MRM) measurements were conducted using electrospray ionization (ESI) with positive polarity.

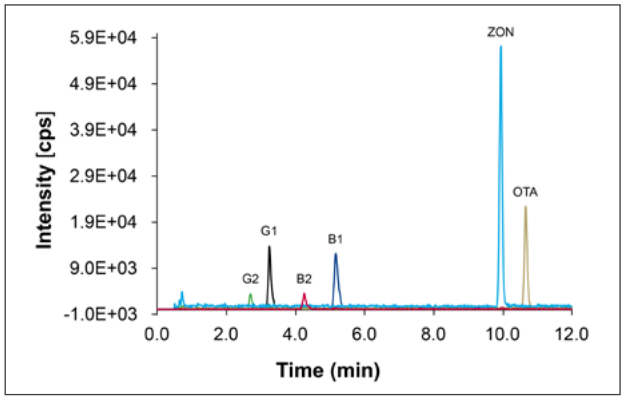

FIGURE 2: Exemplary overlay of Extracted Ion Current (XIC) from quantifier transitions, mycotoxin mix standard

The calculated values for limit of detection (LOD) and limit of quantification (LOQ) for measurements without matrix are below 20 ppb and within the specification of regulations. For LOD a signal to noise ratio (S/N) of 3 was taken as basis. For the LOQ a ratio of S/N = 10 was applied.

TABLE I: Comparison of LOD/LOQ without matrix and limit values in ppb, *valid for animal feeds/foodstuff

Peak	LOD (S/N=3)	LOQ (S/N=10)	FDA*	AOAC	(EC) No 2023/915*
G2	0.160	0.540	20 (sum of G2/G1/B2/B1)	20 (sum of G2/G1/B2/B1)	4-15 (sum of G2/G1/B2/B1)
G1	0.024	0.080			
B2	0.024	0.080		5 (B1)	0.1-12 (B1)
B1	0.018	0.060			
ZON	0.210	0.690	-	-	20 - 400
OTA	0.027	0.090	-	20	0.5 - 80

For recovery rates, the samples were spiked with mycotoxins in a concentration below 20 ppb. With MS detection, recovery rates for all extraction procedures could be determined but matrix suppression was still critical for the complex matrices like hemp seeds and pellets.

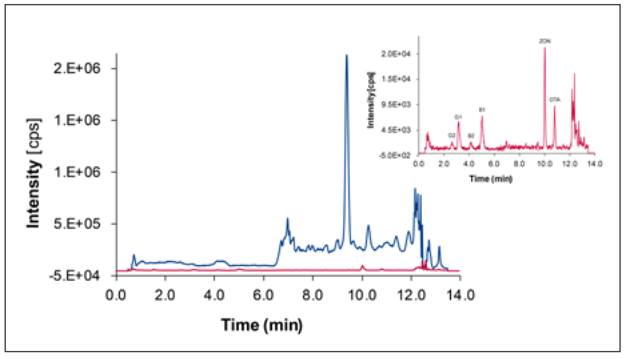

FIGURE 3: Total Ion Current (TIC) of spiked hemp seed sample with different sample preparations, P1 – blue, P4 – red, MS detection, right corner: XIC of P4 for hemp seeds

The most effective sample preparation was the solid phase extraction using immunoaffinity cartridges. Here, all mycotoxins could be detected in all spiked samples.

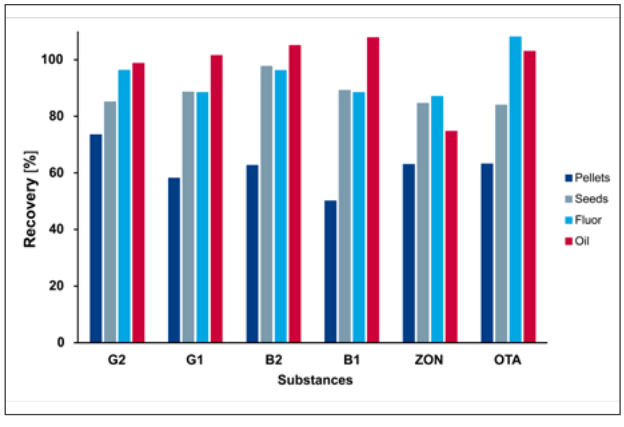

FIGURE 4: Recovery [%] of mycotoxins for all samples with sample preparation P4

More steps in sample preparation require additional consumables, like for QuEChERS or IAC SPE. Therefore, these types of preparations were more time consuming and expensive per sample. However, when it comes to reaching the LODs or LOQs, the more complex procedures were more effective. Simple sample preparation methods, such as SLE/LLE, may result in better matrix suppression and thus in lower recovery rates.

Conclusion

Generally, the limit values were met for all sample preparation procedures. The determination of recovery rates was dependent on the complexity of matrix and complexity of sample preparation. Challenging matrices like hemp and hemp products should be treated with more complex sample preparation procedures.

References

(1) Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed; Docket number: FDA-2020-D-1956-0001

(2) COMMISSION REGULATION (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006.

(3) AOAC SMPR® 2021.010, Standard Method Performance Requirements (SMPRs®) for Quantitative Analysis of Mycotoxins in Cannabis Biomass and Cannabis-Derived Products

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Analysis of chlorinated and brominated acetic acids in different water matrices by HPLC-ICPMS/MS

Ann Marie Rojahn, YMC Europe GmbH, Dinslaken, Germany

This application note presents the analysis of nine haloacetic acids (HAAs) in various water matrices using HPLC-ICPMS/MS. The method demonstrates reduced matrix effects and high sensitivity in detecting chlorinated and brominated acetic acids in tap, groundwater, and river water.

HALOGENATED ACETIC ACIDS (HAAs) are among the most common water disinfection byproducts. Since these are presumably harmful to health, the US Environmental Protection Agency (EPA) regulates the levels of five haloacetic acids (monochloro-, dichloro-, trichloro-, monobromo- and dibromoacetic acid) to an overall maximum of 60 µg/L. The regulations of the European Union include another four haloacetic acids, with a maximum concentration of 80 µg/L for all nine HAAs combined [1].

High performance liquid chromatography coupled with electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS/MS) is commonly used to achieve high sensitivity and selectivity. However, this detection technique is prone to matrix effects due to ion suppression or enhancement in the ionisation source.

Experimental conditions

For this analysis of nine HAAs inductively coupled plasma tandem mass spectrometry (ICPMS/MS) was used for detection, as it is less prone to matrix [2]. Different types of water samples from Austria were

TABLE I: Chromatographic conditions [2].

Column:	YMC- Triart C18 (3 µm, 12 nm) 150 x 3.0 mm ID
Part No.:	TA12S0-1503WT
Eluent:	22 mM oxalic acid in pure water (pH 1.8)
Flow rate:	0.5 mL/min
Temperature:	40 °C
Injection:	50 µL
Samples:	Tap (Graz), ground (Leutschach well) and river (Mur) water Spiked with EPA 552.2 standard 2.0 mg/L of each HAA: chloroacetic acid (CAA), dichloroacetic acid (DCAA), bromoacetic acid (BAA), chlorobromoacetic acid (CBAA), trichloroacetic acid (TCAA), dibromoacetic acid (DBAA), dichlorobromoacetic acid (DCBAA), chlorodibromoacetic acid (CDBAA), tribromoacetic acid (TBAA) all samples: acidified to a final concentration of 50 mM oxalic acid
Detection:	ICPMS/MS

analysed: tap water from Graz, groundwater from the Leutschach well and river water from the Mur.

Chromatographic conditions can be found in Table I.

Results

Figure 1 shows the baseline separation of nine HAAs, each spiked with 2.0 mg/L EPA 552.2 reference material, in about 15 min. A high injection volume is used to achieve the lowest possible limit of detection (LOD). Therefore, the alkaline hard water samples (pH = 7.1–7.9) must be acidified to reduce the pH difference with the mobile phase.

The LOD ranges from 1.8–2.0 µg Cl/L and 1.0–1.5 µg Br/L, while the limit of quantification varies between 4.6 and 12 µg/L.

