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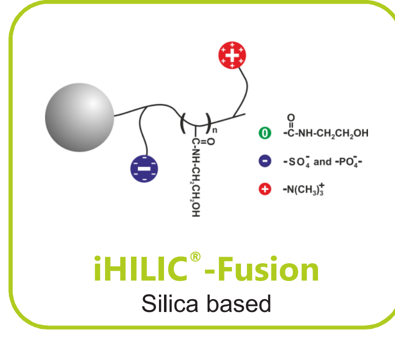


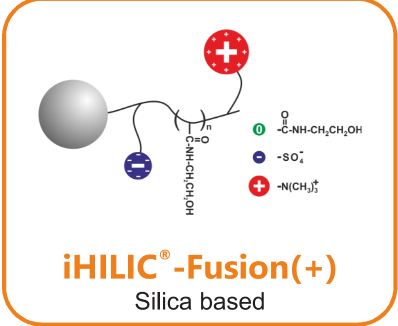


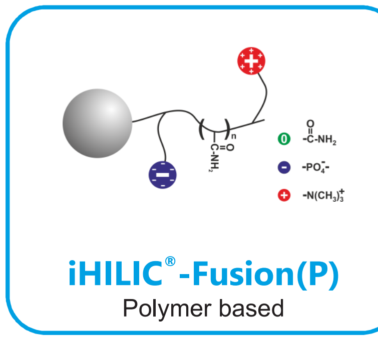


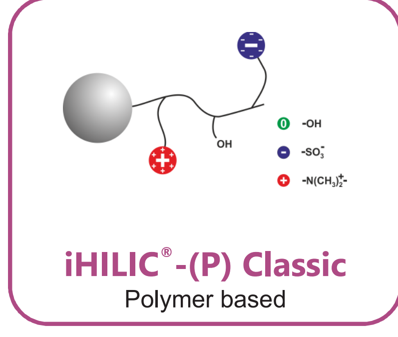


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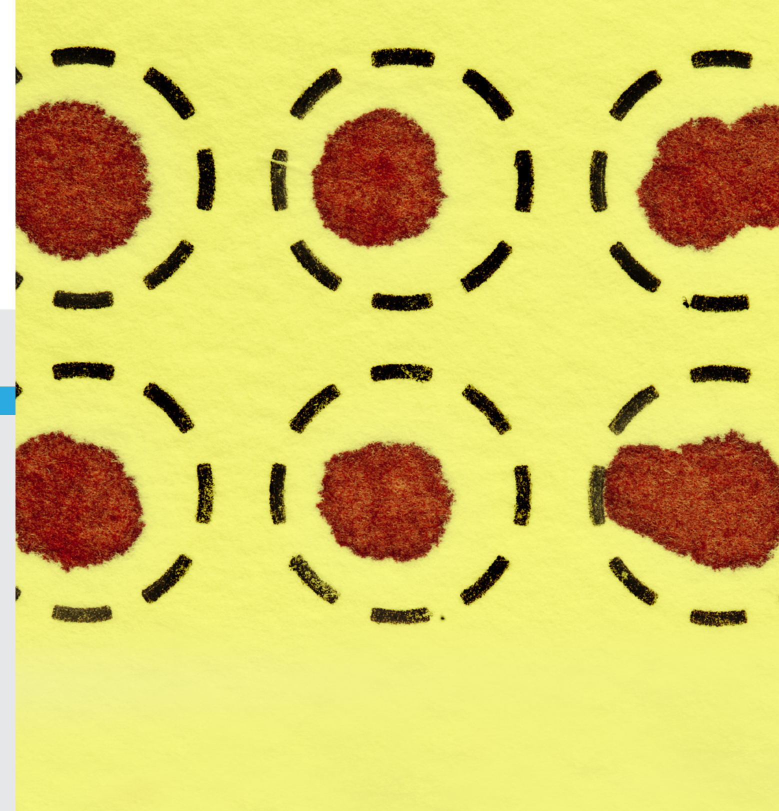

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PEER REVIEW

# Dried Blood Spots in Mass Spectrometry-based Protein Analysis. Recent Developments including Sampling of other Biological Matrices and Novel Sampling Technologies

**Trine Grønhaug Halvorsen[1], Cecilie Rosting[2], Øystein Skjærvø[1], Leon Reubsaet[1],** [1] Section for Pharmaceutical Chemistry, Department of Pharmacy, University of Oslo, Oslo, Norway, [2]STAMI, National Institute of Occupational Health, Oslo, Norway

**The dried blood spot (DBS) sampling technique has been around for decades, predominantly for small molecules and mainly in newborn screening. Although determination of proteins after DBS sampling is usually performed with immunometric assays, the combination with mass spectrometry (MS) is gaining interest. This article provides an overview of DBS sampling for mass spectrometry-based protein analysis. The first part will focus on clinical applications for DBSs and on sampling other biological matrices apart from whole blood, including dried matrix spots (DMSs). The second part will explore the new frontiers of the DBS sampling technology, including novel sampling materials/devices, and novel combinations with mass spectrometry. Examples of use in both qualitative and quantitative protein analysis are highlighted as well as examples using both bottom-up and top-down proteomics approaches.**

## KEY POINTS

- **The past, present and future of paper for blood sampling in protein analysis is reviewed.**
- **The potential and challenges of mass spectrometry-based protein analysis from DBSs are discussed.**
- **The role of DBS sampling and MS for protein analysis in a range of clinical applications is covered.**
- **New frontiers in MS-based protein determination from DBSs are evaluated.**

Dried blood spot (DBS) sampling was introduced in 1913 when Ivar Bang demonstrated this less-invasive sampling strategy in glucose monitoring (1). In 1963 Susi and Guthrie applied DBS sampling for phenylketonuria screening in newborns (2) and the technique gained widespread acceptance. In the beginning, the strategy was mainly used in newborn screening of phenylketonuria and other small molecule metabolic markers. However, as a result of the advantages of DBS sampling, including small sampling volumes, improved stability of analytes, less biohazard risk and easy transport, the technique was also soon evaluated for other analytes. The first reports of DBSs for sampling and storage of proteins are from the early 1970s (3,4). In one work, Thielmann and Aquino (3) demonstrated that blood could be sampled and stored on filter paper prior to electrophoretic separation of hemoglobin S from hemoglobin A. In another work, Laurell (4) described a test for α1-antitrypsin deficiency using DBSs and electrophoretic separation. Since then, the technique has also had widespread use for the analysis of proteins in a wide range of applications (5), the majority of the applications described use immunoassays to determine the proteins.

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**FIGURE 1:** Schematic overview of generic procedure for analysis of proteins in DBS by top-down and bottom-up protein analysis by LC–MS.

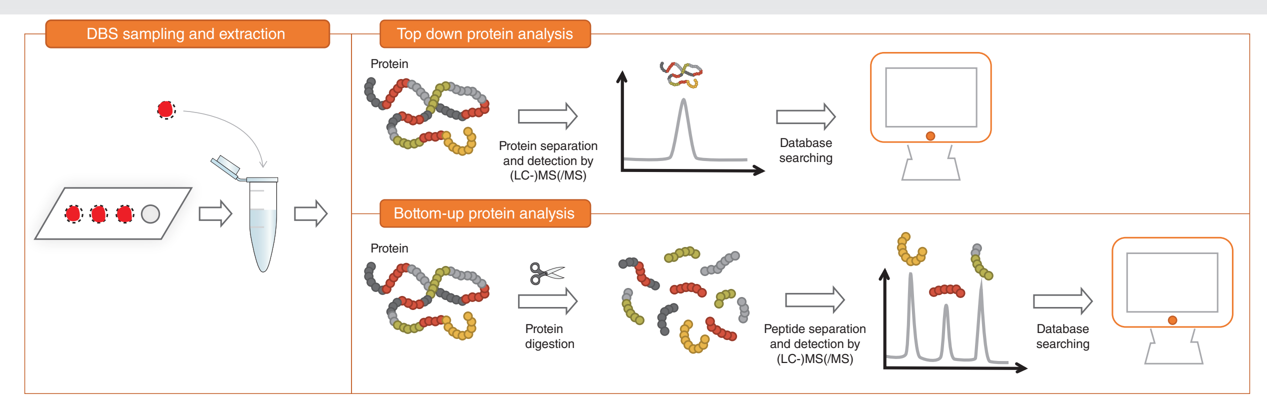



**FIGURE 2:** Schematic overview of different approaches for immunocapture; protein capture (top) versus peptide capture (bottom).

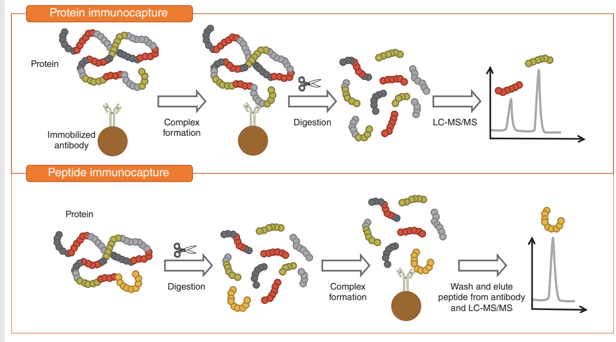
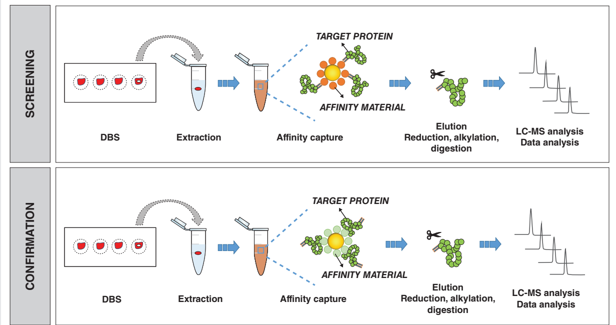



**FIGURE 3:** Overview of a possible set-up for doping analysis. The main steps in the procedure are similar. Complementarity is achieved by applying different affinity capture principles targeting different part of the protein in the screening (upper) and confirmation (lower) procedure.




Although immunoassays are highly sensitive and widely used, mass spectrometry (MS) has certain advantages with respect to specificity and differentiation power. MS is therefore a powerful tool in quantitative protein determination and in the study of the proteome. In the 1980s, the first reports of MS determination of proteins in DBSs can be found. Wada et al. (6) used molecular secondary ion mass spectrometry (SIMS) in a study of DBSs from 80 000 Japanese neonates: A double-focused mass spectrometer with magnetic scanning was used for structural analysis of the detected hemoglobin variants after purification using chromatography and subsequent tryptic digestion of the isolated variants. As the blood samples were obtained from newborns, the use of DBS sampling with its low invasiveness and low sample volumes were advantageous. The large sample set (80 000) combined with the differentiation power of the MS made it possible to easily detect and confirm abnormal globins of low prevalence. Following this, very few reports of mass spectrometric determination of proteins from DBSs can be found throughout the 1990s where the main use of MS for protein analysis of DBSs was to characterize hemoglobin variants.

Overall, the majority of reports on DBS sampling in protein analysis by MS, especially from the earlier days, are on the determination of hemoglobin variants, mainly for use in newborn screening (7–10). Hemoglobin is a high abundance protein and determination of this protein by MS is – even from very small sample volumes such as DBSs – relatively straightforward. The protein can be determined either directly (top-down) or after a simple digestion step (bottom-up) without extensive clean up. This is demonstrated in reference 11. A schematic overview of the top-down and bottom-up procedure after DBS sampling is illustrated in Figure 1.

For many other proteins of interest, such as biomarkers, doping agents or drugs, the situation is more complex. This is because the amount present in the blood sample is extremely low compared to the highly abundant proteins (such as hemoglobin, albumin and immunoglobulins) in blood. For these analytes the applicability

of DBS sampling has been dependent on the development of more sensitive analytical instrumentation as well as on the development of highly selective sample preparation methods.

After the turn of the century, reports on MS-based protein analysis from DBSs have increased. A variety of applications are described from newborn screening (8–10, 12–14), to biomarker analysis (15–21) and discovery (22–26), doping analysis (27–33), and bioanalysis of therapeutic proteins (drug discovery and therapeutic drug monitoring) (34–36). Different sampling materials and devices such as volumetric absorptive microsampling (VAMS) (17,37) and water-soluble sampling material (38,39) have been introduced as well as materials with incorporated functionality for protein pretreatment and enrichment (40,41).

The combination of DBS sampling and MS in the analysis of proteins has been reviewed twice previously, in 2014 and 2017 (42,43). These reviews focus on clinical perspectives in DBS protein quantification using MS, and challenges and opportunities in MS analysis of proteins from DBSs. The present review will give a comprehensive overview of the progress in DBS sampling for MS-based protein analysis with emphasis on the last five years. The first part will focus on clinical applications for DBS sampling (biomarker determination, doping analysis and the analysis of therapeutic proteins) and on the sampling of other biological matrices apart from blood (dried matrix spots [DMSs]). The second part will explore the new frontiers in DBS sampling technology such as novel sampling material/devices, and novel combinations with MS. Examples of use in both qualitative and quantitative protein analysis will be given, as well as examples using both bottom-up and top-down proteomics approaches.

## Clinical applications

### Biomarker determination and discovery

DBS sampling started out as a sampling technique for biomarker determination in newborn screening of inborn errors of metabolism, and is still the method of choice. One important reason for increased and continued use of DBSs in these programmes is the increase in MS sensitivity. In addition, DBS sampling has a large potential for other biomarker applications because blood sampling for screening of diseases or treatment follow-up could easily be performed by the patient, without going to the phlebotomist/hospital/doctor's office. This is especially beneficial when sampling in remote areas or if the goal is to monitor otherwise healthy individuals such as athletes.

Combining DBS sampling with MS is also advantageous for protein biomarker analysis because MS is the analysis method of choice for many of the analytes in the newborn screening programme. In addition, the

**FIGURE 4:** A DBS method diagram for determination of blood doping. Adapted and reprinted with permission from H.D. Cox and D. Eichner, *Mass Spectrometry Method to Measure Membrane Proteins in Dried Blood Spots for the Detection of Blood Doping Practices in Sport, Analytical Chemistry*, **89**, 10029–10036 (2017). Copyright (2017) American Chemical Society.

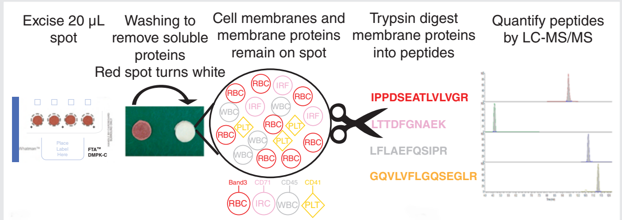
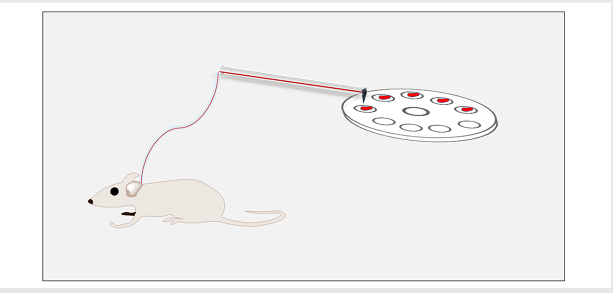



**FIGURE 5:** Illustration of automated DBS sampling that can be combined with MS analysis, as in reference 36.



multiplexing and differentiating capability of the MS will be an extra benefit in protein biomarker analysis.

A majority of the publications applying DBS sampling and MS is within biomarker analysis, either targeted biomarker determination where validated biomarkers are determined or in proteomics studies for biomarker discovery. Most of the biomarker studies published use a bottom-up approach. Top-down studies are also available, but mainly in evaluation of new concepts (discussed in more detail in a separate section). To our knowledge, there are currently still no protein biomarkers routinely analyzed from DBSs by LC–MS. As a consequence of this, limited information about the reproducibility of the published LC–MS methods for analysis of protein biomarkers from DBSs is available. There are a couple of factors that suggests that high reproducibility of DBS LC–MS methods might be a bigger challenge than for other methods, especially for low-abundance biomarkers: In addition to variations arising from DBS sampling itself such as low sample volume, varying hematocrit and non-complete elution from the cards, LC–MS- based protein analysis by the bottom-up approach consists of several steps contributing to the variability such as denaturation, alkylation, proteolysis, immunocapture (especially for low abundance proteins), solid-phase extraction etc. The

A Q&A

# Advances in Field-Flow Fractionation: Supporting Development of Novel Nanomedicines

**Christoph Johann**
*Global Product Manager*
Wyatt Technologies

Field-flow fractionation (FFF) offers a solution for the characterization of sub-micron particles and macromolecules. To discuss flow FFF, multi-angle light scattering (MALS), and more, *LCGC* sat down with Christoph Johann, global product manager at Wyatt Technologies. In this thought-leader series, Johann talks about Wyatt's history with FFF products and methods, what the technology means for the company, and his expectations for FFF. He also discusses the benefits of coupling light scattering online, FFF's role in the pharmaceutical industry, its acceptance by regulatory agencies, the company's new model Eclipse NEON, and more.