The recoveries obtained show that ICPMS/MS detection is less prone to matrix effects compared to ESI-MS/MS because interfering ions with high carbon load (e.g. bicarbonate) are separated from the analytes as they elute with the dead volume. Therefore, an internal standard (IS) is not required unlike ESI-MS/MS, where an isotopically labelled IS for each analyte is preferable.

Furthermore, ICPMS/MS detection can also be performed using non-volatile buffers containing phosphate, sulphate, chloride or sodium.

Conclusion

This method for the analysis of nine haloacetic acids using HPLC-ICPMS/MS proved to be highly effective across different water matrices. It minimises matrix effects compared to traditional techniques, delivering reliable and accurate results without the need for internal standards. The approach shows great potential for routine environmental monitoring of HAAs in compliance with regulatory limits.

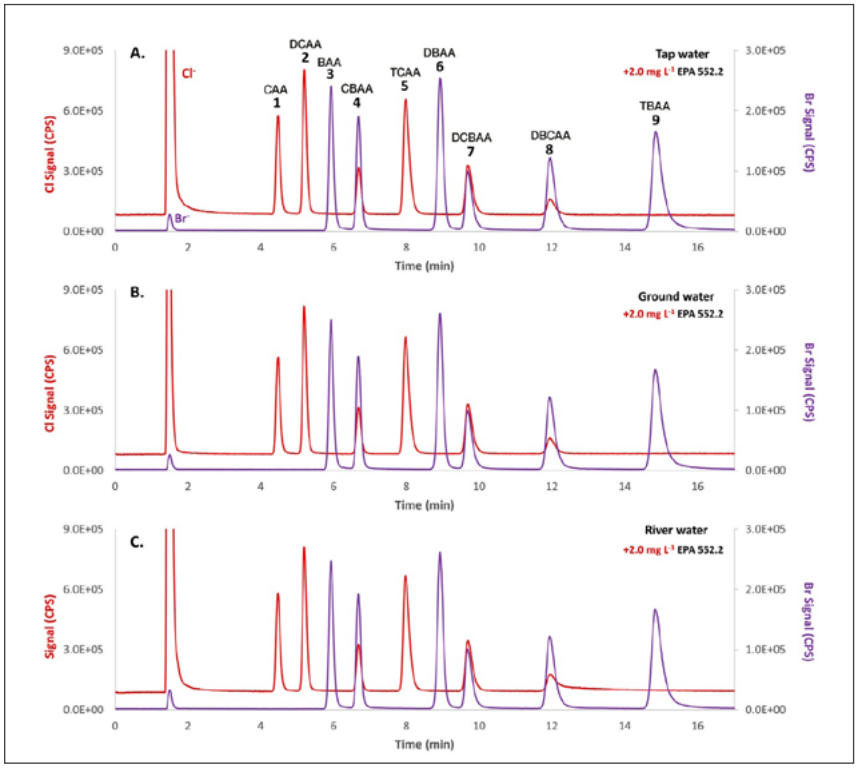

FIGURE 1: separation of haloacetic acids in tap (A), ground (B), and river water (C) samples spiked with the EPA 552.2 certified reference material [2].

References

(1) [1] https://www.europarl.europa.eu/doceo/document/TA-8-2018-0397_EN.html (accessed 08.2024)

(2) [2] Bassam Lajin and Walter Goessler, Analytical Chemistry 2020 92 (13), 9156-9163, DOI: 10.1021/acs.analchem.0c01456.

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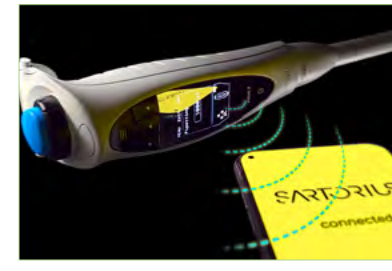

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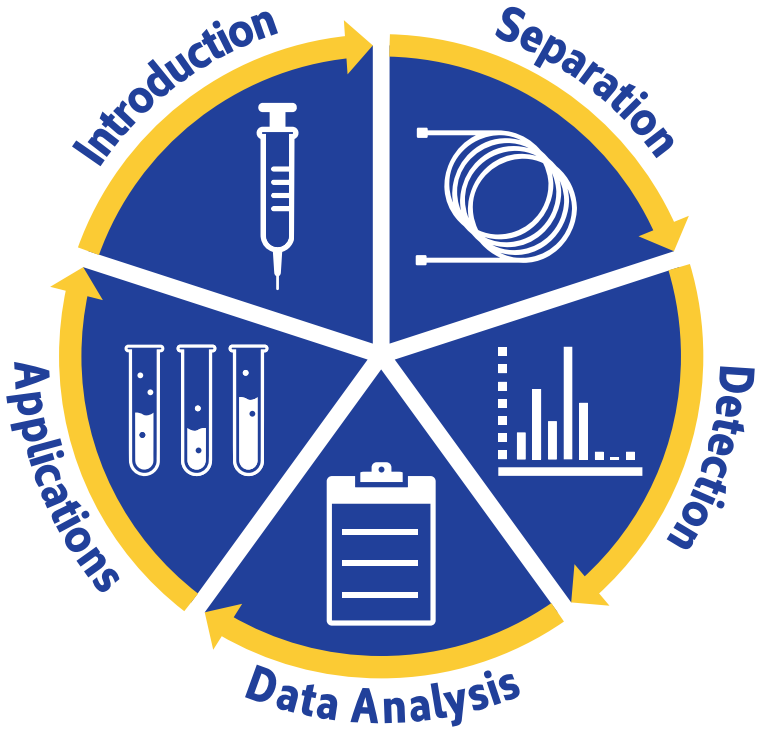

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(CONTINUED FROM PAGE 39)

the peaks begin to overlap, known as *touching bands*, to maximize productivity (23). This overlap, however, does introduce some uncertainty since the start and end of the overlapping peaks cannot be seen (Figure 4, black). To ensure maximum enantiomeric purity (>99.9 %) of the isolated compound, the area of overlap should be separately collected and recycled. To avoid the trial-and-error method when determining where to collect the enantiomers, the peaks can be fit using a model like the Gen2HVL. The Gen2HVL can also manage non-linear peak shapes. Figure 4 shows the fitting of both enantiomers (green and pink) with the Gen2HVL function. The region of enantiomeric overlap that must be recycled is now readily seen between the dotted lines. Note that the residuals here are larger in magnitude than the analytical examples presented above due to the overload profile. Still, using this technique makes it immediately apparent to the chromatographer where to collect the fractions.

Pitfalls in Peak Fitting

As a note of caution, peak fitting should not be used when two or more peaks overlap entirely. As a rule of thumb, the peak maximum or a shoulder must be visually visible before proceeding with the iterative curve fitting process. To confirm the presence of a shoulder, the first or second derivative can be analyzed, looking for splits in the derivatives in the region of interest. The higher the degree of overlap, the lower the statistical confidence will be in the fits. When analyzing overlapping peaks produced by mass spectrometric detection, there is a danger of ion suppression in the region of co-elution, which can give erroneous results. Note that peak fitting is still helpful for LC-MS when there is partial overlap of isobars, as seen in the cases of enantiomers, epimers, and structural isomers. When using generalized functions with several parameters (6 for Gen2HVL, 9 for Gen2HVL<ge>), each parameter may not be statistically significant, meaning a specific parameter is zero. The statistical significance of function parameters can be important for theoretical modeling but has trivial effect on the empirical information (peak area, location, and efficiency), which was the focus of this study.

Conclusions

This article demonstrates a powerful peak model applied to real chromatographic data under analytical (isocratic/gradient) and overload conditions. The newly introduced twice-generalized peak function based on Haarhoff van der Linde (HVL) offers the separation community a versatile function that can solve the frequent peak overlap problems and help them save time, solvents, and costs. Human judgment of the residuals is necessary when assessing GOF and ensuring the fit received is adequate. The *F*-statistic is a robust and reliable metric when deciding what function should be used to model the data and allows the number of parameters in the function to be considered for statistical significance. Applying iterative curve fitting to your workflow will save time, money, and solvent while producing high-quality and statistically confident analytical results for the separation scientist.

Data Availability Statement

Raw data for this manuscript is publicly available in the Harvard Dataverse Repository under a CC0 1.0 DEED license. In the data file, the first column represents time in minutes, and the second column represents signal in mAU. Please see DOI: 10.7910/DVN/UZX46O

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Teknokroma Analitica S.A.
+34 93 669 86 50
YMC Europe
+49 20644270

OTHER COLUMNS, CAPILLARY (<1 MM I.D.)
Axcend
Optimize Technologies
PolyLC Inc.
YMC Europe
+49 20644270

REVERSED-PHASE
GL Sciences BV
Ladybug Scientific LLC
Optimize Technologies
Phenomenex, Inc.
Shimadzu Scientific Instruments
(800) 477-1227
SILEX Chromatography
Teknokroma Analitica S.A.
+34 93 669 86 50
YMC Europe
+49 20644270

COLUMNS, HPLC (>10 MM I.D.)

AFFINITY
KNAUER Wissenschaftliche Geräte GmbH
Tosoh Bioscience GmbH
+49 6155-7043700
Welch Materials, Inc.

CARTRIDGE
Axcend
SiliCycle Inc.

CHIRAL
Regis Technologies, Inc.
Welch Materials, Inc.
YMC Europe
+49 20644270

CUSTOM
Hamilton Company
1 (800) 648-5950

GEL PERMEATION (SIZE-EXCLUSION)
KNAUER Wissenschaftliche Geräte GmbH
PolyLC Inc.
Postnova Analytics
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
Welch Materials, Inc.
YMC Europe
+49 20644270

HYDROPHILIC INTERACTION
Hilicon AB
+46 (90) 193469
PerkinElmer Chromatography Solutions
PolyLC Inc.
Tosoh Bioscience GmbH
+49 6155-7043700
Welch Materials, Inc.
YMC Europe
+49 20644270

HYDROPHOBIC INTERACTION
PolyLC Inc.
Tosoh Bioscience GmbH
+49 6155-7043700
Welch Materials, Inc.
YMC Europe
+49 20644270

ION CHROMATOGRAPHY
Hamilton Company
1 (800) 648-5950

CHROMATOGRAPHY
SiliCycle Inc.

ION-EXCHANGE
KNAUER Wissenschaftliche Geräte GmbH
SiliCycle Inc.
Tosoh Bioscience GmbH
+49 6155-7043700
YMC Europe
+49 20644270

ION-EXCLUSION
Hamilton Company
1 (800) 648-5950

NORMAL-PHASE
GL Sciences BV
Hilicon AB
+46 (90) 193469
KNAUER Wissenschaftliche Geräte GmbH
PerkinElmer Chromatography Solutions
SiliCycle Inc.
Teknokroma Analitica S.A.
+34 93 669 86 50
Tosoh Bioscience GmbH
+49 6155-7043700
Welch Materials, Inc.
YMC Europe
+49 20644270

OTHER COLUMNS, HPLC (>10 MM I.D.)
Hamilton Company
1 (800) 648-5950
Regis Technologies, Inc.
YMC Europe
+49 20644270

POLYMERIC
Hamilton Company
1 (800) 648-5950
Hilicon AB
+46 (90) 193469
KNAUER Wissenschaftliche Geräte GmbH
Polymer Char
Waters Corporation
(508) 478-2000

REVERSED-PHASE
GL Sciences BV
Hamilton Company
1 (800) 648-5950
KNAUER Wissenschaftliche Geräte GmbH
PerkinElmer Chromatography Solutions
Phenomenex, Inc.
Regis Technologies, Inc.
SiliCycle Inc.
Sorbent Technologies, Inc.
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
Welch Materials, Inc.
YMC Europe
+49 20644270

Teknokroma Analitica S.A.
+34 93 669 86 50
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
Welch Materials, Inc.
YMC Europe
+49 20644270

COLUMNS, HPLC (2.1-10 MM I.D.)

AFFINITY

Regis Technologies, Inc.
Tosoh Bioscience GmbH
+49 6155-7043700

CARTRIDGE

Hamilton Company
1 (800) 648-5950
Optimize Technologies
Regis Technologies, Inc.
SiliCycle Inc.
Welch Materials, Inc.

CHIRAL

Regis Technologies, Inc.
Welch Materials, Inc.
YMC Europe
+49 20644270

CUSTOM

Fortis Technologies Ltd
Hamilton Company
1 (800) 648-5950
Ladybug Scientific LLC
Optimize Technologies
Welch Materials, Inc.

GEL PERMEATION (SIZE-EXCLUSION)

Phenomenex, Inc.
Postnova Analytics
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
YMC Europe
+49 20644270

HYDROPHILIC INTERACTION

Develosil
(858) 800-2433
Hilicon AB
+46 (90) 193469
PerkinElmer Chromatography Solutions
Phenomenex, Inc.
PolyLC Inc.
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
YMC Europe
+49 20644270

HYDROPHOBIC INTERACTION

Develosil
(858) 800-2433
PerkinElmer Chromatography Solutions
PolyLC Inc.
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
YMC Europe
+49 20644270

ION CHROMATOGRAPHY

Hamilton Company
1 (800) 648-5950

ION-EXCHANGE

Hamilton Company
1 (800) 648-5950
Phenomenex, Inc.
PolyLC Inc.
SiliCycle Inc.
Tosoh Bioscience GmbH
+49 6155-7043700
Welch Materials, Inc.
YMC Europe
+49 20644270

ION-EXCLUSION

Hamilton Company
1 (800) 648-5950

MONOLITHIC

GL Sciences BV

NORMAL-PHASE

Develosil
(858) 800-2433
GL Sciences BV
Hilicon AB
+46 (90) 193469
KNAUER Wissenschaftliche Geräte GmbH
Optimize Technologies
PerkinElmer Chromatography Solutions
Phenomenex, Inc.
Regis Technologies, Inc.
SiliCycle Inc.
Sorbent Technologies, Inc.
Teknokroma Analitica S.A.
+34 93 669 86 50
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
Welch Materials, Inc.
YMC Europe
+49 20644270

OTHER COLUMNS, HPLC (2.1-10 MM I.D.)

Advanced Materials Technology
Hamilton Company
1 (800) 648-5950
Optimize Technologies
Regis Technologies, Inc.
SiliCycle Inc.

POLYMERIC

Hamilton Company
1 (800) 648-5950
Hilicon AB
+46 (90) 193469
KNAUER Wissenschaftliche Geräte GmbH
Optimize Technologies
Polymer Char
Sorbent Technologies, Inc.
Waters Corporation
(508) 478-2000

REVERSED-PHASE

Develosil
(858) 800-2433
Fortis Technologies Ltd
GL Sciences BV
Hamilton Company
1 (800) 648-5950
HPLC Direct Ltd
KNAUER Wissenschaftliche Geräte GmbH
Optimize Technologies
PerkinElmer Chromatography Solutions
Phenomenex, Inc.
Regis Technologies, Inc.
SiliCycle Inc.
Sorbent Technologies, Inc.
Teknokroma Analitica S.A.
+34 93 669 86 50
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
Welch Materials, Inc.
YMC Europe
+49 20644270

ULTRAHIGH-PRESSURE LC (UHPLC)

Develosil
(858) 800-2433
Fortis Technologies Ltd
Hilicon AB
+46 (90) 193469
Optimize Technologies
PerkinElmer Chromatography Solutions
Phenomenex, Inc.
Princeton Chromatography Inc.
Regis Technologies, Inc.
Shimadzu Scientific Instruments
(800) 477-1227
Sorbent Technologies, Inc.
Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
Welch Materials, Inc.

COLUMNS, LOW-PRESSURE

CARTRIDGE

Hamilton Company
1 (800) 648-5950
Santai Science
SiliCycle Inc.
Sorbent Technologies, Inc.

FLASH

Chromatography Direct
Phenomenex, Inc.
Santai Science
SiliCycle Inc.
Sorbent Technologies, Inc.
Welch Materials, Inc.