**LCGC: You are the product manager for FFF products at Wyatt Technology—why is Wyatt investing in FFF?**

**Christoph:** Wyatt's MALS detectors are widely utilized in conjunction with size-exclusion chromatography (SEC) to analyze the molar mass, size, and conformation of macromolecules. However, SEC has a limited upper size range, and it prevents using SEC-MALS to characterize larger species such as very high-molecular-weight polymers, liposomes, viruses, large protein aggregates, and similarly sized analytes.

On the other hand, FFF is a separation technique that covers a range of 1 to 1000 nanometers, and it can be coupled to Wyatt's online instruments. FFF-MALS determines with high-resolution the absolute and fully quantitative size distribution for various nanoparticles and macromolecules, which is not possible with SEC. Yet, it's not subject to the limitations of small particle numbers that affect other methods such as nanoparticle tracking techniques.

So, FFF greatly expands the range of applications for our light-scattering detectors, and we see some really important markets opening up for these products.

**LCGC: Although FFF has been around for more than 50 years, it's still not a mainstream method. What are your expectations for the future development of FFF?**

**Christoph:** Flow FFF is used in food, pharmaceutical, environmental, and polymer applications, but it has been perceived as a complex method and adoption has not been comparable to GPC. I believe it's ready to break into a wider market as a result of two key factors: the advent of nanoparticle drug and gene delivery technologies that require the capabilities of FFF coupled to MALS and dynamic light scattering (DLS) to bring the promise of these medical advances to patients; and upcoming instrument and software improvements that will eliminate the issues of complexity and user intimidation, so the method can be transferred easily from R&D scientists to quality-control labs.

**LCGC: Where do you see the increasing need for FFF in the pharmaceutical industry?**

**Christoph:** There are two parallel and similar paradigm shifts going on at full speed. In traditional small-molecule pharmaceuticals, more drugs are formulated as nanoparticles, whether as emulsions, nanosolids, or

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encapsulated in liposomes or other nanocarriers, including lipid nanoparticles, polymer micelles, polymersomes, albumin particles, polyplexes, etc.

In the biopharmaceutical world, commercialization of gene therapies—the delivery of DNA or RNA by viral or non-viral gene vectors—is in high gear. SEC-MALS is suitable for small vectors like adeno-associated virus, but larger vectors such as lentivirus or adenovirus require separation by FFF. Non-viral vectors are very similar to small-molecules nanodrug-delivery systems, e.g., lipid nanoparticles or polymersomes. In both cases, the trend is to deliver therapeutic payloads in carrier vehicles that are in the size range of 30 to 300 nanometers, which is very different from the size of current drugs based on small molecules, peptides, proteins, or microparticles.

The standard tools in place for characterizing these new modalities—whether batch DLS or nanoparticle tracking analysis for nano drug delivery systems (DDS) or qPCR and ELISA for gene vectors—are simply insufficient to meet the challenges and analytical needs presented by these complex therapeutics. FFF with MALS, DLS, and spectroscopic detectors provide a powerful and versatile characterization platform that is perfectly matched to these products.

**LCGC: What is new with Wyatt's Eclipse flow FFF products?**

**Christoph:** I am really excited about the next-generation Eclipse NEON. It was redesigned to incorporate Wyatt's advanced NEON platform in order to meet critical requirements for usability, robustness, and performance. All of Wyatt's online detectors, including the DAWN, Optilab, and ViscoStar, have already been released in this platform and the benefits have been enormous in the marketplace.

The most prominent new feature is a front-panel interface with built-in intelligence implemented on a 10-inch multitouch display. The system health indicators eliminate the guesswork—they let the user know whether the instrument is ready for an optimal run, or it provides guidance on what to do to achieve ready status. What's more, the new interface enables manual control of the Eclipse flow FFF controller directly from the instrument display, eliminating the need to use the software for basic setup, cleaning, and maintenance. Our aim is to ensure a perfect analysis each time, with a streamlined workflow and maximum reliability.

On top of that, we have two new hardware features, the dilution control module (DCM) and the Eclipse Mobility. DCM reduces the sample dilution that normally occurs at the outlet of the channel. The dilution can be reduced by the DSM module with a corresponding increase of the detector signal by a factor of up to 10X for higher sensitivity. Fractions that are collected downstream of the last detector, using a standard fraction collector, come out with the same increase in concentration, so the process is much more effective.

Eclipse Mobility combines flow FFF with an electrical field by adding two electrodes to the channel. Applying an electric field changes the retention time of sample components according to their charge, in addition to size-based retention. From the resulting shift in retention time, the electrophoretic mobility and zeta potential can be calculated. We have shown in a peer-reviewed publication that the values are consistent to those obtained by electrophoretic light scattering (ELS).

The advantage of the Eclipse Mobility over standard ELS is that the charge of several populations within a mix can be determined. In a semiquantitative way, it can be established whether the charge is uniform within a broad distributed sample or whether it isn't homogeneous. This helps to understand interactions between nanoparticles that have different or opposing charges. One example is a conjugation of RNA or DNA with a positively charged lipid nanoparticle.

**LCGC: How does the new model relate to your previous offering?**

**Christoph:** The Eclipse NEON replaces the Eclipse Dualtec and Eclipse AF4; they supported different types of separation: center-downstream injection and tip injection. With the Eclipse NEON, both center and tip injection are supported for analytical, semi-prep, and frit-inlet AF4 channels, and tip injection for hollow fiber (HF5) channels or SEC. An automated dual-channel switching configuration is still available.

**LCGC: Where is FFF-DLS-MALS in terms of acceptance by chemistry, manufacturing, and controls (CMC) departments and regulatory agencies?**

**Christoph:** The need for FFF-MALS-DLS in characterization for regulatory filings of drugs, and eventually quality control of nanomedicines, is fully recognized by regulatory agencies and the institutions and organizations developing standards for the pharmaceutical industry. These organizations have been developing protocols and technical documents, as well as publishing reviews and introspection papers, to support the adoption of this method across the pharmaceutical industry. I can say with confidence that it will become an essential characterization tool for nano DDS and gene vectors. The improvements we are making to performance, simplification, robustness, and GMP compliance should meet the needs and expectations of CMC departments, regulatory agencies, as well as quality-control departments.

**FIGURE 6:** MS chromatograms of the hCGβ-T5 peptide after preparation of blank (non-spiked) DMS (lower, red), and DMS spotted with samples spiked at low levels (20 IU/L (58 pM), upper, black) using DMPK-C as sampling material. Adapted and reprinted from C. Rosting, E.V. Tran, A. Gjelstad and T.G. Halvorsen, *Determination of the Low-Abundant Protein Biomarker hCG from Dried Matrix Spots using Immunocapture and Nano Liquid Chromatography Mass Spectrometry, Journal of Chromatography B*, **1077-1078**, 44-51 (2018). Copyright (2018), with permission from Elsevier.

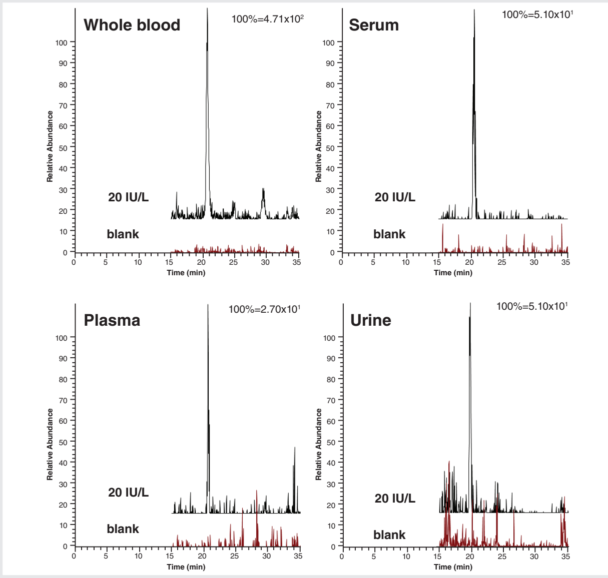
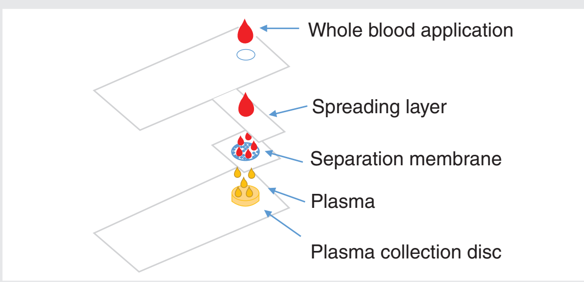



**FIGURE 7:** Schematic of overview of a typical set-up for a plasma separation card.




majority of the published papers circumvent the challenge of varying hematocrit by spotting a fixed volume and using the whole (16,30) or the majority of the spot (19), or by using a normalization step when sampling only a portion of the spotted blood (20). Improving and simplifying both the DBS workflow and the MS-based protein analysis will increase the likelihood for these methods to be used by the routine laboratory in the future.

***Targeted biomarker determination:*** Examples of methods describing biomarker quantification using DBS sampling are the determination of ATP7B protein as a potential screen for Wilson's disease (19) and the determination of *Yersinia pestis* markers (15). In both examples the proteins are determined from DBSs using the bottom-up approach, the most commonly applied approach in biomarker quantification by MS. As the abundance of both ATP7B protein and the *Yersinina pestis* markers in blood is low, a selective sample clean-up step, immunocapture, has been included in the analytical procedure. Immunocapture is a common clean-up and enrichment procedure for low-abundant proteins in biological matrices prior to MS (44), and can be performed both before and after the digestion step (Figure 2). Both approaches are described in combination with DBSs for biomarker analysis. For example, the *Yersinia pestis* markers (15) and human chorionic gonadotropin (hCG; a biomarker for pregnancy, ovarian and testicular cancer as well as doping agent for male athletes) (16) were determined using immunocapture prior to digestion. ATP7B protein (19), and a range of clinically-relevant plasma proteins such as albumin, apolipoproteins and c-reactive protein (20) on the other hand, were determined using immunocapture after digestion. Further sensitivity can be achieved by using nano liquid chromatography (nano LC–) MS/MS for the subsequent analysis (15,16,19): For hCG > 10 times improvement in detection and quantification limits was achieved when transferring a method for the determination of hCG in DBSs from a micro LC–MS/MS system to a nano LC–MS/MS system (16).

***Biomarker discovery:*** Biomarker discovery studies using DBS sampling are also described, both by targeted and non-targeted approaches. A targeted approach with multiple reaction monitoring (MRM) was done for several of the studies: Examples are assessment of schizophrenia risk (23) and screening for primary immunodeficiency disorder (25). In addition, two separate publications demonstrate that a targeted approach can be used for reproducible MS determination of a high number of proteins 97 (22), and 82 (24) from DBSs.

Non-targeted biomarker discovery is described for identification of biomarkers for athletes in different training states (using data independent acquisition). The goal was to identify two different clusters of proteins to define acute versus chronic physiological changes related to functional overreaching (26).

***Determination of protein modifications:*** Another potential application of DBS sampling in protein analysis by MS is the determination of protein modifications. Both post-translational modifications (PTMs) such as site-specific *N*-glycopeptides of serological proteins (45) and environmentally-induced modifications such as the oxidation of cysteine 34 of human serum albumin (46), age-associated methylation of hemoglobin (47), reactive nitrogen and oxygen species-induced modifications in hemoglobin (48,49) are reported. For these analytes it is especially important to evaluate the stability of the modifications

and/or if the modifications studied can be artificially induced during the storage of the DBSs in air.

The main challenge for widespread use of DBSs in MS-based biomarker determination are the quantification of low-abundant proteins from the complex matrix and the low sample volumes used in DBS sampling. This may be resolved using affinity clean-up (for targeted applications) and nano-LC–MS as described for ATP7B protein (19). An additional challenge for use in remote area sampling is that you will need to send the sample to a laboratory with advanced equipment and that the response time will most likely be increased (15).

***Doping analysis:*** DBS sampling has emerged as an interesting sampling alternative in doping analysis (30), and also for protein and peptide doping agents. This is highlighted by the attention the World Anti-Doping Agency (WADA) has focused on alternative sampling matrices such as DBSs. The advantages are linked to the ease of sampling which makes it both possible to directly determine the level of circulating substances immediately prior to (or after) the competition, and the low invasiveness for the athlete. The latter is especially advantageous for "out-of-competition" testing because no trained phlebotomist is necessary for drawing the blood sample. Furthermore, since doping samples sometimes have to be stored for several years (up to 10 years) it is a great advantage that storage of DBS samples is less demanding (both related to temperature and space requirements) than conventional urine and blood samples. Recent examples of protein-doping agents that can be determined by MS from DBS are insulin (33), the synthetic human adrenocorticotropic hormone tretracosactide hexaacetate (29) and the erythropoietin-stimulating protein sotatercept (30). The latter uses a bottom-up approach and high-resolution mass spectrometry (HRMS) determination for both screening and confirmation. Sufficient sensitivity is achieved by affinity clean-up using protein G in the screening procedure and activin A in the confirmation procedure. The screening method can also detect other IgG-based doping agents such as luspatercept and bimagrumab. In addition, as high resolution full-scan MS data is collected retrospective analysis can be performed to detect further IgG-based drugs with performance-enhancing properties. For confirmation a selective extraction of sotatercept was enabled using activin A (30). Figure 3 illustrates a set-up for screening and confirmation in doping analysis using two different affinity approaches for sample clean-up.

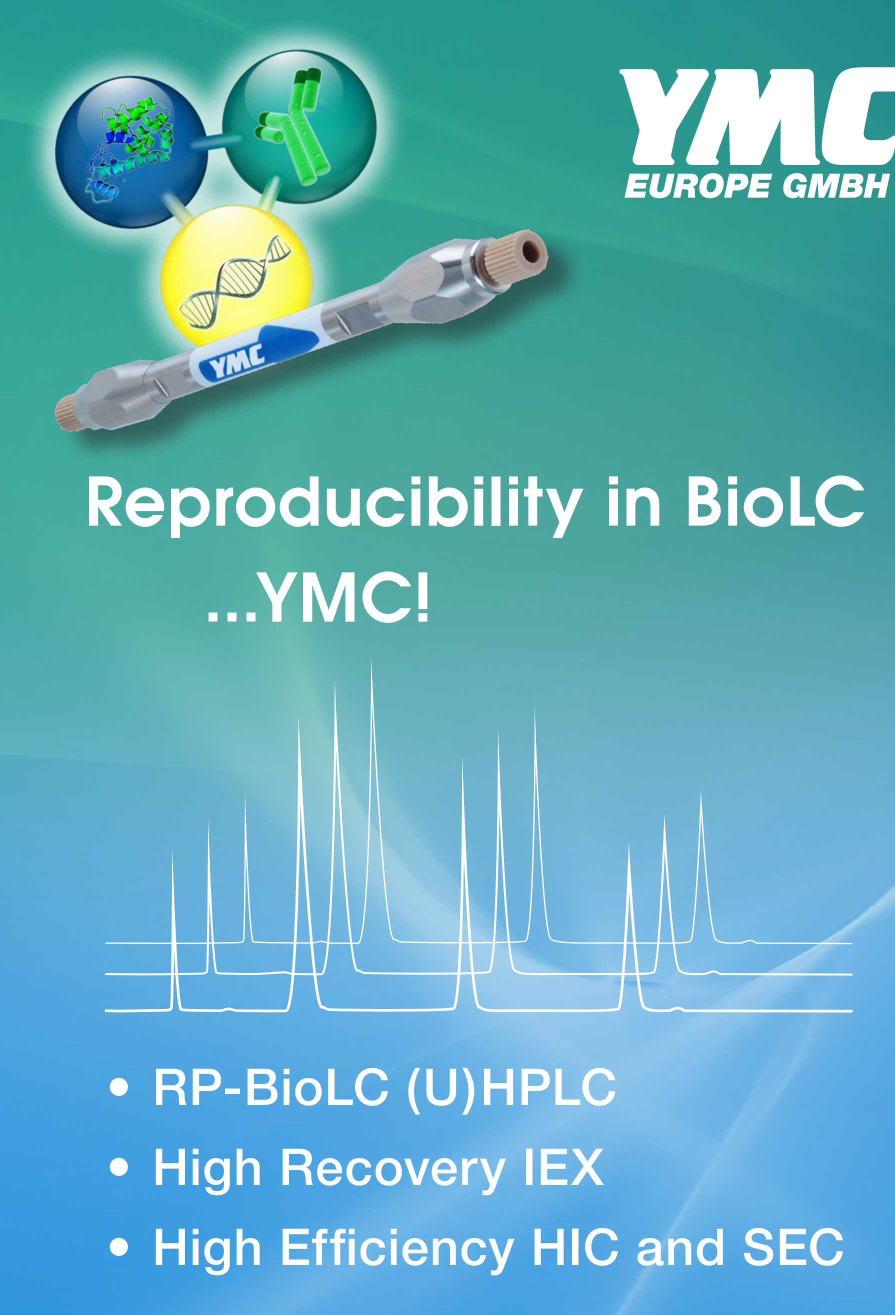


**FIGURE 8:** Schematic overview of the different smart sampling approaches. In step I & II the paper is prepared for immobilization of either enzyme or antibody (step III). In step IV the sample is added, and the proteins either proteolysed (upper) or captured (lower). Step V & VI gives an overview of further sample treatment and analysis. Detailed descriptions of the different approaches can be found for a) in reference 40; for b) in reference 55; for c) in reference 41; and for d) in reference 52.