GEL PERMEATION (SIZE-EXCLUSION)

Waters Corporation
(508) 478-2000

ION-EXCHANGE

Hamilton Company
1 (800) 648-5950
Santai Science
SiliCycle Inc.
Welch Materials, Inc.
YMC Europe
+49 20644270

ION-EXCLUSION

Hamilton Company
1 (800) 648-5950

NORMAL-PHASE

Santai Science
SiliCycle Inc.
Sorbent Technologies, Inc.
Welch Materials, Inc.
YMC Europe
+49 20644270

OTHER COLUMNS, LOW-PRESSURE

Hamilton Company
1 (800) 648-5950
Santai Science
SiliCycle Inc.

REVERSED-PHASE

Hamilton Company
1 (800) 648-5950
Santai Science
SiliCycle Inc.
Sorbent Technologies, Inc.
Waters Corporation
(508) 478-2000
YMC Europe
+49 20644270

RIGID SIZE-EXCLUSION

Waters Corporation
(508) 478-2000

COLUMNS, MICROBORE (1-2 MM I.D.)

CAPILLARY

Greyhound Chromatography and Allied Chemicals Ltd
LC Services Ltd

CUSTOM

Hamilton Company
1 (800) 648-5950

GEL PERMEATION (SIZE-EXCLUSION)

Tosoh Bioscience GmbH
+49 6155-7043700
Waters Corporation
(508) 478-2000
YMC Europe
+49 20644270

ION-EXCHANGE

Hamilton Company
1 (800) 648-5950
PolyLC Inc.
YMC Europe
+49 20644270
Hamilton Company
1 (800) 648-5950

NORMAL-PHASE

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(858) 800-2433
Hilicon AB
+46 (90) 193469
Teknokroma Analitica S.A.
+34 93 669 86 50
YMC Europe
+49 20644270

OTHER COLUMNS, MICROBORE (1-2 MM I.D.)

Hamilton Company
1 (800) 648-5950
Hilicon AB
+46 (90) 193469

REVERSED-PHASE

Develosil
(858) 800-2433
Hamilton Company
1 (800) 648-5950
Phenomenex, Inc.
Regis Technologies, Inc.
Teknokroma Analitica S.A.
+34 93 669 86 50
YMC Europe
+49 20644270

ULTRAHIGH-PRESSURE LC (UHPLC)

Develosil
(858) 800-2433
Hilicon AB
+46 (90) 193469
Optimize Technologies
YMC Europe
+49 20644270

COLUMNS, SPECIALTY

AMINO ACIDS

Develosil
(858) 800-2433
Hilicon AB
+46 (90) 193469
Regis Technologies, Inc.
Teknokroma Analitica S.A.
+34 93 669 86 50
Welch Materials, Inc.

BASIC COMPOUNDS

Develosil
(858) 800-2433
Teknokroma Analitica S.A.
+34 93 669 86 50
Welch Materials, Inc.
YMC Europe
+49 20644270

BIOPOLYMERS

Develosil
(858) 800-2433
Hamilton Company
1 (800) 648-5950
PSS GmbH - Perfect Separation Solutions

CARBOHYDRATES

Hamilton Company
1 (800) 648-5950
Hilicon AB
+46 (90) 193469
Teknokroma Analitica S.A.
+34 93 669 86 50
Welch Materials, Inc.

MONOCLONAL ANTIBODIES

Develosil
(858) 800-2433
Hamilton Company
1 (800) 648-5950
Novilytic
PSS GmbH - Perfect Separation Solutions

Tosoh Bioscience GmbH
+49 6155-7043700
YMC Europe
+49 20644270

NUCLEOSIDES AND NUCLEOTIDES

Develosil
(858) 800-2433
Hamilton Company
1 (800) 648-5950
Hilicon AB
+46 (90) 193469
Welch Materials, Inc.
YMC Europe
+49 20644270

ORGANIC ACIDS

Develosil
(858) 800-2433
Hamilton Company
1 (800) 648-5950
Hilicon AB
+46 (90) 193469
Welch Materials, Inc.

OTHER COLUMNS, SPECIALTY

Hamilton Company
1 (800) 648-5950
Optimize Technologies
Regis Technologies, Inc.

PEPTIDES

Develosil
(858) 800-2433
Hamilton Company
1 (800) 648-5950
Hilicon AB
+46 (90) 193469
Phenomenex, Inc.
SiliCycle Inc.
Teknokroma Analitica S.A.
+34 93 669 86 50
Welch Materials, Inc.

POLYNUCLEAR AROMATIC HYDROCARBONS

Hamilton Company
1 (800) 648-5950
Teknokroma Analitica S.A.
+34 93 669 86 50

POLYSACCHARIDES

Develosil
(858) 800-2433
Hamilton Company
1 (800) 648-5950
Hilicon AB
+46 (90) 193469
PSS GmbH - Perfect Separation Solutions
YMC Europe
+49 20644270

PROTEINS

Develosil
(858) 800-2433
Hamilton Company
1 (800) 648-5950
Phenomenex, Inc.
SiliCycle Inc.
Teknokroma Analitica S.A.
+34 93 669 86 50
YMC Europe
+49 20644270

TRICYCLIC ANTIDEPRESSANTS

Develosil
(858) 800-2433
Teknokroma Analitica S.A.
+34 93 669 86 50

DETECTOR ACCESSORIES

FLOW CELLS

ECOM spol. s r.o.
Phone +420221511310
E-mail info@ecomsro.cz
www.ecomsro.com
See ad on page 23

LC Services Ltd
Waters Corporation
(508) 478-2000

LAMPS

Chromatography Direct
HPLC Direct Ltd
LC Services Ltd

OTHER ACCESSORIES

LC Services Ltd

DETECTORS

AMPEROMETRIC

KNAUER Wissenschaftliche Geräte GmbH
Verulam Scientific Ltd

CHARGED AEROSOL

Karin_Aspen

CHIRAL

KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations

CONDUCTIVITY

Karin_Aspen
KNAUER Wissenschaftliche Geräte GmbH
Verulam Scientific Ltd

ELECTROCHEMICAL

KNAUER Wissenschaftliche Geräte GmbH
Verulam Scientific Ltd
Wyatt Technology

EVAPORATIVE, LC (LIGHT-SCATTERING)

KNAUER Wissenschaftliche Geräte GmbH
Postnova Analytics
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000
Welch Materials, Inc.

FLAME IONIZATION

PerkinElmer Chromatography Solutions

FLUORESCENCE

Karin_Aspen
KNAUER Wissenschaftliche Geräte GmbH
PerkinElmer Chromatography Solutions
Postnova Analytics
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd

INFRARED

PerkinElmer Chromatography Solutions
Polymer Char

LIGHT-SCATTERING

KNAUER Wissenschaftliche Geräte GmbH
Postnova Analytics
PSS GmbH - Perfect Separation Solutions
Tosoh Bioscience GmbH
+49 6155-7043700
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000
Wyatt Technology

MASS SPECTROMETRIC

KCA Laboratories
KNAUER Wissenschaftliche Geräte GmbH
McKinley Scientific
PerkinElmer Chromatography Solutions
Shimadzu Scientific Instruments
(800) 477-1227
Waters Corporation
(508) 478-2000

PH-METERING

Verulam Scientific Ltd

RADIOACTIVITY

KNAUER Wissenschaftliche Geräte GmbH
Verulam Scientific Ltd

REFRACTIVE INDEX

ECOM spol. s r.o.
Phone +420221511310
E-mail info@ecomsro.cz
www.ecomsro.com
See ad on page 23

KNAUER Wissenschaftliche Geräte GmbH
PerkinElmer Chromatography Solutions
Postnova Analytics
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000
Wyatt Technology

UV–VIS, FIXED-WAVELENGTH OR FILTER

D-Star Instruments, Inc.

ECOM spol. s r.o.
Phone +420221511310
E-mail info@ecomsro.cz
www.ecomsro.com
See ad on page 23

LC Services Ltd
Postnova Analytics
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd

UV–VIS, PHOTODIODE-ARRAY

ECOM spol. s r.o.
Phone +420221511310
E-mail info@ecomsro.cz
www.ecomsro.com
See ad on page 23

KNAUER Wissenschaftliche Geräte GmbH
PerkinElmer Chromatography Solutions
Postnova Analytics
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000

UV–VIS, SCANNING

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Phone +420221511310
E-mail info@ecomsro.cz
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Postnova Analytics
Verulam Scientific Ltd

UV–VIS, VARIABLE-WAVELENGTH

D-Star Instruments, Inc.
ECOM spol. s r.o.
+42 0221511310
KNAUER Wissenschaftliche Geräte GmbH
PerkinElmer Chromatography Solutions
Verulam Scientific Ltd
Welch Materials, Inc.