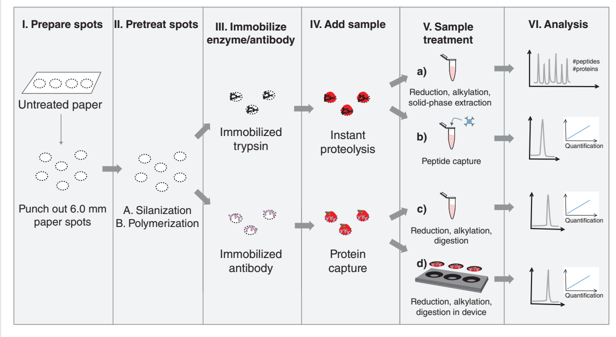



**FIGURE 9:** Schematic overview of the combination of DBS with paper-spray MS.

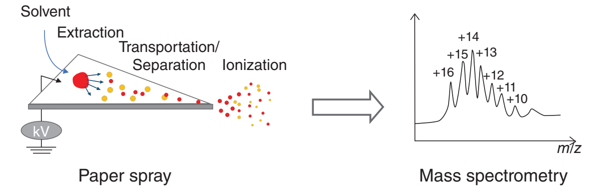



**TABLE 1:** Limit of detection (LOD) and repeatability (RSD, %) of hCG from different sample matrices after spotting of 15-µL on DMPK-C cards. Adapted and reprinted from C. Rosting, E.V. Tran, A. Gjelstad and T.G. Halvorsen, *Determination of the Low-Abundant Protein Biomarker hCG from Dried Matrix Spots using Immunocapture and Nano Liquid Chromatography Mass Spectrometry, Journal of Chromatography B*, **1077-1078**, 44-51 (2018). Copyright (2018), with permission from Elsevier.

| Sample matrix | Limit of detection[1] (IU/L (pM)) | Repeatability[2] (RSD %) |
|---|---|---|
| Whole blood | 5.0 (14.5) | 22.5 |
| Plasma | 8.9 (25.8) | 152.2 |
| Serum | 7.8 (22.6) | 14.9 |
| Urine | 10.5[2] (30.5)[3] | 10.0[3] |

[1]n =5 and based on S/N = 3; [2]n = 5 and spiking level 20 IU/L (58 pM) [3]n = 3

Another recent progression in doping analysis where proteins are monitored from DBSs is to determine blood doping by autologous blood or recombinant human erythropoietin (31,32). In the described method (31,32) the membrane proteins are isolated and subsequently protein digested before LC–MS/MS determination of two reticulocyte membrane proteins (Figure 4); one of the two membrane proteins is only present in immature reticulocytes while the other is present on both red blood cells and reticulocytes. The ratio of these two proteins is more sensitive to changes in erythropoiesis than the conventional way of only monitoring percentage reticulocytes (31,32). Hence the method has a potential in blood doping analysis.

The major challenge for increased use of DBS sampling in doping analysis of proteins is most likely the current need of specific (affinity based) sample enrichment procedures which makes targeted screening of multiple analytes more difficult than for small molecule doping agents.

***Bioanalysis of therapeutic proteins:*** In drug discovery, there has been an increasing focus on using microsampling techniques to reduce the volume of blood and hence the number of animals as test subjects in drug development (50). This trend is also valid in the development of biopharmaceuticals, such as for therapeutic proteins/antibodies, which is a fast-growing class of drugs on the market. The use of both DBS sampling and MS for analysis of therapeutic proteins during drug discovery has been described, although not extensively. A recent example is an automated system based on DBS sampling using serial microsampling (36). The system was used for studying the pharmacokinetics (PK) of a therapeutic protein, and only one mouse was necessary to collect a full PK curve as opposed to one mouse per data point using conventional sampling methods (blood serum). An illustration of an automated DBS sampling system is shown in Figure 5. The major difference compared to traditional PK studies will be that the curves are obtained for whole blood and not serum or plasma which commonly is the matrix of choice to establish dose response curves. After elution from the DBSs the target protein was enriched using protein A and digested prior to analysis.

## Other sampling matrices/dried matrix spots

Most bioanalytical methods are developed and validated using serum or plasma as the biological matrix, and for biomarkers reference levels are established using the same matrices. A challenge with DBS sampling for use in biomarker analysis is therefore a lack of reference levels using capillary blood as sample matrix. As a result of this, the advantages of small sample volume and increased stability of dried analytes makes it worthwhile in some occasions to also use the dried sampling format for other biological matrices than whole blood. In addition, for analytes that are mainly distributed in plasma (such as plasma proteins), the sensitivity might be higher when using serum or plasma compared to whole blood. As increased stability is often seen for

dried matrices, another advantage is that demanding and costly sample freezing necessary for storage and shipping of liquid samples can be avoided (51). Synovial fluid, cerebrospinal fluid, saliva, tears and urine, as well as the processed blood matrices serum and plasma are all biological matrices that can be used for dried matrix spotting. In analysis of proteins by MS, the use of other matrices is scarce, but has been described for diagnostic proteins such like hCG (16) as well as for two biomarkers for *Yersinia pestis* (F1 antigen and low calcium response V antigen) (15), site-specific *N*-glycopeptides of serological proteins (45), and serum proteins in a study on patients with intrauterine growth restriction (a pathological pregnancy condition) (51).

DMS sampling for protein analysis by MS is described mainly for serum (16,41,45,51,52). Other matrices used are plasma (both human [16] and mouse [15]), urine (16) and (mouse) spleen (15).

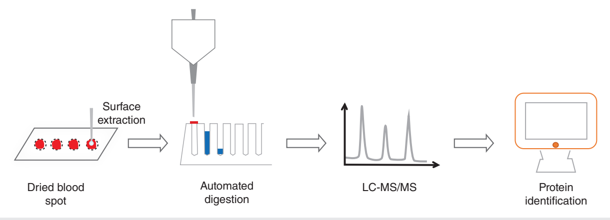
The analyte determined from most different matrices is hCG. It has been determined from dried plasma, serum and urine spots in addition to from DBSs (16). In addition, it has been determined from dried serum spots using a smart sampling device combining the sampling step with immunoaffinity capture (41,52). Figure 6 shows MS chromatograms at the limit of detection (LOD) of hCG from four different matrices spotted on conventional DBS cards.

LODs in the lower IU/L-level (corresponding to 14.5–30.5 pM) were observed for hCG from all matrices (Table 1). The repeatability at this low level was satisfactory (relative standard deviation [RSD] < 25%) for all matrices except plasma (Table 1). The reason for the high RSD observed from plasma was speculated to be due to the anticoagulant because a similar effect was observed for whole blood drawn using different anticoagulants previously (38). This suggests that urine and serum might be more suitable alternative matrices for spotting, and that attention has to be paid to the anticoagulant when using plasma as the sample matrix and during method development for DBSs when whole blood is collected in tubes containing anticoagulant.

## Exploring New Frontiers in DBS Sampling

**New sampling materials and devices:** Recently there has been a focus on new sampling materials and devices that can overcome some of the challenges often associated with DBS sampling. These new materials and devices may improve the repeatability and reproducibility of LC–MS methods for proteins from DBSs, and hence increase the likelihood for these methods to be used in a routine laboratory. Traditionally, pure cellulose cards (most commonly non-impregnated to avoid denaturation) are used for DBS sampling of proteins. The major challenges are poor extraction recoveries from blood spots and the effect of hematocrit

**FIGURE 10:** Limit of detection (LOD) and repeatability (RSD, %) of hCG from different sample matrices after spotting of 15-µL on DMPK-C cards. Adapted and reprinted from, N.J. Martin, J. Bunch and H.J. Cooper, *Dried Blood Spot Proteomics: Surface Extraction of Endogenous Proteins Coupled with Automated Sample Preparation and Mass Spectrometry Analysis, Journal of the American Society for Mass Spectrometry* **24**, 1242-1249 (2013).




on quantification. The issue of poor extraction recoveries has been approached by introducing water-soluble sampling materials: In 2015 water-soluble carboxymethyl cellulose was introduced as a potential sampling material in protein analysis from blood spots (38). Rosting et al. evaluated the material for the analysis of hCG from DBSs. The method was later refined and evaluated for other sample matrices (plasma, serum, urine) and it was demonstrated that lower LODs were obtainable for all matrices (except whole blood where comparable LODs were seen) using the water-soluble material compared to conventional sampling cards (16).

Other materials such as volumetric absorptive microsampling (VAMS) focus on the possibility to sample an accurate volume to avoid the potential issue of varying blood hematocrit. Blood hematocrit will have an impact on quantification when only a portion of the blood spot is used for further analysis, hence sampling using VAMS eliminates the volume variation and is expected to improve the reproducibility of the analysis. VAMS has been evaluated for proteins by MS in both a generic set-up using six non-human model proteins (37), and in quantification of circulating cardiovascular risk associated apolipoproteins (17). Despite VAMS being described to be less affected by blood hematocrit because of the sampling of accurate volume, an effect of the hematocrit on the recovery was seen for two out of six model proteins at low hematocrit (20%) in the work using non-human model proteins and a quite wide hematocrit range (20-60%) (37). The reasons for this are not clear but it is described that although the VAMS collect a constant volume independent of hematocrit, extreme hematocrit levels might influence extraction recoveries from VAMS (53,54).

Another way of ensuring accurate volume is by using plasma separation cards. These cards separate the blood cells from the blood plasma and sample an accurate volume of the blood plasma. Although applied in protein analysis from dried serum spots (51) after directly spotting serum on the base sheet of the plasma separation card, these cards have not yet been used for spotting whole blood in protein analysis by MS. A schematic overview of a typical set-up for a plasma separation card can be found in Figure 7.

*So-called* smart sampling materials have recently been introduced. These sampling materials integrate protein relevant sample preparation to the sampling step and hence reduce the sample preparation time consumption significantly (40,52,55). The main advantage of this concept is to utilize the sample drying time to perform part of the sample preparation. This will also reduce the number of manual steps in the procedure and could have a positive effect on the reproducibility of the analyses. Both proteolytic enzymes (trypsin) and antibodies have been covalently immobilized to paper polymerized with 2-hydroxyethyl methacrylate-co-2-vinyl-4,4-dimethyl azlactone (pHEMA-VDM), and good performance has been described in quantitative proof-of-concept studies (52,55). A schematic overview of the different applications employing smart materials is shown in Figure 8.

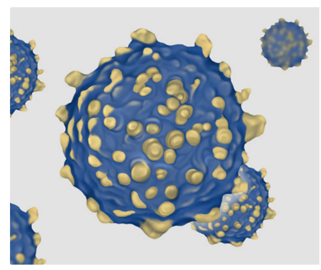
**Novel combinations with mass spectrometry:** Most described methods for MS-based protein analysis of DBSs uses liquid chromatography coupled to ESI-MS. Novel approaches of protein analysis, DBS sampling and MS are the use of paper spray (56,57), and the use of liquid extraction surface analysis (LESA) in combination with direct electrospray MS (58,59). The combination of DBS sampling with paper-spray MS is convenient because in DBS sampling the sample is already spotted on paper. Nevertheless, only a couple of top-down examples of this combination are described, in these applications the paper is modified prior to use to reduce the hydrophilicity of the paper and hence reduce the interaction between the analytes and the paper (56,57). A schematic overview of the process is shown in Figure 9.

Applying LESA to DBSs allows for direct electrospray analysis from the dried sample after the addition of a small volume of extraction solvent on top of the DBSs. The technique has been combined with MS both with and without high field asymmetric waveform ion mobility spectrometry (FAIMS). The top-down approach is especially advantageous in combination with LESA because the extraction drop can then be directly injected to the MS (59–61). Additionally, non-targeted bottom-up proteomics examples are also described (58). Possibilities for automating the digestion process after LESA are also available (62). A schematic overview of an automated process for digestion of DBSs after LESA can be found in Figure 10. Automated workflows are advantageous to ensure robust sample handling and increased sample throughput.

DBS as a sampling format is also well suited for combinations with other direct ionization techniques such as desorption

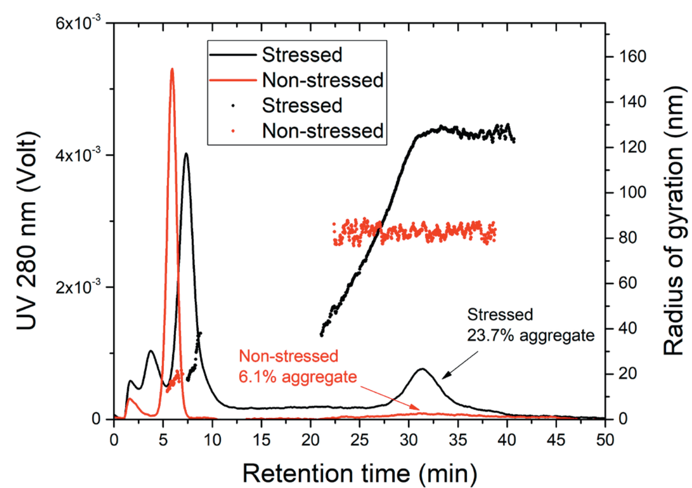
# Quantification of Adeno-Associated Virus Aggregation

## The Field-Flow Fractionation Platform



Adeno-associated viruses (AAVs) are increasingly used for gene therapy due to their versatility and safety. One of the biggest concerns for manufacturing a uniform AAV suspension is the presence of viral aggregates, which can create problems with transduction efficiency, biodistribution, and immunogenicity. These large AAV aggregates are challenging to separate and characterize by traditional column-based chromatography techniques such as size exclusion chromatography (SEC).

Asymmetrical Flow Field-Flow Fractionation with Multi Angle Light Scattering (AF4-MALS) can separate and size large AAV aggregates, and discern a difference in aggregate concentration due to the stressing protocol. Some or all of the large aggregates would be filtered out by SEC, resulting in incorrect determination of the aggregate content or the false conclusion that no aggregates are present.



For more details visit www.postnova.com and search for 'virus'.


Contact us for more information: **www.postnova.com**

electrospray ionization (DESI). However, for full utilization of this concept to occur the potential issue of matrix effects, that is, ion suppression, which is also seen with paper spray, must be resolved.

## Conclusion

Recently, there has been an extensive increase in the use of DBS as a sampling method prior to protein analysis by mass spectrometry. The sampling technique has been applied to a wide range of analytes in a variety of applications followed by LC–MS detection. The use, however, of the sampling technique in MS analysis of proteins is not widespread. The main reasons for this are most likely the quantification challenges observed as a result of varying hematocrit as well as correlation with already established methods using serum or plasma as the sample matrix. In addition, the small sample volumes used in DBS sampling can be a challenge for low-abundant proteins, where pre-concentration of a larger sample volume is often necessary to obtain sufficient sensitivity. The first issue can be circumvented using novel sampling materials /formats that ensures sampling of a fixed volume while still being able to use blood from a finger or heel prick. The second is also solvable, but will need reference levels for the biomarkers/doping agents/drugs to be established in DBSs and correlated to existing levels and matrices. The latter can be solved by selective sample preparation techniques and sensitive instrumentation. Development of new *so-called* smart sampling materials for protein analytes will potentially increase the advantages of DBS sampling to other sampling formats because part of the sample preparation can be performed simultaneously with sampling. However, the time-reducing advantages of the smart sampling approaches will first be fully utilized when combined with direct MS techniques, which is something that will require improvements in these techniques to reduce matrix effects and improve the sensitivity of the analysis.

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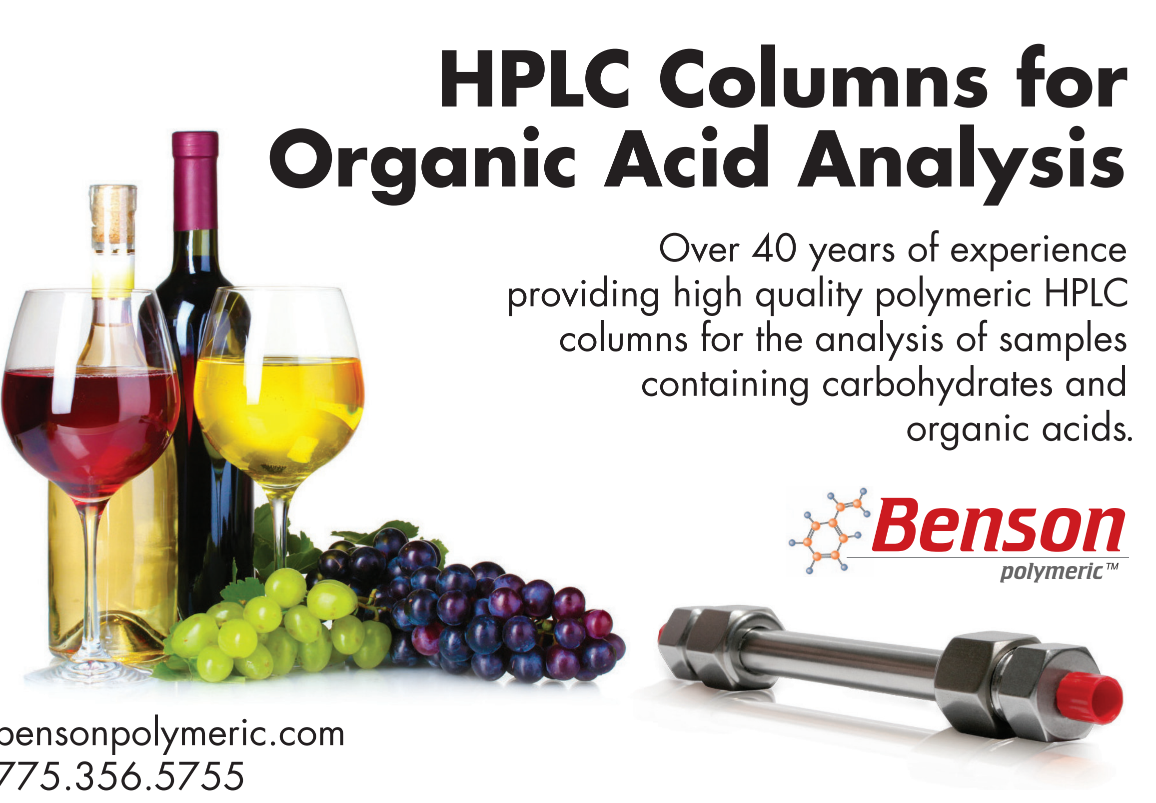

# Liquid Chromatography at Room Temperature: When Should the Column Temperature Be Specified in a Method?

**Dwight R. Stoll,** LC Troubleshooting Editor

**In reversed-phase liquid chromatography, increasing the column temperature usually results in a decrease in analyte retention. Will this cause problems if a method does not call for control of the column temperature but the room temperature of the laboratory environment changes?**

In the early days of liquid chromatography (LC), precise control of the column temperature was not a primary consideration in many instrument setups. Unlike gas chromatography (GC), where the column temperature is the single most important experimental variable that dictates retention, in LC the column temperature has a smaller influence on retention compared to the mobile-phase composition (that is, the organic:aqueous ratio for reversed-phase and hydrophilic interaction chromatography (HILIC) separations, or cation:anion concentration for charge-based separations). As the community's knowledge about the effects of temperature on LC separations has increased, the need for accurate and precise control of column temperature has increasingly been recognized. For example, we now know much more about the effects of column temperature on the retention of different analyte types (1), the importance of matching the temperature of the mobile phase at the column inlet to the temperature of the column itself (2), and the effects of heat flow in columns on apparent column efficiency (3). As a result, users generally pay attention to the control of temperature in their LC instruments (and the designs of instrument modules for doing so), and specify a particular temperature or temperature range when developing and validating a method. However, there are still many methods in use that have been developed for use at "room temperature," where there is no provision for the LC instrument to control the column temperature, and in some cases no precise specification of what is meant by "room temperature" in the method. This leads to the obvious question: with such a method, can one reasonably expect to obtain the same separation in Anchorage, Alaska, as in Mumbai, India? In this installment of "LC Troubleshooting", I discuss some of the basics of the effect of temperature on retention in LC, and then discuss some experimental data that helps us understand when some variation in room temperature might be okay, and when it might seriously affect the performance of a separation.

## Basics of the Effect of Column Temperature on Retention in Reversed-Phase LC

In reversed-phase LC, the influence of temperature on retention is most commonly described using a Van 't Hoff type of relationship, like that shown in equation 1:

$$\ln (k) = A + B * \frac{1}{T} \qquad [1]$$

where $k$ is analyte retention factor, and $T$ is temperature (in K). The variable $A$ is a condition-specific parameter related to the entropy

of transfer of the analyte from the mobile phase to the stationary phase and the phase ratio (the relationship of stationary phase volume to mobile phase volume), and *B* is related to the enthalpy of transfer of the analyte from the mobile phase to the stationary phase. Figure 1 shows experimental retention data for several neutral small molecules obtained with a C18 column, an acetonitrile:water mobile phase, at different temperatures. The data follow a roughly linear trend, as predicted by equation 1. Recent work by the Weber group has examined a large body of experimental measurements of this type, and concluded that a non-linear version of equation 1 frequently produces a better fit of the data than a linear one (1). Other researchers have argued that the apparent linearity of the data is more of a happy accident than a thermodynamic inevitability (4).

The slope of each line in Figure 1 is related to the enthalpy of transfer of the analyte from the mobile to the stationary phase. For molecules that are chemically similar, the slopes are numerically similar, as shown by the data for toluene, ethylbenzene, and propylbenzene in Figure 1a. In a separation of a mixture of molecules like these, increasing the column temperature will decrease retention, but the relative spacing of the peaks (that is, the selectivity) will not change much, because all of the peaks move in the same direction at about the same rate. However, for molecules with different functional groups, the slopes can be quite different, as shown in Figure 1b for the analytes p-nitrobenzyl chloride and anisole. In a case like this, increasing column temperature will decrease retention for both analytes, but at different rates, such that selectivity and resolution will change. In extreme cases this can even lead to a reversal of elution order (5). Readers interested in exploring these phenomena on their own can do so easily using our web-based simulator that allows the user to change mobile-phase composition and column temperature, and observe the impact on retention and resolution (www.multidlc.org/hplcsim).

### Let’s Look at Some Experimental Data

In preparation for writing this instalment of “LC Troubleshooting,” I created a microenvironment for


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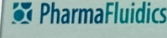

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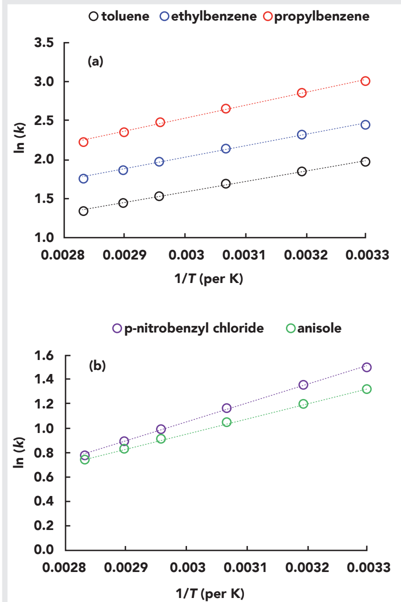
**FIGURE 1:** Effect of changing column temperature ($T$) on retention factor ($k$) for several neutral small molecules obtained using a C18 column and mobile phase of 50:50 ($v/v$) acetonitrile/water: (a) shows toluene, ethylbenzene, and propylbenzene, while (b) shows p-nitrobenzyl chloride and anisole. Dashed lines are linear regression lines. Data from reference (6).




one of the LC instruments in my laboratory. I first disconnected the column from the flow path going through the column thermostat compartment we normally use to control the column temperature, such that the mobile phase flow went directly from the autosampler to the column, and then directly on to the detector. Then, I heated or cooled the microenvironment to simulate the impact of changing room temperature on my separation, and measured the ambient air temperature near the column inlet using a small thermocouple. Finally, I recorded the chromatograms for separations of a simple mixture of small molecules (one acid, one base, and two neutrals) at different "room temperatures" after equilibrating the system for about 30 min for each temperature. Three of these chromatograms—recorded with "room temperatures" of 24, 28, and 42 °C—are shown in Figure 2. The vertical dashed line is added as a visual aid, to help quantify the shift in retention that occurs as the temperature is increased. Although there is a shift that is discernable by eye, it is instructive to plot the resulting data as a function of temperature to more precisely understand the effect, particularly for changes in selectivity. Figure 3 shows the effect of temperature on the retention factors of all four probe compounds. In Figure 3a, I have plotted the absolute values of the retention factors, and, in Figure 3b, I have plotted the percent change in retention relative to average retention for each compound over the range of temperatures measured. As expected, the retention of all compounds decreases as temperature increases, and here we have our first important point from this experiment. That changes in "room temperature" can definitely be enough to cause a change in retention on the order of 2% for small molecules, even over a modest range of laboratory room temperatures that might be experienced in the United States. The data point at 42 °C is admittedly a bit extreme, but there are LC laboratories across the globe that do experience this type of extreme condition.

In Figure 3b, we see that the slopes of the trends (% change in $k$ vs. $T$) are pretty similar, with the exception of nortriptyline, which exhibits a much steeper slope. As discussed earlier, if the retention of all compounds changed at the same rate in response to a change in temperature, this would not be too much of a problem for method implementation, because resolution would not be affected too much. However, it appears from Figure 3b that the rate of retention for nortriptyline is on the order of two-fold higher than that of the other compounds. By plotting the selectivity of the separation against temperature we can obtain a more precise view of these changes in relative retention. Figure 4a shows $\alpha$ values for several pairs of the probe compounds, and Figure 4b shows the percent changes in these values vs. temperature. Here we see that there is no obvious change in the selectivity of the separation toward the neutral compounds (acetophenone and butyrophenone) as the temperature is increased from 24 to 42 °C. Even the selectivity of the separation for butyrophenone and n-butylbenzoic acid does not change, despite the fact that these are chemically quite different molecules. Even though the acid is ionogenic, at pH 3.2 it is mostly protonated and neutral. On the other hand, we see that the selectivity of the separation toward nortriptyline changes substantially over the range of temperature studied here. The alpha values for nortiptyline and butyrophenone (red circles) or butylbenzoic acid (blue circles) change by almost 5%.

## Implications for Method Development and Implementation

So far, we have observed that a change in room temperature can have a significant effect on the selectivity of a reversed-phase

separation for some, but not all, types of molecules. The practically important question, then, is: Under what circumstances do changes of this kind really matter? The molecules I chose for this study are well separated (Figure 2), and even a 5% change in selectivity is not enough to bring them close enough together that they are no longer separated. Obviously, this will not always be the case. For separations where two neighbouring peaks are just barely resolved at one room temperature, changing to a different room temperature could have a big effect on the resolution of that pair. We can quantify this effect using the Purnell equation for resolution, shown in equation 2.

$$R_S = \frac{\sqrt{N}}{4}\frac{\alpha\text{-}1}{\alpha}\frac{k}{1+k} \qquad [2]$$

If we consider a situation with a plate number ($N$) of 15 000, a retention factor ($k$) of 5 for the later eluted of two peaks, and an $\alpha$ of 1.1, the resolution ($R_S$) of the two peaks will be about 2.3. We can use equation 2 to calculate the effect of a change in $\alpha$ on the resolution. Figure 5 shows how the resolution decreases as $\alpha$ decreases by up to 5% (that is, from 1.1 down to 1.045). Whereas a resolution of 2.3 corresponds to a nice separation where the valley between the peaks goes to baseline, at a resolution of 1.1 there is significant peak overlap. This overlap can significantly compromise quantitative accuracy of the method, particularly when one of the members of the peak pair is present at a concentration much higher than the other.

**FIGURE 2:** Effect of changing column temperature ($T$) on retention factor ($k$) for several neutral small molecules obtained using a C18 column and mobile phase of 50:50 ($v/v$) acetonitrile/water: (a) shows toluene, ethylbenzene, and propylbenzene, while (b) shows p-nitrobenzyl chloride and anisole. Dashed lines are linear regression lines. Data from reference (6).

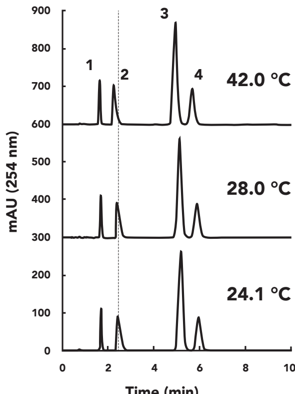
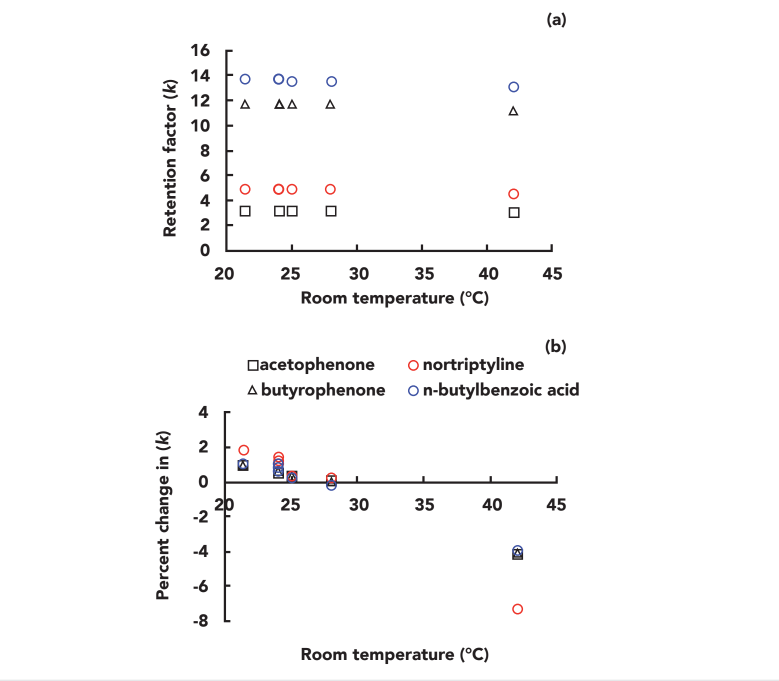



**FIGURE 3:** (a) Retention factor and (b) percent change in retention factor for the test compounds over the range of room temperatures tested (21–42 °C).




## Summary

In this installment of "LC Troubleshooting", we have examined the impact of a change in room temperature on the performance of reversed-phase LC separations when the column temperature is not controlled, such that the column takes on the temperature of the ambient room air. In most cases, an increase in temperature leads to a decrease in retention for reversed-phase separations. This will not affect resolution of neighbouring peaks too much as long as the degree of

**FIGURE 4:** (a) Selectivity and (b) percent change in selectivity for the test compounds over the range of room temperatures tested (21–42 °C).