HPLC

Advanced Materials Technology
Axcend
Berthold Technologies GmbH & Co.KG
Conquer Scientific LLC
D-Star Instruments, Inc.
Dr. Maisch HPLC GmbH
ECOM spol. s r.o.
+42 0221511310
GL Sciences BV
KCA Laboratories
KNAUER Wissenschaftliche Geräte GmbH
KRSS Ltd
Ladybug Scientific LLC
Optimize Technologies
PDR-Separations
PerkinElmer Chromatography Solutions
Sciencix
(952) 895-8292
Shimadzu Scientific Instruments
(800) 477-1227
SunChrom GmbH
Waters Corporation
(508) 478-2000
Wilmington PharmaTech

LC ACCESSORIES AND SUPPLIES

COUNTERCURRENT CHROMATOGRAPHY ACCESSORIES

CC Biotech LLC
DWK Life Sciences GmbH

FITTINGS FOR HIGH-PRESSURE LC

Analytical Components International
Hilicon AB
+46 (90) 193469
Optimize Technologies
Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

FITTINGS FOR LOW-PRESSURE LC

Analytical Components International
Optimize Technologies
Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

FLASH CHROMATOGRAPHY ACCESSORIES

DWK Life Sciences GmbH
Santai Science

FRITS

DWK Life Sciences GmbH
Optimize Technologies
Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

MIXING TEES

Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

SOLVENT RESERVOIRS AND ACCESSORIES

DWK Life Sciences GmbH
Optimize Technologies

SYRINGES

Greyhound Chromatography and Allied Chemicals Ltd
Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

TRAPS

Optimize Technologies

TUBE CONNECTORS

Analytical Components International
Hilicon AB
+46 (90) 193469
Optimize Technologies
Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

TUBING

Analytical Components International
Chromatography Direct
Greyhound Chromatography and Allied Chemicals Ltd
Hilicon AB
+46 (90) 193469
Optimize Technologies
Postnova Analytics

PUMPS AND ACCESSORIES

DEBUBBLERS

PerkinElmer Chromatography Solutions

GRADIENT

ECOM spol. s r.o.
+42 0221511310
PerkinElmer Chromatography Solutions
Verulam Scientific Ltd

ISOCRATIC

ECOM spol. s r.o.
+42 0221511310
Eldex Corporation
PerkinElmer Chromatography Solutions
Postnova Analytics

Verulam Scientific Ltd

LOW-FLOW-RATE

ECOM spol. s r.o.
+42 0221511310
Eldex Corporation
GL Sciences BV

METERING

Eldex Corporation

OTHER PUMPS AND ACCESSORIES

GenTech Scientific LLC
VICI Valco Instruments Co. Inc.
1(800) 367-8424

PERISTALTIC

Postnova Analytics

PISTON

Analytical Components International
Eldex Corporation
Optimize Technologies
PerkinElmer Chromatography Solutions
Postnova Analytics
Verulam Scientific Ltd

PULSE-DAMPENING DEVICES

Eldex Corporation
PerkinElmer Chromatography Solutions
Verulam Scientific Ltd

PUMP SEALS AND SPARE PARTS

Bal Seal Engineering
Eldex Corporation
HPLC Direct Ltd
Optimize Technologies
PerkinElmer Chromatography Solutions
Postnova Analytics
Quantum Analytics
Verulam Scientific Ltd

SYRINGE

PerkinElmer Chromatography Solutions
Postnova Analytics

TITANIUM OR NONMETALLIC

Optimize Technologies
PerkinElmer Chromatography Solutions
Verulam Scientific Ltd

SYSTEM COMPONENTS

AUTOSAMPLERS

Axcend
GenTech Scientific LLC
KNAUER Wissenschaftliche Geräte GmbH
LC Services Ltd
Postnova Analytics
Quantum Analytics
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd

COLUMN SELECTION AND SWITCHING SYSTEMS

KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations
Verulam Scientific Ltd
VICI Valco Instruments Co. Inc.
1(800) 367-8424

DEGASSING EQUIPMENT

ECOM spol. s r.o.
+42 0221511310
KNAUER Wissenschaftliche Geräte GmbH
Postnova Analytics
Verulam Scientific Ltd

FLOW CONTROLS AND FLOW PROGRAMMERS

Hoffer Flow Controls Inc.
VICI Valco Instruments Co. Inc.
1(800) 367-8424

FLOWMETERS, LIQUID

Hoffer Flow Controls Inc.

FRACTION COLLECTORS, AUTOMATED

ECOM spol. s r.o.
+42 0221511310
Gilson, Inc.
KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations
Postnova Analytics
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd

FRACTION COLLECTORS, DROP COUNTERS FOR

Postnova Analytics

FRACTION COLLECTORS, REFRIGERATED

Postnova Analytics
Verulam Scientific Ltd

GRADIENT MIXERS

PDR-Separations

GRADIENT PROGRAMMERS

PDR-Separations

INTERFACES, LC-MS

Waters Corporation
(508) 478-2000

LEAK DETECTORS, SOLVENT

Verulam Scientific Ltd

NITROGEN GENERATORS, LC-MS

F-DGSi
GenTech Scientific LLC
McKinley Scientific

PRESSURE MONITORS

Eldex Corporation

PRESSURE REGULATORS

Optimize Technologies
VICI Valco Instruments Co. Inc.
1(800) 367-8424

RECONDITIONED EQUIPMENT

Jaytee Biosciences Ltd
Quantum Analytics

SAMPLE INJECTORS, MANUAL

KNAUER Wissenschaftliche Geräte GmbH
Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

SAMPLE INJECTORS, MOTORIZED

GenTech Scientific LLC
KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations
Postnova Analytics

SAMPLE INJECTORS, MOTORIZED

VICI Valco Instruments Co. Inc.
1(800) 367-8424

TEMPERATURE CONTROLLERS

VICI Valco Instruments Co. Inc.
1(800) 367-8424

VALVES, MANUAL

ECOM spol. s r.o.
+42 0221511310
KNAUER Wissenschaftliche Geräte GmbH
Optimize Technologies
Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

VALVES, MOTORIZED

KNAUER Wissenschaftliche Geräte GmbH
Postnova Analytics
VICI Valco Instruments Co. Inc.
1(800) 367-8424

VALVES, PROPORTIONAL

Postnova Analytics

SYSTEMS

AMINO ACID

908 Devices
PerkinElmer Chromatography Solutions
Shimadzu Scientific Instruments
(800) 477-1227

AMINO ACID

Verulam Scientific Ltd

COUNTERCURRENT

AECS-QuikPrep Ltd
CC Biotech LLC
PDR-Separations

FLASH CHROMATOGRAPHY

Advion Interchim Scientific
ECOM spol. s r.o.
+42 0221511310
KRSS Ltd
Santai Science
Verulam Scientific Ltd

GRADIENT

D-Star Instruments, Inc.
ECOM spol. s r.o.
+42 0221511310
GIBNIK Analytical Solutions SL
KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations
PerkinElmer Chromatography Solutions
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000

HIGH-PRESSURE (CLOSED-COLUMN)

Axcend
Chromatography Direct
ECOM spol. s r.o.
+42 0221511310
KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations
PolyLC Inc.
Verulam Scientific Ltd
Welch Materials, Inc.

HIGH-TEMPERATURE

chillers, air cooled chillers, water cooled chillers
ECOM spol. s r.o.
+42 0221511310
Shimadzu Scientific Instruments
(800) 477-1227
Tosoh Bioscience GmbH
+49 6155-7043700

ION

GIBNIK Analytical Solutions SL
KNAUER Wissenschaftliche Geräte GmbH
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd

ISOCRATIC

D-Star Instruments, Inc.
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+42 0221511310
GenTech Scientific LLC
KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd

LC-MS (COMPLETE SYSTEMS)

908 Devices
Advion Interchim Scientific
Axcend
Conquer Scientific LLC
GenTech Scientific LLC
GIBNIK Analytical Solutions SL
Gilson, Inc.
Jaytee Biosciences Ltd
KNAUER Wissenschaftliche Geräte GmbH
KRSS Ltd
McKinley Scientific
PerkinElmer Chromatography Solutions
Polymer Char
Postnova Analytics
Quantum Analytics
Shimadzu Scientific Instruments
(800) 477-1227
Waters Corporation
(508) 478-2000

LOW-PRESSURE (OPEN COLUMN)

PerkinElmer Chromatography Solutions
Santai Science

MICRO LC

Axcend
GenTech Scientific LLC
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd

MULTIDIMENSIONAL SYSTEMS

GIBNIK Analytical Solutions SL
PSS GmbH - Perfect Separation Solutions
Shimadzu Scientific Instruments
(800) 477-1227
Waters Corporation
(508) 478-2000

ON-LINE ANALYZERS

Postnova Analytics
Verulam Scientific Ltd

OTHER COMPLETE SYSTEMS

KRSS Ltd
PolyLC Inc.
Polymer Char
Postnova Analytics

PARTITION CHROMATOGRAPHY EQUIPMENT

AECS-QuikPrep Ltd
GIBNIK Analytical Solutions SL
Gilson, Inc.

PREPARATIVE

AECS-QuikPrep Ltd
D-Star Instruments, Inc.
ECOM spol. s r.o.
+42 0221511310
GIBNIK Analytical Solutions SL
KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations
Santai Science
Shimadzu Scientific Instruments
(800) 477-1227
Tosoh Bioscience GmbH
+49 6155-7043700
Verulam Scientific Ltd
Welch Materials, Inc.

PROCESS

AECS-QuikPrep Ltd
ECOM spol. s r.o.
+42 0221511310
Santai Science
Tosoh Bioscience GmbH
+49 6155-7043700

SIMULATED MOVING BED

AECS-QuikPrep Ltd
KNAUER Wissenschaftliche Geräte GmbH
Verulam Scientific Ltd

SIZE-EXCLUSION

KNAUER Wissenschaftliche Geräte GmbH
Polymer Char
Postnova Analytics
PSS GmbH - Perfect Separation Solutions
Shimadzu Scientific Instruments
(800) 477-1227
Tosoh Bioscience GmbH
+49 6155-7043700
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000
Wyatt Technology

ULTRAHIGH-PRESSURE LC (UHPLC)

Advion Interchim Scientific
Axcend
GenTech Scientific LLC
GIBNIK Analytical Solutions SL
Jaytee Biosciences Ltd
KNAUER Wissenschaftliche Geräte GmbH
KRSS Ltd
Optimize Technologies
PerkinElmer Chromatography Solutions
Shimadzu Scientific Instruments
(800) 477-1227
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000
Wyatt Technology

GAS CHROMATOGRAPHY

Products and Services for Chromatography

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Welch Materials, Inc.

STATIONARY PHASES, ADSORBENTS

Welch Materials, Inc.

SUPPORTS, DIATOMACEOUS-EARTH

Porvair Sciences
Phone +44 1978 661144
E-mail hello@porvairsciences.com
www.microplates.com
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SUPPORTS, OTHER

Porvair Sciences

COLUMNS

BLANKS

Frontier Laboratories Europe
InnovaQuartz

CAPILLARY (OPEN-TUBULAR)

Ladybug Scientific LLC
PerkinElmer Chromatography Solutions
VICI Valco Instruments Co. Inc.
1(800) 367-8424

CAPILLARY, CHIRAL

VICI Valco Instruments Co. Inc.
1(800) 367-8424

CAPILLARY, ENVIRONMENTAL

GL Sciences BV
PerkinElmer Chromatography Solutions
Phenomenex, Inc.
Sorbent Technologies-SorbTech
Teknokroma Analitica S.A.
+34 93 669 86 50
VICI Valco Instruments Co. Inc.
1(800) 367-8424

CAPILLARY, HIGH-TEMPERATURE

Frontier Laboratories Europe
LECO Corporation
PerkinElmer Chromatography Solutions
Phenomenex, Inc.
VICI Valco Instruments Co. Inc.
1(800) 367-8424

CUSTOM-MADE

Frontier Laboratories Europe
Gilson
Ladybug Scientific LLC
Phenomenex, Inc.
Teknokroma Analitica S.A.
+34 93 669 86 50
VICI Valco Instruments Co. Inc.
1(800) 367-8424

OTHER COLUMNS

VICI Valco Instruments Co. Inc.
1(800) 367-8424

PACKED, ANALYTICAL

Biotage AB, Sweden
Gilson
PerkinElmer Chromatography Solutions
Porvair Sciences
Sorbent Technologies-SorbTech

PACKED, CAPILLARY (MICROPACKED)

PerkinElmer Chromatography Solutions
Teknokroma Analitica S.A.
+34 93 669 86 50
Welch Materials, Inc.

PACKED, PREPARATIVE

Biotage AB, Sweden
Gilson

DETECTORS, DETECTOR ACCESSORIES

DETECTOR ACCESSORIES

Gilson

ELECTRON-CAPTURE

PerkinElmer Chromatography Solutions
Shimadzu Scientific Instruments
(800) 477-1227
VICI Valco Instruments Co. Inc.
1(800) 367-8424

FLAME IONIZATION

PerkinElmer Chromatography Solutions
Shimadzu Scientific Instruments
(800) 477-1227
VICI Valco Instruments Co. Inc.
1(800) 367-8424

FLAME PHOTOMETRIC

PerkinElmer Chromatography Solutions

FLUORESCENCE (MERCURY SPECIFIC)

PerkinElmer Chromatography Solutions

HELIUM IONIZATION

VICI Valco Instruments Co. Inc.
1(800) 367-8424

ION MOBILITY SPECTROMETERS

Gilson

IR (FTIR) SPECTROMETERS

PerkinElmer Chromatography Solutions

MASS SPECTROMETERS, HIGH-RESOLUTION

CD Bioparticles
Gilson
LECO Corporation
Markes International GmbH
+49 (0)69 6681089-10
Markes International Inc.
1 866-483-5684
SepSolve Analytical Ltd
Shimadzu Scientific Instruments
(800) 477-1227
Syft Technologies
+64-3-338 6701

MASS SPECTROMETERS, LOW-RESOLUTION

Jaytee Biosciences Ltd
LECO Corporation
PerkinElmer Chromatography Solutions
Syft Technologies
+64-3-338 6701

MASS SPECTROMETERS, RESIDUAL GAS ANALYSIS, MAGNETIC

Gilson
Syft Technologies
+64-3-338 6701

MASS SPECTROMETERS, RESIDUAL GAS ANALYSIS, QUADRUPOLE

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KCA Laboratories
PerkinElmer Chromatography Solutions
Syft Technologies
+64-3-338 6701

NITROGEN-SELECTIVE

DETector Engineering & Technology
PerkinElmer Chromatography Solutions

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Gilson
Syft Technologies
+64-3-338 6701

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VICI Valco Instruments Co. Inc.
1(800) 367-8424

SULFUR-SELECTIVE

PerkinElmer Chromatography Solutions

THERMAL CONDUCTIVITY

PerkinElmer Chromatography Solutions
Shimadzu Scientific Instruments
(800) 477-1227
VICI Valco Instruments Co. Inc.
1(800) 367-8424

THERMIONIC (N-P SELECTIVE)

DETector Engineering & Technology

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CARRIER GASES

Air Products PLC
F-DGSi
Parker Hannifin
PEAK Scientific

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VICI Valco Instruments Co. Inc.
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VICI Valco Instruments Co. Inc.
1(800) 367-8424

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LC Services Ltd
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NITROGEN GENERATORS

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Jaytee Biosciences Ltd
Nitrogen & Hydrogen Generators
Parker Hannifin
PEAK Scientific

PUMPS

Gilson
LC Services Ltd

REGULATORS

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1(800) 367-8424

SEPTA

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Phenomenex, Inc.
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SYRINGES