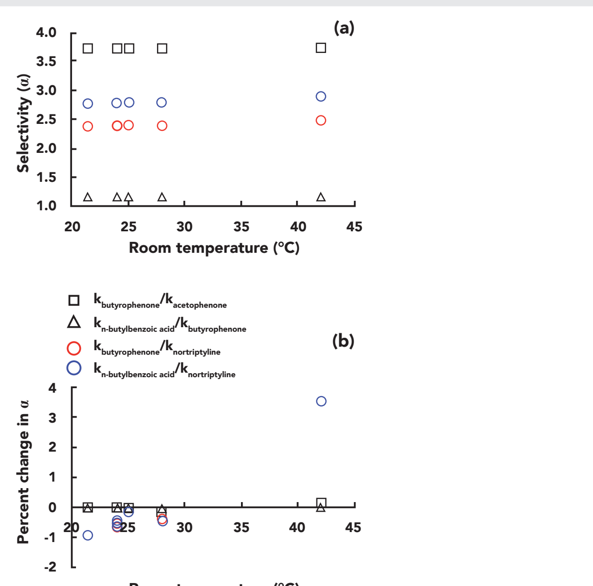
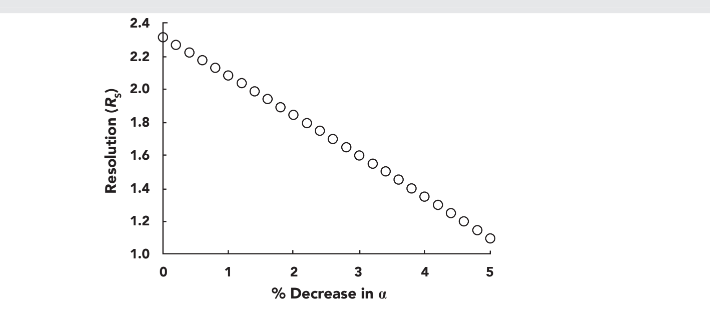



**FIGURE 5:** Effect of a decrease in selectivity (α) on the resolution for a pair of closely eluted peaks. Resolution is calculated using the Purnell equation (equation 2) with $N$ = 15000, $k$ = 5, and starting α = 1.1.




shift in retention is similar for both peaks. However, this is not always the case, and significant changes in the selectivity of the separation on the order of several percent can occur. These changes in selectivity can in turn lead to a significant decrease (or increase) in the resolution of closely eluting peaks, on the order of 50%! Taken together, this means that:

1. Specifying what constitutes "room temperature" during the method development will help prevent problems during method implementation, particularly if the method will be deployed in different laboratory environments where actual room temperatures may be very different.
2. During method development, the robustness of the method should be characterized to help understand what the sensitivity of the method performance is to changes in column temperature.
3. Controlling the column temperature with some means of thermostatting can help avoid these problems altogether, albeit at the cost of adding an instrument module for this purpose.

## Acknowledgment

I would like to thank Ravi Ravichandran for bringing his thought provoking questions about room temperature LC to me, and many subsequent helpful conversations on this topic.

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# New Sample Preparation Products and Accessories

**Douglas E. Raynie,** Sample Preparation Perspectives Editor

**This yearly report on new products introduced in the preceding year, since May 2019, covers sample preparation instrumentation, supplies, and accessories.**

New sample preparation technologies introduced in the past year were more bountiful than in previous years. Most product introductions were aimed at improved performance of existing technologies, or aimed at specific applications. Sorbents and accessories for solid-phase extraction (SPE) led the way, as did automated, multiplatform integration of sample preparation.

Our annual review of sample preparation products covers the previous year. In late 2019, the *LCGC* staff submitted a survey to vendors of sample preparation products. Responses to this survey are compiled in this review, as are new product introductions observed during the past 12 months. Additionally, after a keyword search (using the terms "sample preparation equip", "extraction equipment", "blend/grind/mix/shake/stir", "evaporators/ evaporation", "filtration and purification", and "pipettes/pipetters") of the online Pittcon 2020 vendor list, each of these vendors were visited during the conference.

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

## Solid-Phase Extraction

Mixed-mode SPE has been gaining in popularity in recent years. Phenomenex introduced a new product in that space, under its Strata line, combining reversed-phase sample cleanup and matrix removal. The Strata-X Pro SPE is available in cartridge (1, 3, and 6 mL) and 96-well plate or microelution formats. The company claims time savings of up to 40% via elimination of the conditioning and equilibration steps. Table 1 summarizes the Strata-X Pro, and other SPE products.

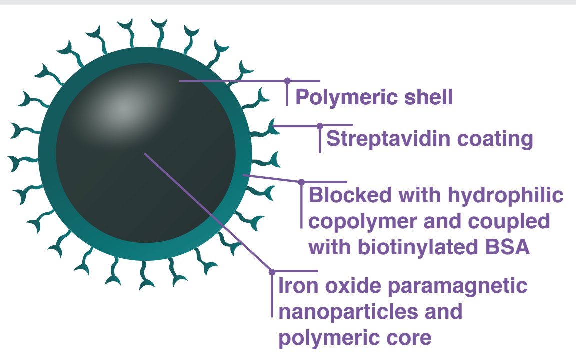
Orochem introduced two families of SPE products. The Agility Deluxe line of polymeric ion-exchange products is based on divinylbenzene (DVB) with strong- or weak- cation- or anion-exchange capabilities, and is available in cartridge, plate, and microelution formats. The company also introduced a series of SPE disks, featuring its Panthera Deluxe reversed-phase DVB. The thin disks with high sorbent capacity allow fast flow rates and use less elution solvent. The disks are designed for pesticides, herbicides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PCAHs), and total petroleum hydrocarbons (TPHs) from aqueous samples.

Restek introduced several new products in its Resprep SFE line for method-specific performance. The Resprep PLR SPE is available in cartridge and well-plate formats, with proprietary material for sample cleanup. SPE materials feature graphitized carbon for organochlorine pesticide cleanup in soil and water, CarboPrep adsorbent for general utility use, EPH silica for petroleum hydrocarbons, florisil for pesticides, and a multipurpose silica. A novel co-sintered frit design, with the proprietary sorbents immobilized into the rigid, porous structure, allows a stabilized sorbent with high manufacturing control and performance uniformity.

CDS Analytical acquired the Empore line from 3M during the past year. Styrene-DVB ion-exchange disks and cartridges and 96-well plates with C8, C18, styrene-DVB ion exchange, and a universal resin are available. The key feature of the original disk format is trapping of the sorbent particles within an inert matrix of polytetrafluoroethylene (PTFE) for a denser, more uniform extraction bed.

Biotage developed the Atlantic ReadyDisk as single-use (disposable) disks with silica or DVB base material

FIGURE 1: Overview of bioZen streptavidin-coated magnetic beads introduced by Phenomenex. A summary of the product composition is presented in Table 3.




and trifunctional C18 or DVB for absorption or sample cleanup of aqueous samples according to the EPA 500, 600, or 8000 series methods.

Porvair Sciences announced the Microlute PLR as a next generation microplate for phospholipid and protein removal from plasma and serum samples.

Trapping columns for liquid chromatography and mass spectrometry (LC–MS) analysis were introduced in a variety of dimensions by Optimize Technologies. Opti-Lynx Micro, Opti-Trap, Exp 2 Nano, and Exp 2 Stem traps are placed into LC injector valves for sample concentration or purification. Various sizes accommodate a range of load rates and a number of phases are available.

With the growing interest in cannabis testing, due to legalization of medicinal marijuana and industrial hemp, Agilent developed cannabis pesticide and mycotoxin testing kits consisting of 6-mL SPE cartridges and associated consumables. The kits include consumables for processing 400 samples and method guidelines.

In addition to SPE products, instrumentation to support or perform SPE was also introduced throughout the year. With the acquisition of the Empore SPE products, CDS Analytical introduced a number of related SPE processing equipment. For example, for working with 96-well plates, positive pressure and vacuum manifolds, centrifuges, and liquid handling robots are available. Orochem also introduced a family of positive-pressure processors under the Ezypress name. Sorbent bed drying, as well as faster analyte processing, are the advantages of this processing mode, especially with viscous samples.

At the end of March 2020, Thermo Scientific-Dionex came out with the AutoTrace 280 per- and polyfluoroalkyl substances (PFAS)SPE instrument. The system is designed to simultaneously process up to six water samples according to EPA method 537.1 in 2 to 3 h, with only about 15 min of manual intervention. Minimization of PTFE-coated components reduces the risk of interferences.

The Biotage VacMaster Disk is an SPE unit with a single port vacuum manifold to assist extraction of aqueous samples. Multiple units, up to eight, can be combined in parallel. Existing vacuum pumps can be used, and any disk holder or cartridge with luer fittings are allowed. Suggested applications of the VacMaster include EPA methods 625.1, 1664B, and 8270. Biotage also developed the Extrahera GLP software to manage the good laboratory practice (GLP) workflow with audit trail tracking.

The EZprep123 from Fluid Management Systems processes six samples in parallel in less than 45 min for the isolation of dioxins, polychlorinated biphenyls, and polybrominated diphenyl ethers (PBDEs). This system is a companion to the EZSPE for water and waste water analysis, and the EZPFC for the extraction of polyfluorinated compounds in water and waste water.

## Automated, Multiplatform Instrumentation

Given the number of steps in a typical sample preparation scheme (1), the role of automated, multiplatform instrumentation is of strong interest to suppliers of chromatography and sample preparation materials, and to the end users working at the laboratory bench. However, defining which sample preparation steps to include, and development of such equipment at an affordable cost, remain significant hurdles in this area. As such instrumentation continues to emerge, issues of reproducibility, labour intensity, and laboratory safety are addressed. The past year saw important new products in this area.

Last year, Markes International introduced the Centri, a multiplatform sampling and concentration system for gas chromatography. This year, an expansion of the Centri platform was presented. Trap-enabled preconcentration, extraction, and enrichment makes the system attractive to a wider range of laboratories. High capacity sorptive extraction, headspace sampling, thermal desorption, and solid-phase microextraction (SPME), including the SPME Arrow, allow collection and concentration of a broad range of volatile and semivolatile organic compounds. On-board diagnostics accommodates system self-checking. Table 2 presents a compilation of the automated, multiplatform product introductions. Improvements are also noted in the CTC Analytics PAL3 system, particularly in the area of automation. Smart SPME fibers contain a microchip

to monitor extraction parameters and usage history. Polydimethylsiloxane (PDMS), acetate, DVB, and carbon fibre coatings are available. The Smart chip is also used in SPME Arrow and liquid handling syringes. The Sirius Automation MicroTasker is a modular platform with a laboratory information management system interface featuring vial sorting and weighing, liquid handling, capping and uncapping, and barcode reading.

## Sample Preparation Accessories and Related Products

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

### Magnetic Bead Extractions

Isolation of analytes via dispersion of an SPE-like particle is widely used and enhanced when magnetic particles are used. Phenomenex introduced bioZen streptavidin-coated paramagnetic beads, as depicted in Figure 1. The bead is activated with a biotinylated capture antibody to prepare for immunocapture of monoclonal antibody targets. After analyte isolation, the beads are recovered from the liquid sample by magnetic attraction. Table 3 provides an overview of the sample preparation accessories and related products.

### Centrifugation

While standard centrifuges are available for test tubes and well-plates, MicroSolve Technology developed the Vial Centrifuge for use with 12 x 32 mm glass autosampler vials for applications in metabolomics, LC–MS, and bioanalysis. Heavy-walled glass vials have 1.2 or 1.8 mL capacity, and an air-flow system keeps the sample cool during centrifugation.

### Pyrolysis

The Gerstel PyroVial was introduced to the European market this past year, and is slated for release in other geographies. The PyroVial performs at up to 800 °C, and can be used as a microscale reaction chamber prior to LC–MS or GC–MS analysis.

### Solid-Phase Microextraction

While SPME featured prominently in the automated, multiple-platform instruments previously discussed, stand-alone SPME products were also featured this year. Restek developed a triple-phase coating with DVB, carbon, and PDMS in standard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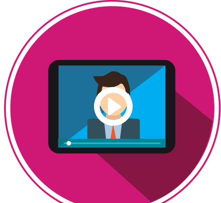

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| **TABLE 1:** Solid-phase extraction products | | |
|---|---|---|
| **Vendor Name** | **Product Name** | **Description** |
| Phenomenex | Strata-X Pro | SPE cartridges (1, 3, or 6 mL tubes), 96-well plates, or microelution plates featuring polymeric base material that works with matrix-removal technology and proprietary functional groups for application to acidic, basic, polar, or apolar analytes. Removal of phospholipids reduces ion suppression in mass spectrometric analysis. |
| Orochem | Agility Deluxe | Polymeric-based ion-exchange particles have narrow-size distribution, and accommodate the full range of pH from 1 to 14. The high capacity material allows use of small amounts of sorbents and correspondingly lower elution volumes. |
| | SPE Disks | Panthera Deluxe DVB, C8, and C18 phases for analysis of drinking water, ground water, and wastewater by EPA methods. Flow rates of 1 L in 10 m, with over 90 % recovery. |
| | Ezypress Positive Pressure Processor | Modular rack design allows quick exchange of 48, 96, or 144 cartridge format or 96-well plate format, with collection into 75 or 100 mm test tubes. |
| Restek | Resprep PLR SPE | 1 mL cartridges and 2 mL 96-well plates with 25 mg proprietary sorbent for sample cleanup through removal of phospholipids and proteins |
| | Resprep CarboPrep Plus | Graphitized carbon, 95 mg x 3 mL, cartridges for organochlorine pesticides. Treatment of carbon allows consistent selectivity. |
| | Resprep Polymeric | Numerous formats of cartridges and 96-well plates in hydrophilic-lipophilic balance (HLB), mixed- and weak- anion- and cation-exchange. Bonded polymeric material is free of silica interactions, and is water wettable and stable from pH 1–14, with higher loading capacity compared with silica sorbents. |
| CDS Analytical | Empore | A variety of configurations, including 47 and 90 mm disks with 12 µm particles, 1, 3, and 6 mL cartridges, and 96-well plates. A variety of processing accessories, including manifolds, centrifugation, and liquid handling, are available. |
| Thermo Scientific – Dionex | AutoTrace 280 | Single instrument automates all steps of EPA method 537.1 for determination of polyfluoroalkyl substances in drinking water. Can be configured to accommodate cartridges with 1, 3, or 6 mL plungers or 47 mm disks. |
| Biotage | Atlantic ReadyDisk | Preassembled, disposable disk holder, with 47 mm extraction disks with silica or DVB base material, and trifunctional C18 or DVB for analysis of pesticides, herbicides, bisphenol A, carbonyls, dioxins, furans, phenols, polyfluoroalkyl substances, and other pollutants from large volume water samples. |
| | VacMaster Disk | Hardware modularity is the key feature, with a simple three-way valve to separate aqueous, chlorinated, and organic solvent waste and collection into 125 mL Erlenmeyer flasks or 40 mL VOA vials. Either 47 mm or 90 mm SPE disks may be used. |
| | Extrahera GLP Software | Software with LAN or LIMS connectivity, for sample tracking in combination with the Extrahera automated extraction system. Multiple layers of system control, management, and security are featured. |
| Porvair | Microlute PLR | Even pore distribution in a solid network allows consistent flow of biofluids through microelution plate. |
| Optimize Technologies | Opti-Lynx Micro<br>Opti-Trap<br>EXP 2 Nano<br>EXP 2 Stem | Online sample purification and concentration of LC–MS with load rates from 0.005 to 20 mL/min. C4, C8, C18, hydrophobic interaction, phenyl-hexyl, cyano, amide, ion exchange, and bioanalysis specific phases available. |
| Agilent Technologies | Cannabis Pesticides and Mycotoxins Kit | Encapped C18 cartridges (6 mL) and all accessories for processing up to 400 samples are provided in one kit. |
| Fluid Management Systems | EZprep123 | Automated, high-throughput extraction of dioxins, polychlorinated biphenyls, and polybrominated diphenyl ethers from toluene, hexane, or dichloromethane extracts, with simultaneous fat removal |

fibre and SPME Arrow formats. A broad selectivity range allows analysis via headspace sampling or direct immersion.

Agilent also developed a family of SPME fibres to complement its SPME Arrow line. Applications include the determination of a variety of environmental pollutants from water samples using a wide selection of stationary phase coatings.