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1(800) 367-8424

TUBING

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VALVES

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ZERO-AIR GENERATORS

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Jaytee Biosciences Ltd

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GL Sciences BV

PYROMETERS
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GL Sciences BV
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VICI Valco Instruments Co. Inc.
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THERMAL-DESORPTION DEVICES
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GL Sciences BV
LECO Corporation
Markes Instruments (Shanghai) Co., Ltd.
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Markes International GmbH
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Markes International Inc.
1 866-483-5684

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GenTech Scientific LLC
GIBNIK Analytical Solutions SL
Jaytee Biosciences Ltd
KRSS Ltd
Parker Hannifin
PerkinElmer Chromatography Solutions
Shimadzu Scientific Instruments
(800) 477-1227
Syft Technologies
+64-3-338 6701
VICI Valco Instruments Co. Inc.
1(800) 367-8424

GAS ANALYZERS
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PerkinElmer Chromatography Solutions
Syft Technologies
+64-3-338 6701
VICI Valco Instruments Co. Inc.
1(800) 367-8424

GC–FTIR (COMPLETE SYSTEMS)
PerkinElmer Chromatography Solutions
Conquer Scientific LLC
GenTech Scientific LLC
GIBNIK Analytical Solutions SL
Greyhound Chromatography and Allied Chemicals Ltd
LECO Corporation
PerkinElmer Chromatography Solutions
Quantum Analytics
Shimadzu Scientific Instruments
(800) 477-1227

MULTIDIMENSIONAL
GIBNIK Analytical Solutions SL
LECO Corporation
SepSolve Analytical Ltd
Shimadzu Scientific Instruments
(800) 477-1227

ON-LINE ANALYZERS
Syft Technologies
+64-3-338 6701

OTHER SYSTEMS
Gilson
VICI Valco Instruments Co. Inc.
1(800) 367-8424

PORTABLE
Kindwell Inc.
Parker Hannifin
PerkinElmer Chromatography Solutions
Syft Technologies
+64-3-338 6701

PREPARATIVE
Biotage AB, Sweden
Gilson

PROCESS
Syft Technologies
+64-3-338 6701
VICI Valco Instruments Co. Inc.
1(800) 367-8424

SUPERCRITICAL FLUID CHROMATOGRAPHY

Products and Services for Chromatography

COLUMNS

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Dr. Maisch HPLC GmbH
Gilson
Hilicon AB
+46 (90) 193469

PACKED
Gilson
Hilicon AB
+46 (90) 193469
Regis Technologies, Inc.
SiliCycle Inc.
YMC Europe
+49 20644270

SPECIALTY (CHIRAL, LIQUID-CRYSTAL, ETC.)
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Regis Technologies, Inc.
YMC Europe
+49 20644270

DETECTORS, DETECTOR ACCESSORIES

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Parker Hannifin

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Gilson

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Gilson
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DETector Engineering & Technology

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+42 0221511310
Gilson

UV–VIS, PHOTODIODE-ARRAY
ECOM spol. s r.o.
+42 0221511310
GenTech Scientific LLC
Waters Corporation
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UV–VIS, VARIABLE-WAVELENGTH
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+42 0221511310
GenTech Scientific LLC
Gilson

SFC ACCESSORIES

FERRULES
VICI Valco Instruments Co. Inc.
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PUMPS
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FRITSCH GmbH , Milling and Sizing

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1(800) 367-8424

STATIONARY PHASES
Hilicon AB
+46 (90) 193469

SYSTEMS

CAPILLARY COLUMN
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Shimadzu Scientific Instruments
(800) 477-1227

OTHER SYSTEMS
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ECOM spol. s r.o.
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Gilson
Shimadzu Scientific Instruments
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SFC–MS (COMPLETE SYSTEMS)
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Waters Corporation
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THIN LAYER CHROMATOGRAPHY

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Miles Scientific

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SiliCycle Inc.

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DWK Life Sciences GmbH
Miles Scientific

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DWK Life Sciences GmbH
Miles Scientific
SiliCycle Inc.

DIPPING CHAMBERS
DWK Life Sciences GmbH
Miles Scientific

DRYING RACKS
Miles Scientific

HPTLC ACCESSORIES
Miles Scientific

HPTLC EQUIPMENT
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Miles Scientific

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Chromatography Direct
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Miles Scientific

Porvair Sciences
Phone +44 1978 661144
E-mail hello@porvairsciences.com
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Sorbent Technologies, Inc.

PREPARATIVE EQUIPMENT AND ACCESSORIES
Gilson
Miles Scientific
Porvair Sciences

SCRAPING DEVICES
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SORBENTS
BRADY Europe, Middle East & Africa
Miles Scientific
SiliCycle Inc.
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SPRAYING EQUIPMENT
Miles Scientific

UV LAMPS FOR TLC
Miles Scientific

VIEWING CABINETS
Miles Scientific

VISUALIZATION DEVICES
Miles Scientific

ZONE COLLECTORS
Miles Scientific

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Products and Services for Chromatography

CAPILLARY ELECTROPHORESIS

COMPLETE SYSTEMS
908 Devices

GEL ELECTROPHORESIS

ACCESSORY EQUIPMENT
Hamilton Company
800-648-5950

REAGENTS, SOLVENTS, STANDARDS

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AMINO ACIDS
Acrolab
Verulam Scientific Ltd

AMPHOLYTES
Acrolab

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Acrolab
Biovenic
Cayman Chemical Company

BIOLOGICAL-GRADE
Acrolab
Creative BioMart Chromatography

BUFFERS
Acrolab
PreOmics
PreOmics GmbH

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Regis Technologies, Inc.

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Acrolab
Regis Technologies, Inc.
Verulam Scientific Ltd

DYES
Acrolab

ENZYMES
Acrolab
Cayman Chemical Company

GENERAL LABORATORY CHEMICALS
Acrolab
Emerald Scientific
Greyhound Chromatography and Allied Chemicals Ltd
HPLC Direct Ltd

ION CHROMATOGRAPHY BUFFERS
Acrolab
Regis Technologies, Inc.

ION-PAIR REAGENTS
HPLC Direct Ltd
Regis Technologies, Inc.

PEPTIDE SYNTHESIS REAGENTS
SiliCycle Inc.

PROTEINS
Acrolab
Cayman Chemical Company

SOLVENTS
Acrolab
Emerald Scientific
Greyhound Chromatography and Allied Chemicals Ltd
Regis Technologies, Inc.

VISUALIZATION REAGENTS
Cayman Chemical Company

WATER, HIGH-PURITY
Acrolab
Emerald Scientific

STANDARDS

CLINICAL
Acrolab
Cayman Chemical Company
Chiron AS
ChromaDex Reference Standards and Services
LGC
MocDoc - Dental Practice Management Software
Verulam Scientific Ltd

ENVIRONMENTAL
Acrolab
Cayman Chemical Company
Chiron AS
ChromaDex Reference Standards and Services
Greyhound Chromatography and Allied Chemicals Ltd
LGC
Phenomenex, Inc.
Planta Analytica

ION CHROMATOGRAPHY
Acrolab
ChromaDex Reference Standards and Services
LGC

OTHER STANDARDS
Acrolab
Cayman Chemical Company
Chiron AS
ChromaDex Reference Standards and Services
Emerald Scientific
Jaytee Biosciences Ltd
LGC
Planta Analytica

PEPTIDE-PROTEIN
Acrolab
ChromaDex Reference Standards and Services
LGC

PESTICIDE AND HERBICIDE
Acrolab
Cayman Chemical Company
Chiron AS
ChromaDex Reference Standards and Services
Greyhound Chromatography and Allied Chemicals Ltd
LGC
Planta Analytica

PHARMACEUTICAL
Acrolab
ASAS LAbor GmbH
Cayman Chemical Company
Chiron AS
ChromaDex Reference Standards and Services
Emerald Scientific
Greyhound Chromatography and Allied Chemicals Ltd
IJEST
LGC
Planta Analytica
Roots Analysis

POLYMERS
Acrolab
ChromaDex Reference Standards and Services
LGC
Polymer Char
Waters Corporation
(508) 478-2000

PRIMARY
Acrolab
Cayman Chemical Company
ChromaDex Reference Standards and Services
LGC
Planta Analytica