Advion worked with Oak Ridge National Laboratory to create the Touch Express Open Port Sampling Interface, which uses a low volume, open port of continuously swept solvent that flows directly into its Expression compact mass spectrometer. Direct assays, screening, and large molecule determinations are possible.

**QuEChERS**

QuEChERS, the quick, easy, cheap, effective, rugged, and safe extraction

| **TABLE 2:** Automated, multi-platform products | | |
|---|---|---|
| **Vendor Name** | **Product Name** | **Description** |
| Markes International | Centri | Extension of the Centri platform features intelligent diagnostics for valve operation and leak detection, sampling accessories for headspace, SPME, and related techniques, automated liquid handling for addition of derivatizing reagents or internal standards, and automated gas chromatography injection. |
| CTC Analytics | PAL3 | Extensions to the GC autosampler-like sampling platform include microprocessor monitoring of total number of strokes, temperatures, date of installation, and preventive maintenance warnings for syringe, SPME fiber, and SPME Arrow configurations, resulting in increased productivity and process safety. PDMS, acetate, DVB, and carbon SPME fiber coatings and a range of film thicknesses are available. |
| Sirius Automation | MicroTasker | High-speed (6 s from sample to weighing) automation platform handles mini-tubes, standard test tubes up to 40 mL, and up to eight microplate racks. Syringe volumes from 50 µL to 5.0 mL can be used. Weighing uses a four-place Mettler-Toledo analytical balance. |

technique originally created for the screening of pesticide residues in produce, continues to spawn new product introductions for food safety and other applications. Orochem developed a family of QuEChERS products designed for AOAC method 2007.01 products or specialty areas like fruits, pigmented proteins, hemp, and stevia.

Shimadzu also created a QuEChERS kit designed for microscale analysis of biological or forensic samples. The Micro Volume kits use only 100 mg of extraction salts preloaded into 2.0 mL tubes.

**Supported Liquid Extraction**

Supported liquid extraction combines features of liquid–liquid extraction with the surface area advantages of SPE. Agilent has been a major player in this field, and this year introduced Chem Elut S with a synthetic sorbent to replace diatomaceous earth supports. This more-consistent support material is available in 96-well plate, cartridge, and bulk formats. Applications include removal of salts and phospholipids from sample matrices.

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One of Leman Instruments' newest innovations is its combined technology gas generator, the GC STATION 60LC. The single unit incorporates an inboard compressor and is a concept unlike any other currently offered in the marketplace. This advancement is unique to our brand and has been appreciated by our customers for being incredibly practical. Combining high purity hydrogen, zero air and nitrogen generators, this product acts as a fully automated solution.

"Further developments include new communication protocols which we have established for the benefit of our customers, who will want to be provided with all of the necessary information. All of our products are part of this system; the technology and protocols can be easily integrated with systems all over the globe."

Another notable product from Leman Instruments is the range of HYDRO 50L 50 ml/min to 15 l/min High Purity Hydrogen Generators for FID and CARRIER GAS to fit almost any type of application which needs High purity H2 production close to the consumer in laboratory environment.

Based on the field proven Solid Polymer Electrolyte (PEM) cell technology, pure Hydrogen is produced at low pressure from electricity and high quality distilled water (ZEROWATER). This process can be started on demand and does not require any caustic solution. The produced Hydrogen is available 24/7 with constant minimum purity superior to 99.995 % and maximum at 99.99995 % at output flows of 50 ml/min to 15 l/min (depending on the model.) The H2 output pressure is regulated electronically and can be set from 0.5 to 10 bar. (7 to 140 psig).

Each instrument is equipped with high performance communication interfaces (RS485, Ethernet, WLAN) to create a very flexible gas network with local or central control. Due to the software being focused on safety, automatic regulation and reliable communications, the Leman's products are easy to install, reliable, safe and pleasant to operate.

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**TABLE 3:** Sample preparation accessories and related products

| Vendor Name | Product Name | Description |
|---|---|---|
| Phenomenex | BioZen Streptavidin Coated MagBeads | Iron-coated 1 µm beads blocked with tosyl-activated hydrophilic copolymer, and coated with streptavidin. Product has biotin binding capacity of >200 pmol biotin/mg, and is available in 25, 50, and 500 mg formats. |
| MicroSolve Technology | Vial Centrifuge | Centrifugation at 1000 to 16,800 xg for up to eight samples per batch. Timed for 1 to 99 min. Cold room safe and air flow design keeps samples cool. Footprint is 9 x 12 x 7.75 inches. |
| Gerstel | PyroVial | Fully automated pyrolysis at up to 800 °C when coupled with the Gerstel MultiPurpose Sampler. Headspace vapors can be sampled for GC–MS determination and less volatile pyrolysis products can be analyzed by GC–MC or LC–MS. |
| Restek | Triple-Phase SPME | DVB–wide-range carbon–PDMS SPME coating for standard fused-silica fibers or SPME Arrow. Available in a number of dimensions. |
| Agilent Technologies | SPME Fibers | Fiber-based SPME with PDMS, polyacrylate, DVB/PDMS, C-WR/PDMS, and DVB/C-WR/PDMS coatings. |
| | Chem Elut S | Supported liquid extraction with synthetic sorbent to replace traditionally used diatomaceous earth. Available in 200 µL, 400 µL, 1 mL, 3 mL, 5 mL, 10 mL, and 20 mL tubes, 96-well plates, and bulk formats. |
| | Captiva Filter Vials | Polypropylene filter vials of 1 mL volume with nylon, PES, PTFE, and RC filter in 0.2 µM or 0.45 µM pore sizes. |
| Advion | Touch Express Open Port Sampling Interface | Combines SPME with a solvent-swept port coupled to electrospray ionization MS with a resulting broad spectrum of application areas. |
| Orochem | OroQuest QuEChERS Kit | QuEChERS consumables are packed directly into 2, 15, or 50 mL centrifuge tubes for pesticide residue and food ingredient analysis. Primary-secondary amine, graphitized carbon, C18, and other sorbents are utilized for AOAC compatible analysis. |
| Shimadzu | Micro Volume QuEChERS Kit | Small volume kits for rapid analysis of biological samples. Extraction salts (100 mg) are preloaded into 2.0 mL vials. |
| MilliporeSigma | Millicup FLEX | Disposable propylene vacuum filtration unit with 250 mL sample capacity. Uses 47 mm membrane filters. |
| Polymer Char | External Filtration System | Designed to clarify polymer samples for gel permeation chromatography. Accommodates six 10 or 20mL vials in the dissolution oven prior to the automated filtration. |
| Pall | Acrodisk Syringe Filters | Available in 13, 25, and 32 mm diameter water-wettable PTFE filters with an optional asymmetric glass-fiber filter for higher particulate samples. |
| Cornerstone Scientific | Antarisz Filter Media | Binderless glass microfiber disks, 2.1 to 30 cm, with a unique multidepth surface for high throughput. |
| Ecodyst | Ecochyll High-Speed Evaporators | Fast solvent evaporation and recovery under mild conditions. Units can handle solvent volumes of 5 to 200 L. |
| DWK Life Sciences | Kimble volumetric glassware | Borosilicate glass volumetric flasks featuring heavy-duty, wide-mouth openings, conformed to ASTM E438 standards for ease of liquid transfer, including calibration and regulatory compliance. |
| Fritsch Milling and Sizing | Pulverisette 14 Premium Line | Impact and cutting milling up to 22,000 rpm, with high airflow cooling allows particle fineness below 40 µm with sample throughput on the order of 15 L per h. Accessories support applications in biology, chemistry, agriculture and forestry, foods, plastics, and pharmaceuticals. |
| SPEX Sample Prep | Genolyte | Homogenization of up to three 2 mL vials, with temperatures of 0 to 10 °C maintained. Three speeds (2000, 2500, and 3000 rpm) and optional lysis vials are available, with a variety of grinding media. |
| Organomation | Nitro-Gen nitrogen generator | Hollow-fiber membrane based generation of 95–99% pure nitrogen from compressed air for blanketing, gas blowdown, LC–MS, and other uses. The generator runs off of compressed air; no electricity is required. |

**Filtration**

A number of filtration products were introduced in the past year. Agilent produced the Captiva Filter Vials with nylon, PES, PTFE, and RC filters in 0.2 µm or 0.45 µm pore sizes. The product will allow easier and faster filtration of LC or GC samples than using syringes, syringe filters, or standard vials.

MilliporeSigma created a disposable polypropylene vacuum filtration unit for sample processing.

Polymer Char's External Filtration System is an automated device for eliminating carbon black, catalyst residue, and other small particles in polymer samples. The apparatus extends the lifetime of in-line filters and

gel permeation columns and reduces noise in light scattering detectors.

Pall introduced water-wettable PTFE membranes in its Acrodisk syringe filter line designed to protect LC columns from particulate build-up.

Cornerstone Scientific presented the Antarisz filter media, glass microfibre disks for botanical extracts.

**Solvent Recovery**

Ecodyst marketed a family of Ecochyll high-speed solvent evaporators to aid solvent recovery in extraction laboratories. Models range from 5 to 200 L, and are energy efficient. Volatile or gaseous solvents, like butane or carbon dioxide, can be accommodated along with traditional organic solvents.

**Volumetric Glassware**

We are all familiar with volumetric glassware, but Kimble advanced the state-of-the-art with the first ever heavy-duty, wide-mouth flasks that reduce glass-to-glass contact during dilution workflows. ISO-certified manufacturing and flasks covering the standard range of volumetric flasks are available, with tight tolerances for accuracy and precision.

**Grinding and Homogenization**

Particle size reduction is often needed in the preparation of solid and semi-solid samples to shorten diffusion pathways for extraction, and to increase sample homogeneity. Recent new products in this area include the Pulverisette 14 line from Fritsch. This new sample grinder combines impact and cutting mill processes, with uniform production of particles down to 40 µm. The inclusion of pre-grinding and fine grinding into a single unit allows comminution of soft to medium hard, brittle, fibrous, and temperature-sensitive materials.

SPEX introduced the Genolyte multipurpose homogenizer to accommodate plant and animal tissues, seeds, soils, minerals, and other samples in 2–12 mL vials. Sample temperatures between 0 and 10 °C are maintained.

**Gas Generators**

Organomation introduced the Nitro-Gen nitrogen generator. This instrument uses a hollow-fibre membrane to convert compressed air into 95–99% pure nitrogen gas. The generator couples with any of the company's gas blowdown evaporators.

## Conclusion and Future Directions

No clear trends in new product introductions were readily observed, which is unsurprising, given the diversity of the field. On that basis, one can only expect the trend in coming years to be some combination of tool building and problem solving for bioanalysis, food, environmental, and related areas.

## References

1) D.E. Raynie, *LCGC Europe* **29**(3), 142–152 (2016).

**Douglas E. Raynie** "Sample Prep Perspectives" editor Douglas E. Raynie is a Department Head and Associate Professor at South Dakota State University, USA. His research interests include green chemistry, alternative solvents, sample preparation, high-resolution chromatography, and bioprocessing in supercritical fluids. He earned his PhD in 1990 at Brigham Young University under the direction of Milton L. Lee. Raynie is a member of LCGC's editorial advisory board. Direct correspondence about this column via e-mail to LCGCedit@mmhgroup.com

# New Gas Chromatography Products for 2019–2020

**Nicholas H. Snow,** Department of Chemistry and Biochemistry, Seton Hall University, New Jersey, USA

**"GC Connections" presents the column's annual review of new developments in the field of gas chromatography seen at Pittcon and other venues in the past 12 months.**

I am pleased to present our annual review of new products in gas chromatography (GC), introduced between spring 2019 and spring 2020. Many of these products were showcased at the 71st meeting of the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) at McCormick place in Chicago, Illinois, from 29 February to 5 March, 2020. As has become tradition, the exposition lasted three days. Overall attendance was down significantly, due to coronavirus COVID-19 concerns. The exposition included 542 exhibitors and there were over 9000 attendees. Several international exhibitors cancelled due to travel concerns. Four major players in the GC market, Agilent Technologies, Perkin Elmer, Thermo-Fisher Scientific, and Waters did not exhibit.

Pittcon 2021 will be held from 6 to 10 March, 2021 in New Orleans, Louisiana, USA. I strongly encourage all readers to consider attending a GC-related conference or participate in your local or regional chromatography discussion group in the next year. As you know, 2020 is a rough year for conference organizers and for all of the vendors, contractors, and economies that conferences support. They need your help. Just as importantly, conference and meeting attendance is still the most effective and efficient way to train and educate yourself about GC, and improve your skills and performance.

Again this year, the exposition included vendor booths displaying new instrumentation and products, along with educational exhibits including Pittcon Park, the Laboratory Gauntlet, a planetarium, demonstration spaces, and a TED-style presentation theatre on the exposition floor. Pittcon still has excellent content to offer to students and scientists at all levels of expertise and in all roles. In the demonstration spaces, many vendors performed working demonstrations of their products.

The technical programme at Pittcon 2020, which ran over five days from Sunday to Thursday, included a very strong GC presence. Pittcon continues to be a leading showcase for the latest GC and comprehensive two-dimensional gas chromatography (GC×GC) related research. Three major awards were presented to gas chromatographers this year. The Chromatography Forum of the Delaware Valley presented the Dal Nogare Award to Ron Majors, retired from Agilent Technologies and the former "Sample Prep Perspectives" and "Column Watch" editor for *LCGC*. Throughout his career, Majors made numerous GC-related contributions. The *LCGC* Lifetime Achievement Award went to Daniel W. Armstrong of the University of Texas at Arlington, USA. Among Armstrong's many accomplishments is the development of ionic liquid stationary phases for capillary GC. Finally, the American Chemical Society Division of Analytical Chemistry Satinder Ahuja New Investigator Award in Separation Science was presented to Katelynn Perrault from the University of Honolulu, Hawaii. Perrault is an up-and-coming leader in GC×GC, and a renowned expert in the application of GC×GC in forensic science.

In addition to the award sessions, there were several symposia, oral sessions, poster sessions, and networking events with significant GC presence, enough to keep attendees very busy on all days.