RADIOCHEMICAL
ChromaDex Reference Standards and Services
LGC

SPECTROCHEMICAL
ChromaDex Reference Standards and Services
LGC

DATA HANDLING, CALIBRATION

Products and Services for Chromatography

HARDWARE

ANALYTICAL SYSTEMS AND CONTROLLERS FOR GC

Servicios Integral en Cromatografía - SISCHROM

ANALYTICAL SYSTEMS AND CONTROLLERS FOR LC

ECOM spol. s r.o.
+42 0221511310
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000

AUTOMATED WORKSTATIONS, IBM- COMPATIBLE

Biotage AB, Sweden

CALIBRATION SYSTEMS, LC

Waters Corporation
(508) 478-2000

DATA ACQUISITION AND DATA HANDLING

ACD/Labs (Advanced Chemistry Development)
(416) 368-3435
RotaChrom Technologies PLC
Solutions for LC/GC-MS data and applications
Ultraview Corporation
Verulam Scientific Ltd
Waters Corporation
(508) 478-2000

DATA CONVERTORS, ANALOG-TO-DIGITAL

Ultraview Corporation
Verulam Scientific Ltd

DATA CONVERTORS, DIGITAL-TO-ANALOG

Ultraview Corporation

INSTRUMENT–COMPUTER INTERFACES

Verulam Scientific Ltd

PRINTERS

BRADY Europe, Middle East & Africa

PROCESS CONTROL

Hoffer Flow Controls Inc.

SOFTWARE

BARCODE SYSTEMS/SOFTWARE

Autoscribe Informatics LIMS
BRADY Europe, Middle East & Africa
MocDoc - LIMS Software

CALIBRATION

Infometrix, Inc.
Jaytee Biosciences Ltd
Solutions for LC/GC-MS data and applications
Waters Corporation
(508) 478-2000

CE PREDICTION AND MODELING

S-Matrix Corporation

CHEMOMETRICS

AIST Software Inc
Infometrix, Inc.
Markes International GmbH
+49 (0)69 6681089-10
Markes International Inc.
1 866-483-5684
Solutions for LC/GC-MS data and applications
Wiley Science Solutions

CUSTOM, FOR CHROMATOGRAPHY

AIST Software Inc
Infometrix, Inc.
LECO Corporation
RotaChrom Technologies PLC
Solutions for LC/GC-MS data and applications
Waters Corporation
(508) 478-2000

DATA ACQUISITION

AIST Software Inc
Autoscribe Informatics LIMS
BRADY Europe, Middle East & Africa
SepSolve Analytical Ltd
Solutions for LC/GC-MS data and applications
Waters Corporation
(508) 478-2000

DATA ANALYSIS AND REPORTING

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AIST Software Inc
Autoscribe Informatics LIMS
CD ComputaBio
Infometrix, Inc.
Markes International GmbH
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Markes International Inc.
1 866-483-5684
Northwest Analytics Inc
S-Matrix Corporation
SepSolve Analytical Ltd
Shimadzu Scientific Instruments
(800) 477-1227
Solutions for LC/GC-MS data and applications
Waters Corporation
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Wiley Science Solutions

EXPERT SYSTEMS

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S-Matrix Corporation
SepSolve Analytical Ltd
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GC METHOD DEVELOPMENT

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LECO Corporation
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Solutions for LC/GC-MS data and applications

GRAPHICS OR MANUSCRIPT PREPARATION

ACD/Labs (Advanced Chemistry Development)
(416) 368-3435
Solutions for LC/GC-MS data and applications

INSTRUMENT CONTROL

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KNAUER Wissenschaftliche Geräte GmbH
SepSolve Analytical Ltd
Waters Corporation
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LABORATORY INFORMATION MANAGEMENT SYSTEMS

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Autoscribe Informatics LIMS
BRADY Europe, Middle East & Africa
Waters Corporation
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Wiley Science Solutions

LC METHOD DEVELOPMENT

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Jaytee Biosciences Ltd
RotaChrom Technologies PLC
S-Matrix Corporation
Solutions for LC/GC-MS data and applications
Waters Corporation
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ROBOTICS SYSTEM CONTROL

Autoscribe Informatics LIMS

SFC METHOD DEVELOPMENT

S-Matrix Corporation

SIZE EXCLUSION

PSS GmbH - Perfect Separation Solutions
S-Matrix Corporation
Waters Corporation
(508) 478-2000

STATISTICAL ANALYSIS

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LECO Corporation
Markes International GmbH
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Northwest Analytics Inc
S-Matrix Corporation
SepSolve Analytical Ltd
Solutions for LC/GC-MS data and applications

CHROMATOGRAPHY SERVICES

Products and Services for Chromatography

CHROMATOGRAPHY SERVICES

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Cayman Chemical Company

ANALYSES, ENVIRONMENTAL

GERSTEL
Syft Technologies
+64-3-338 6701
Utility Testing Laboratory

ANALYSES, FORENSIC

Cayman Chemical Company
GERSTEL
Solutions for LC/GC-MS data and applications

ANALYSES, GC, GC–FTIR

Cayman Chemical Company

ANALYSES, GC, GC–MS

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Solutions for LC/GC-MS data and applications
Syft Technologies
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Utility Testing Laboratory

ANALYSES, LC, LC–MS

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Axcend
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GenTech Scientific LLC
GERSTEL
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KRSS Ltd
LC Services Ltd
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Waters Corporation
(508) 478-2000

ANALYSES, SEC

PSS GmbH - Perfect Separation Solutions
Waters Corporation
(508) 478-2000

ANALYSES, SFC, SFC–MS

Waters Corporation
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ANALYSES, SPE

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CHROMATOGRAPHY DATA SYSTEM VALIDATION

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COLUMN REPACKING, LC

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SiliCycle Inc.

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CONSULTING, GC

Anthias Consulting Ltd
Infometrix, Inc.

CONSULTING, GENERAL

Anthias Consulting Ltd
Planta Analytica
Quantitative in silico analytical chemistry

CONSULTING, LC

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Fortis Technologies Ltd
Infometrix, Inc.
KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations

Planta Analytica
SiliCycle Inc.

CONSULTING, SPE

Anthias Consulting Ltd
SiliCycle Inc.

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S-Matrix Corporation
SiliCycle Inc.
Wilmington PharmaTech
Wilmington PharmaTech Company

EQUIPMENT QUALIFICATION OR VALIDATION

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LEASING OR RENTING, GC EQUIPMENT

Quantum Analytics
Syft Technologies
+64-3-338 6701

LEASING OR RENTING, HPLC EQUIPMENT

Gilson
McKinley Scientific
Quantum Analytics

METHOD DEVELOPMENT, CE

Advanced Materials Technology
RotaChrom Technologies PLC
Wilmington PharmaTech

METHOD DEVELOPMENT, GC, GC–MS

Anthias Consulting Ltd
Cayman Chemical Company
Jaytee Biosciences Ltd
LECO Corporation
Regis Technologies, Inc.
Solutions for LC/GC-MS data and applications
Wilmington PharmaTech

METHOD DEVELOPMENT, LC, LC-MS

Anthias Consulting Ltd
Cayman Chemical Company
Fortis Technologies Ltd
Jaytee Biosciences Ltd
KNAUER Wissenschaftliche Geräte GmbH
PDR-Separations
Planta Analytica
Regis Technologies, Inc.
RotaChrom Technologies PLC
S-Matrix Corporation
SiliCycle Inc.
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Wilmington PharmaTech
YMC Europe
+49 20644270

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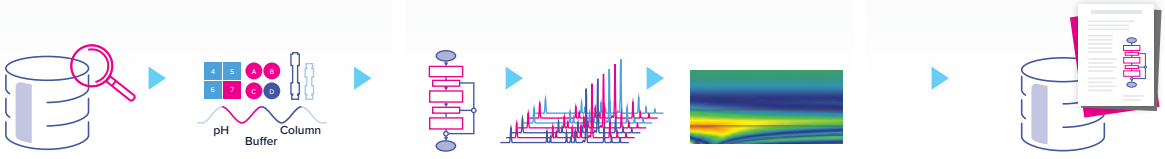

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