**TABLE 1:** New instruments and systems in 2019–2020

| Company | Product | Description |
|---|---|---|
| Agilent Technologies | Intuvo 990 Micro-GC | Agilent 990 Micro GC systems provide features designed to deliver the quality and speed that are critical to gas analysis. Up to four channels of separation and detection, micro-machined TCD detection, and the use of only 10% of power and carrier gas compared to conventional laboratory gas chromatography (GC) systems. This system is used for analysis of gas samples only, includes up to 4 channels of separation and detection, micro-machined TCD detection, a small footprint in dimensions and environmental. He, $H_2$, $N_2$, and Ar carrier gas options are included; a wide range of micro-GC column phases are available and also a wide range of accessories, sample handling, reporting and result exporting tools. |
| Chromatotec | airmoScan Xpert | Due to the large number of volatile organic compounds (VOCs), there is a need to combine their separation and quantification by GC with flame ionization detection (FID) and their individual identification by mass spectrometry (MS). The airmoScan Xpert is a combination of two instruments: a dual GC-FID system for the monitoring of $C_2$ to $C_{16}$ compounds and a process quadrupole MS instrument. The instrument allows the monitoring of at least 123 VOCs at ppt, ppb, ppm, and % levels in only 30 min. Thanks to this dual detector combination, users can avoid the risk of saturated signal on the FID. Thanks to its advantages (easy to use, fully automatic, intrinsically linear, precise and very stable system with data validation) this solution has been implemented in fixed and mobile laboratories for urban and industrial field analysis all over the world. |
| Gow Mac | Series 5900 GC | The Series 5900 Isothermal gas chromatograph features a patented discharge ionization detector (DID) that detects trace gas impurities in the part per billion (ppb) range. This second-generation DID gas chromatograph features a patented detector, LCD touchscreen control, custom valve configurations, and a corrosion resistant option. Manufacturers, distributors, and users of specialty, electronic, and industrial bulk gases can rely on the Series 5900 for superior detection limits, reproducibility, and accuracy. Each Series 5900 GC is custom built to a particular application. |
| Spectra Analysis | DiscovIR-GC | The new Spectra Analysis DiscovIR System is a solution for identifying the components in a complex mixture. Infrared spectroscopy provides each compound's unique fingerprint, making it quick and easy to identify each peak by comparison to the extensive solid-phase IR libraries. The DiscovIR system is particularly useful in identifying structural isomers, which can be a challenging area for mass spectrometry. DiscovIR's unique temperature-controlled, vacuum-deposition method ensures that results are accurate and reproducible. Eluted peaks are first deposited in a film onto a rotating IR-transparent disc. The disk rotates the concentrated spot into the infrared beam and the spectrum is collected. |
| Thermo Fisher Scientific | Dioxin Analyzer | The Thermo Scientific Dioxin Analyzer provides food safety laboratories with an advanced analytical workflow for the accurate detection of dioxins and other persistent organic pollutants (POPs) in food and feed. The new workflow brings together the TSQ 9000 triple quadrupole GC–MS/MS system with the advanced electron ionization (AEI) source, capillary GC column, system suitability check, standard solutions, software, and comprehensive user guidelines to create a simple-to-use and cost-effective solution, specifically designed for the effective routine screening of dioxins and POPs. The new AEI source enables the sensitive detection of trace levels of dioxins and POPs in complex sample matrices, providing reliable analysis even with lower sample weights, reducing the cost of sample preparation and system maintenance. The workflow includes a comprehensive suite of software tools and pre-loaded acquisition, calculation, and reporting templates to help laboratories meet European Union requirements for the confirmatory analysis of food and feedstuff. The Dioxin Analyzer facilitates the analysis of a large variety of samples, delivering laboratories with a flexible system, and by providing setup guidance documentation and a quality check standard, it enables out-of-box implementation and reduced training needs. |
| Wasson-ECE and VUV Analytics | Monomer Analyzer and Eclipse E-VUV | The catalyst required to produce ethylene, propylene, and butadiene monomers is poisoned by common byproducts such as $H_2S$, COS, mercaptans, oxygenates, arsine, and phosphine. These impurities also affect the final quality of the product and, consequently, the price that can be demanded for it. Wasson-ECE Instrumentation has designed a process GC system capable of detecting ppm to ppb levels of these analytes. To resolve and quantify such diverse analytes, Eclipse is configured with seven capillary columns housed in two temperature-programmable ovens and one isothermal oven. Two chromatography methods direct eluates to three multiplexed detectors (FID, PDHID, and MSD) for unambiguous compound identification and quantification in 30 min. |

Continued on Page 250

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**TABLE 1:** New instruments and systems in 2019–2020 (continued)

| Company | Product | Description |
|---|---|---|
| Wasson-ECE and VUV Analytics | Monomer Analyzer and Eclipse E-VUV | Eclipse PGCs are sensitive and capable online analyzers. The cumulative effort between Wasson-ECE and VUV Analytics has resulted in the Eclipse E-VUV, the first ever process gas chromatograph (PGC) capable of delivering laboratory-quality chromatography with vacuum ultraviolet detection technology. Eclipse PGC were specifically designed to perform laboratory-quality capillary chromatography in hazardous or general-purpose process environments. The Eclipse E-VUV is also equipped with the VGA-100, the world's first benchtop detector capable of vacuum ultraviolet (VUV) detection, from VUV Analytics. All gas-phase molecules absorb strongly in the vacuum ultraviolet region (125–240 nm). This strong absorption provides compound-specific spectra that delivers both quantitative and qualitative data. |
| Young In Chromass | ChroZen GC | The ChroZen GC system is equipped with state-of-the-art technology in both hardware and software that enhances user convenience and data reliability. The ChroZen GC features substantially enhanced sensitivity and reproducibility with new powerful UPC (ultimate pneumatic control) and an intuitive LCD display, enabling more reliable and accurate data. |
| Young In Chromass | YL 6900 GC–MS | The YL6900 GC–MS system is a new powerful mass spectrometer developed for gas chromatography and mass spectrometry technologists backed by 22 years of experience in this field. This innovative GC–MS instrument supports a huge number of spectra with the highest scanning speed (20 000 amu/sec) across every GC peak, provides very wide mass range (1–1200 u) and the lowest instrument detection limit (< 10 fg of OFN), and the highest sensitivity. This GC–MS instrument offers operational simplicity, powerful tuning protocols, and the highest productivity with automation. |

**TABLE 2:** New GC columns introduced in 2019–2020

| Company | Product | Description |
|---|---|---|
| Abel Industries | AbelBonded Columns | AbelBonded (AB) GC columns are among the products of Abel Industries Canada Ltd. They offer complete stationary phase selections, including polysiloxane, polyethylene glycol (PEG), and porous layer open tubular (PLOT); many dimensions (variations in length, internal diameter, and film thickness), low bleed, high inertness, high efficiency, consistent retention times, repeatability, and competitive price. |
| Millipore Sigma (Supelco) | Columns for the Intuvo GC system | These are first GC column alternatives for use with the Agilent Intuvo GC system. From workhorse SLB-5ms and unique selectivity ionic liquid columns to specialty columns like the Chiraldex column, customers now have a full portfolio of alternatives to choose from when using the Agilent Intuvo GC system. Intuvo-compatible GC columns install in < 1 min with click-and-run connections, require no maintenance or trimming, use leak-free connections, use high speed or high throughput GC with fast heating and cool downtime, and eliminate unplanned downtime, time-intensive tasks, and user technical limitations. The full line of GC columns is made specifically for use with the Agilent Intuvo GC system. These columns allow users to complete analyses faster, easier, and with more accuracy, sensitivity and reproducibility. |
| Phenomenex | ZB-PAH-EU and ZB-PAH SeleCT | Designed for PAH testing, the Zebron ZB-PAH-EU and ZB-PAH-SeleCT offer excellent performance through the power of targeted selectivity when analyzing polycyclic aromatic hydrocarbons (PAHs) in the food, environmental, electronics, and fuel industries. The ZB-PAH-EU column offers up to 70% faster PAH analysis, elevated temperature stability (340/360 °C), excellent resolution, and accurate quantitation of European regulated EU 15+1 and EPA 610 PAHs. Zebron outperforms popular GC columns for the separation of EU 15+1 and EPA 610 PAHs and separates the 18 PAH isomers within 12 min, resolving all critical pairs while demonstrating consistent column inertness. The ZB-PAH-SeleCT column offers enhanced resolution for chrysene and triphenylene (PAH Interferences) and increased Benzo[b,k]fluoranthene separation. The Zebron ZB-PAH-SeleCT column is designed to achieve complete resolution of chrysene from triphenylene along with other EU 15+1 PAH compounds. Its unique selectivity helps eliminate false positives while resolving PAH isomers, providing easy, fast, and accurate quantification of PAHs in environmental and food samples. |

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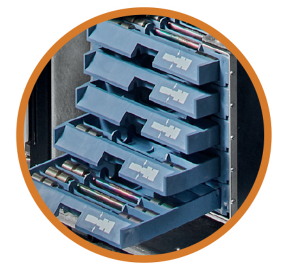

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**TABLE 3:** New GC Accessories and Consumables for 2019–2020

| Company | Product | Description |
|---|---|---|
| CTC Analytics | PAL 3 Series II Autosampler | Fast, safe, and reliable sample preparation is a key factor for high productivity and reduced costs per sample. The PAL3 Series II with smart technology in combination with smart consumables provides the required process safety and effectivity. All PAL certified smart consumables are equipped with a unique smart chip containing parameters, ranges, and usage history enabling full traceability of sample preparation down to the syringe and perfectly matched with the PAL3 Series II. |
| Cerno Bioscience | GC/ID Version 1.1 | GC/ID is fully automated data processing software that enables dramatic improvements for GC–MS qualitative analysis. By incorporating Cerno's TrueCal calibration technology for accurate mass formula ID of the molecular and fragment ions, retention index (RI) confirmation utilizing NIST RI values, along with conventional NIST library search with mixture and background deconvolution of co-eluting peaks, a significant improvement in compound ID certainty is achieved on single quadrupole GC–MS systems. |
| Data Apex | Data Analysis Software | Clarity is an advanced chromatography data system (CDS) with optional software modules for data acquisition, processing, and instrument control. Its wide range of data acquisition interfaces (A/D converters, LAN, USB, RS232) allows connection to any GC or LC system. Up to four independent chromatography systems can be simultaneously connected, and each can acquire up to 32 signals. Languages include English, French, German, Russian, Spanish, and Chinese localization. Control modules provide integrated control of selected instruments such as GC instruments, pumps, detectors, autosamplers.<br><br>Extensions provide functions for specific separation techniques such as PDA, GPC analysis, mass spectrometry, or SST. Clarity2Go in an iPhone or Android-powered mobile devices allows users to monitor running analyses from multiple installations of Clarity. Clarity comes with extensive free support from DataApex as well as from the growing community of users in the Clarity Forum. Unlike many large corporations, the DataApex Company is in close contact with its users. |
| DWK Life Sciences (Wheaton brand) | Wheaton BlueMag caps | The 9 mm and 18 mm magnetic screw caps replace any screw thread cap designed for magnetic vial handling for standard 9 mm GC screw top vials and 18 mm headspace vials. A new design solves poor magnetic capture and/or alignment that can result in automation interruption of off-centered vials or dropped vials. Bimaterial design offers precision tactile feedback to help ensure user complete closure with less than one full turn. Used with X-Y-Z autosamplers with magnetics (HPLC, LC, GC, GC–MS) and all headspace applications. Quantities available in packs of 10, 50, and 100 caps per bag. |
| HTA | GC Autosamplers | HTA offers different models of autosamplers for liquid or headspace techniques and all-in-ones (liquid, headspace, and SPME capabilities on the same instrument). Top mounted on instruments, they save valuable bench space. HTA autosamplers fit every GC and GC–MS system, every brand, from oldest to newest models, by a dedicated interface. They ensure maximum compatibility and top performance. |
| Gerstel | Advanced Olfactory Detection Port ODP 4 | The ODP sniff port allows the sensing of compounds by the human nose as they are eluted from the gas chromatograph. The effluent is split as it leaves the column so that it arrives simultaneously at the nose and at the detector. Additional information is gained on compounds that are responsible for specific odors. Parallel operation with most standard detectors, including MSD, is provided and a humidifier for convenient usage is included. Voice recording and voice recognition capability allow spoken comments to be transferred and added automatically to the chromatogram as peak annotations. The ODP 4 provides a heated mixing chamber in which humidified make-up gas and column effluent are mixed without risk of condensation. |
| Gerstel | MPS robotic autosampler | As a new member of the MPS family, the MPS robotic is a highly efficient GC–MS autosampler with extended robotic functionality. The MPS robotic provides reliable processing of complex tasks. Syringe holders and syringes are integrated in special syringe modules, which can be exchanged automatically within a running sequence when using the MPS robotic pro for maximum flexibility. The MPS robotic is controlled by the Gerstel Maestro software in a simple and efficient manner. Maestro provides full flexibility by allowing an ongoing analysis sequence to be modified to incorporate priority samples. The built-in, context-sensitive help function enables simple sequence and method development and setup. |

Continued on Page 253

Continued from Page 252

**TABLE 3:** New GC Accessories and Consumables for 2019–2020 (continued)

| Company | Product | Description |
|---|---|---|
| Phenomenex | Zebron Gas Management filters | Higher capacity filters extend a GC column's lifetime. The high adsorption capacity of the Zebron Gas Management filters removes contaminants from the mobile phase gases, which reduces potential column bleed, increases the column lifetime, and protects sensitive GC detectors from damage. With the new dual wall Zebron Gas Management filters, users get more protection, better safety, and less contamination. These plug-in GC and LC gas filters are easy to use, incorporate a dual filter housing to prevent diffusion and increase safety, have very high capacity, ensure high gas purity (99.9999% pure), and are available in universal, moisture, oxygen, and hydrocarbon filters. Zebron Gas Management trap cartridge systems make changing gas traps quick and easy. Spring-loaded check valves close when the filter is removed and open only when a new filter has been locked in place. With the Zebron gas traps there is no need for loosening or tightening fittings every time a trap is changed and one can eliminate contamination during the process. |
| Phenomenex | Zebron Inlet liners | Optimized for trace and active compound analysis, Zebron Plus GC liners undergo a unique chemical deactivation treatment resulting in a remarkably inert GC inlet liner. Zebron Essential liners undergo a vapour deposited deactivation process resulting in a thin film liner that is ideal for routine analysis. Though the upfront cost of self-packing your liner may seem attractive, the time and headaches caused by resulting tailing or irreproducible peaks can be sizeable. Self-packed wool fibres commonly break during installation and any existing deactivation on the liner can also be scratched or damaged. Prepacked Zebron Plus liners undergo the deactivation process with the quartz wool already in place, which ensures that any active sites that form during packing are not exposed. Easy to install, touchless packaging allows users to protect themselves from cuts or breakage and eliminates contamination that may occur during the installation process. |
| Shimadzu | Carrier Gas Selector | Helium has been used as the standard carrier gas for GC for many years, but due to the recent rise in prices, alternatives such as nitrogen and hydrogen, which are inexpensive and readily available, have become the new standard. With the new Carrier Gas Selector option, a laboratory can not only reduce helium gas during standby and overnight operation by 70% or more, but also help to automate the switch to an alternative gas for method development or analysis with an alternate carrier gas. Because this gas switching is electronically controlled, it prevents gas switching work errors and achieves complete data integrity by recording parameters, including the type of gas used. The addition of Analytical Intelligence functions to automate switching of the gas makes this once challenging change simple enough for operators at any level. In addition, leaving the diagnosis of the equipment status to the software ensures greater system up-time and faster ROI. |
| Shimadzu | Packed Column Functionality | The Nexis GC-2030 can now be equipped with both a capillary column and a packed column at the same time, making it possible to construct systems for various applications. For example, combining it with the HS-20 headspace sampler makes it possible to realize a system in which headspace analysis is performed using a capillary column while also performing analysis using a sample introduced via a gas sample valve using a packed column. Switching between headspace analysis and different sample introduction techniques for continuous analysis despite the type of column required enables more efficient analysis by unattended operation. |
| Shimadzu | ClickTek Tool Free Maintenance | When using GC, the most common tasks are maintenance of the inlet and replacement of the column. The Nexis GC-2030 using ClickTek takes the confusion of out installing liners and columns for operators of all skillsets, helping to eliminate the risk of installation errors and leading to more uptime. ClickTek was recently improved to standardize the fixed length of the ferrule on the column, which in the past needed to be changed based on the detector being used. Now, using one single length for all detectors, the confusion of what should be set is removed, making this the simplest Shimadzu system to maintain. |

**TABLE 4:** New training and education opportunities for 2019–2020

| Company | Product | Description |
|---|---|---|
| Axion Training Institute | HPLC/GC Bootcamps | Axion Training Labs has trained over 12 000 scientists in HPLC and GC. Axion Analytical Labs, Inc. has provided scientific services to every major pharmaceutical, chemical, and petroleum company in the United States since 1997. They have also provided similar services to most of the larger government labs (FDA, FBI, DEA, USDA, CDC, DOE, DOD, Homeland Security), along with many other Fortune 500 companies. |
| *LCGC* Magazine's ChromAcademy | New Platform | ChromAcademy as you've never seen it before. Learn on any device, with greatly improved functionality. With a vast scientific knowledge and a decade in providing e-learning, they know how to make learning easier. The new ChromAcademy features customizable learning pathways and advanced reporting tools, ensuring that employees only receive the training that they need. With an advanced user-experience design and infrastructure, enjoy seamless learning that's intuitive and fresh. Find the content that's relevant and learn when it suits, with improved responsiveness, navigation, search, and bookmarking. |
| LECO | GC×GC Symposium and Workshop | The 1st North American GC×GC Symposium & Workshop is a free three-day seminar sponsored by LECO Corporation from 15 to 17 September, 2020 at the LECO Corporation Customer Experience Center in Saint Joseph, Michigan, USA. Users who have a GC–MS analytical challenge will want to see what they have been missing! Attendees will learn from key opinion leaders in the field of GC and GC×GC technology as to how these solutions can seamlessly fit into their workflow and benefit their laboratory. This complimentary symposium and workshop will include two days of presentations featuring a diverse range of applications by global experts in GC×GC technology (LECO's Pegasus brand of products, including the classic Pegasus 4D-C, BT 4D, and GC-HRT+ 4D). The third day will feature hands-on laboratory sessions using GC×GC solutions and allow for one-on-one conversations with LECO's in-house experts. The speaker lineup includes Robert Synovec from the University of Washington, Christopher Reddy from Woods Hole Oceanographic Institute, and many more. A social program will also be included. |
| JEOL USA, Inc | msFineAnalysis version 2 | GC–MS reporting software combines data from electron ionization (EI) and soft ionization to include database search, exact mass, and isotope information for molecular ions and fragments into an integrated report. Colour coding indicates confidence levels for compound assignments. Analysis can be carried out on EI data alone, or EI data together with field ionization, photoionization, or chemical ionization. |
| John Wiley and Sons | Book – *Basic Gas Chromatography*, 3rd. Edition | *Basic Gas Chromatography*, Third Edition, provides a brief introduction to GC following the objectives for titles in this series. It should appeal to readers with varying levels of education and emphasizes a practical, applied approach to the subject. This book provides a quick need-to-know introduction to gas chromatography—still the most widely used instrumental analysis technique—and is intended to assist new users in gaining understanding quickly, and as a quick reference for experienced users. The new edition provides updated chapters that reflect changes in technology and methodology, especially sample preparation, detectors, and multidimensional chromatography. The book also covers new detectors recently introduced and sample preparation methods that have become much more easily accessible since the previous edition. |

Overall, sessions related to cannabis analysis, biofuels, food and beverages, forensics, and general applications of both GC and GC×GC were offered.

The information presented in this article is based on vendors' responses to questionnaires and additional information from press releases, websites, and product literature, not on actual use or experience of the author. Every effort has been made to collect accurate information, but because of the preliminary nature of some of the material, *LCGC Europe* cannot be responsible for errors or omissions. This column cannot be considered a complete record of all new GC products introduced in the past year, because not all vendors chose to respond to the questionnaire, and not all had a strong presence at Pittcon, nor is all of the submitted information necessarily included here, because of the limited available space and the editors' judgment as to its suitability. The index in this article provides a listing of the vendors whose new GC products are covered in this review. For a more complete picture of the GC space today, I encourage you to review the two previous years' reviews by Hinshaw (1,2).

In new instruments, trends seen over the past few years toward smaller, more automated systems, systems that are more specialized, and spectrometric detectors continue with the product introductions this year. Table 1 shows a list of new instruments introduced over the past year.

Capillary columns continue to be updated by most of the column vendors. The three vendors highlighted in Table 2 provide examples of three important trends in column development: lower prices, specialized columns for specific systems, and new column chemistries geared towards specific problems.

Developments in new accessories and consumables trend toward simplifying analysis, and include a range of devices from sample vials and sampling devices through data system software. For more information on sample preparation, see the specific sample preparation article by Raynie in this issue of *LCGC Europe* (3). As with the GC reviews, it is

| INDEX: Companies introducing new GC products in 2019–2020 |
|---|
| **Company** |
| Abel Industries |
| Agilent Technologies |
| Axion Training Institute |
| Cerno Bioscience |
| Chromatotec |
| CTC Analytics |
| Data Apex |
| DWK Life Sciences |
| JEOL USA |
| Gerstel |
| Gow Mac |
| HTA |
| LECO |
| Millipore Sigma (Supelco) |
| Phenomenex |
| Shimadzu |
| Spectra Analysis |
| Thermo Fisher Scientific |
| Wasson-ECE and VUV Analytics |
| Young In Chromass |

worthwhile to review the past two years as well for a complete picture of sample preparation products (4,5). Table 3 provides a listing of accessories and consumables launched over the past year.

Besides several short courses associated with Pittcon, additional training and educational resources are available for 2020. Opportunities for in-person short courses, seminars, and training are offered by most major instrument vendors and through many conferences and organizations, including the American Chemical Society, Eastern Analytical Symposium (Princeton, New Jersey, USA, November 2020) and local and regional chromatography discussion groups. *LCGC*'s online training partner, ChromAcademy, has launched a completely revamped online platform. A new edition of McNair and Miller's classic book "*Basic Gas Chromatography*", with Nicholas Snow added as an author, was published this year. New developments in training and education for 2019–2020 are shown in Table 4.

As GC approaches 70 years since its invention, developments and inventions are still going strong. As seen here and in 2020, GC-related developments and innovations are ongoing and include all areas: sampling and supplies, new instruments, columns, detectors, data systems, and educational opportunities. I look forward to more innovation and advancements as the decade progresses, and I hope to see many of you at EAS 2020 in my home state of New Jersey, or at Pittcon 2021 in New Orleans.

## References


1) J.V. Hinshaw, *LCGC Europe* **32**(5), 250–257 (2019).
2) J.V. Hinshaw, *LCGC Europe* **31**(5), 266–272 (2018).
3) D.E. Raynie, *LCGC Europe.* **33**(5), 241–247 (2020).
4) D.E. Raynie, *LCGC Europe.* **32**(5), 258–263 (2019).
5) D.E. Raynie, *LCGC Europe* **31**(5), 274–279 (2018).



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# How Do You Write User Requirements for Chromatographs and Chromatography Data Systems?

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**One of the biggest failures with purchasing chromatograph systems and chromatography data system (CDS) software is either the total lack of or poorly written user requirements. So, how can you write acceptable requirements? Is specifying a chromatograph the same as software?**

When it comes to the purchase of chromatographs or chromatography data system (CDS) software, the worst possible task for a user is to specify what they want it to do. Users either "can't be bothered" or "know what they want". With chromatographers like this, the world will always need consultants, if not to help them do the job properly in the first place then to dig them out of the hole that they dug themselves. In this instalment of "Questions of Quality" the writing of a user requirements specification (URS) for both a liquid chromatograph system and CDS software is discussed.

## Why Did You Buy This Rubbish?

Be honest, have you ever bought a chromatograph system that was an absolute lemon or CDS that failed to meet your expectations? I have. This column is written for all those readers who lied when answering the question in the first sentence. Let's look at some of the miserable excuses for this sorry state of affairs:

- It's a chromatograph—it works
- I was too busy
- I was too busy so I went with my friend's suggestion
- I was never given time by my boss
- I believed the supplier's literature
- The salesman bought me a nice meal and it would be impolite not to order
- My boss said we must spend the money before the end of the month or lose it.

The root cause of this is the abject failure to plan and make the time available to specify your requirements adequately for instruments and software. With an adequate URS you can evaluate the software or chromatograph objectively. You only have one chance to get a purchase right, otherwise you'll have to live with your lemon for several years. Amazon returns are not available for chromatograph systems or CDS software.

The way out of this quagmire is to write meaningful user specifications that will enable you and your laboratory to spend money wisely and get the right instrument and CDS for the job. There is a caveat: buying only on price can be a false economy in the long run.

## Why Do We Need Specifications?

There are two main reasons for writing user specifications.

1. *Investment protection:* You want the right tool for the right job. Buying the wrong item will give you more problems over the lifetime of the instrument than spending the time to write down what you want in the first place. Buying the wrong item wastes scarce resources and makes you look an idiot with management.
2. *Compliance with regulations or quality standards*: The laboratory or organisation is required to do this to meet their legal requirements or quality commitments.

You may think that these are two entirely different areas but you are wrong. If you approach the writing of user requirements with a business-driven attitude but with a compliance or quality wrapper, you can kill the two proverbial birds with one stone. If you write down your requirements with adequate document controls and approve them, then this meets both reasons for writing specifications. Note, I mentioned the business rationale for

writing requirements first as this must be the main driver for writing a URS.

### Further Business Rationale for Writing a URS

Don't forget the real reasons for writing a URS, especially for CDS software, are the following (1):

- It serves as a reference against which commercial products are selected, evaluated in detail, and any enhancements are defined. You avoid being seduced by technology or buying a poor system using this approach.
- It identifies gaps between your requirements and the CDS applications offered by suppliers. This allows you to seek enhancement of the selected system or to review and possibly adjust your requirements to match software on the market.
- It reduces the total system effort and costs, since careful review of the document should reveal omissions, misunderstandings, or inconsistencies in your specification and this means that they can be corrected easily before you purchase an instrument or application.
- It provides the input to user acceptance test specifications and qualification of the system.
- It defines what procedures need to be written, modified, or archived for both the users and administrators of the system.

You will have noticed that I have not mentioned any regulations or quality guidelines, merely described what has happened in many laboratories when chromatograph systems and software are purchased. However, I don't wish to disappoint you, so here are the quality standard requirements and pharmaceutical regulations you may need to consider.

### ISO 17025 Quality Standard

ISO 17025: 2005, a quality standard for testing and calibration laboratories, presents in Section 5.5 the following requirements for analytical instruments:

*5.5.2 Equipment and its software used for testing, calibration, and sampling shall be capable of achieving the accuracy required and shall comply with specifications relevant to the tests and/or calibrations concerned.…*

*Before being placed into service, equipment (including that used for sampling) shall be calibrated or checked to establish that it meets the* ***laboratory's specification requirements*** *and complies with the relevant standard specifications* (2).

Note the highlighted text "laboratory's specification requirements". Not the supplier's but the laboratory's specification. This implies that there can be a difference between the supplier's specification and that required by the laboratory. The important point is that a laboratory does not have to follow the supplier's specification to the letter; the key point is what does the laboratory want an instrument to do?

The 2017 update of ISO 17025 (3) has omitted this requirement and simply asks in section 6.4.1 for *access to equipment ... that is required for the correct performance of laboratory activities ...* It is a pity that guidance for laboratory specifications has been lost from this standard.

### Good Laboratory and Manufacturing Practice Regulations

Let us move onto US Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) Regulations for Equipment and see what they say about specifications. The US GMP Regulations for Equipment are found in section 21 CFR 211.63 (4) and state:

*Equipment used … shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance.*

The corresponding regulations for GLP are in section 21 CFR 58.61 (5) and are as follows:

*Equipment used … shall be of appropriate design and adequate capacity to function according to the protocol and shall be suitability located for operation, inspection, cleaning and maintenance.*

Both US GMP and GLP require appropriate design suitable for intended use or function for the protocol, respectively. Intended use has been interpreted as documenting requirements, otherwise how can you determine what the use will be and verify that it works? However, both of these regulations were originally drafted in the 1970s and are relatively vague—is there anything more up-to-date and informative?

### General Principles of Software Validation

If you think that all this writing requirements is rubbish and a waste of time, Section 5.2.2 of this FDA Guidance states simply (6):

*It is not possible to validate software without predetermined and documented software requirements.*

Now that I have your undivided attention, please read on.

### *USP* <1058> Analytical Instrument Qualification (AIQ)

The latest version of *USP* <1058> on Analytical Instrument Qualification has the following statements (7):

*The first activity is the generation of a user requirements specification (URS), which defines the laboratory's particular needs and technical and operational requirements that are to be met.*

*The subsequent qualification activities necessary to establish fitness for purpose may be grouped into four phases: design qualification (DQ), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ).*

Therefore, writing the URS for an analytical instrument is a totally separate activity than the design qualification (DQ) phase or selecting the instrument and supplier.

DQ is an activity that confirms that the instrument(s) you propose purchasing meet the requirements in your URS. This is consistent with requirements 3.2 and 3.3 in EU GMP Annex 15 on Qualification and Validation (8). You test the user requirements in the operational qualification (OQ) phase of work. This was discussed in past instalments of "Questions of Quality" focused on the new version of *USP* <1058> (9) and in the three papers co-authored with Paul Smith (10–12).

The problem now comes with a further statement in the new *USP* <1058>:

*It is expected that DQ requirements will be minimal for commercial, off-the-shelf instruments.*

Note these words well: requirements will be minimal. Not zero, not virtual, not imaginary, not verbal, but minimal. Minimal means written down, documented, recorded, and formal (approved).

### Minimal Instrument Requirements But What About Software?

While instrument specifications may be minimal, this is certainly not the case for software. Here the functions need to be specified for instrument control, data acquisition, processing, calculation of results, and reporting. CDS applications are configurable and the configuration needs to be documented as either part of the overall specification, for example, system policies, user roles, use of electronic signatures. Alternatively, more dynamic aspects of the system, such as specifying, building, testing, and maintenance of custom calculations and reports, should be controlled by procedure. This is not an exercise in minimalism.

### Different Approaches for Chromatograph and CDS Specifications?

From the discussion above, we appear to have a dichotomy with our URS documents. On the one hand the chromatograph specification is expected to be minimal, but should be much more detailed for the CDS application software. To help you with this crucial task we'll have a look at practical approaches to specifying both components. We'll start with our exercise in minimal high performance liquid chromatography (HPLC) user requirements. For many, the first response is to quote the supplier's specification verbatim.

### Why Can't I Use a Supplier's Specification?

Yes, I know you are lazy and have analyses to perform, but this is not the way to write your specification. There are several reasons for this:

- A supplier's specification covers the entire operating range of the instrument. Do you really want an HPLC pump specification to cover the range of 0.05–10.0 mL/min, when you only use 1.0–2.0 mL/min? If you specify the whole range, you will have to test it to show it complies. Similarly, a UV detector may have an operating range from 190–800 nm. What do you use normally? Perhaps 210–280 nm, for example? Focus on what you actually need now rather than what you could use. I know there are those that suggest a wider operating range—just in case. However, resist this suggestion and use change control once the instrument is operational to adjust your approach. You know it makes sense.
- A supplier's specification will have operating parameters measured under highly-controlled environmental conditions that your laboratory cannot hope to match. Therefore *USP* <1058> wants suppliers to generate meaningful specifications (7) so that they can be reproduced in customers' laboratories.

### What Could a Minimal HPLC Specification Look Like?—Isocratic HPLC

An example of a simplified and minimal specification for an isocratic HPLC is shown in Table 1. It details a supplier's operating range for each component in the middle column and then in the right-hand column are the laboratory's requirements, which are selected from the supplier's operating range. It took me about five minutes to write this outline specification. It's not that hard to write a specification, is it?

Hold on, is there something missing from this specification? Of

**TABLE 1:** A simplified minimal laboratory specification for an isocratic HPLC system

| HPLC Component | Supplier's Specification | Laboratory Requirements |
|---|---|---|
| Isocratic pump | 0.05–10.0 mL/min | 1.0–2.0 mL/min |
| Autosampler | 100 sample capacity | 50 samples |
| Injection volume | 1–1000 µL volume | 20–100 µL |
| Oven / column compartment | 25–100 ˚C | 40–50 ˚C |
| UV detector | 190–800 nm | 210–280 nm |

course, the acceptance criteria for each parameter are missing and these are an integral part of any laboratory instrument specification. Otherwise, how can you test or qualify a component to demonstrate that it is fit for intended use?

How should these acceptance criteria be derived?

1. The overriding requirement is that any acceptance criterion must be scientifically sound as required by 21 CFR 211.160(b) (4) and ICH Q7(R1) clause 11.1 (13).
2. There are acceptance criteria for many analytical instruments in the general chapters of the pharmacopoeias.

One piece of advice I would offer is use the pharmacopoeial acceptance criteria as written and not to make them tighter. They have been specified for a reason following discussion and debate across industry.

### What Could a Minimal HPLC Specification Look Like?—Gradient HPLC

Table 1 shows the simplified specification for an isocratic HPLC. What would happen if you wanted a gradient chromatograph? How would you specify this? For example, you could have a simple binary system or would you want a quaternary gradient system? Let's assume the Gods of Finance have been kind and bestowed upon you the cash to splash on a quaternary system. How do you envision using the system? I appreciate the hotshots in R&D are itching to develop a quaternary gradient separation to show off their superior chromatography skills to the mere mortals in the quality control department, however, let's get real. To have a robust method remember the KISS principle: keep it simple, stupid.

When developing a method the principle should always be isocratic separation first, gradient separation second. If a gradient separation is required, we should use a binary system and not a tertiary or a quaternary system. How do we normally use a quaternary HPLC pump? Typically, A and B will be the solvents for a binary gradient, C will be an aqueous wash, and D will be an organic wash such as methanol or acetonitrile. In our minimal specification we need to state this. Consider what acceptance criteria would you want. Obviously, you'll need to look at the accuracy of mixing A and B solvents along with the overall performance of the mixed mobile phase flow rate accuracy. However, do you need to specify any acceptance criteria for solvents C and D? If you take a risk-based approach, probably not. All done?

Not quite, how would you mix the gradient? Low or high pressure mixing? Does it really matter? Yes, it does, especially if you are transferring a method from one laboratory to another because how the gradient is mixed could potentially impact a separation. If one laboratory has low pressure mixing and the other high, there could be problems reproducing the original gradient.

The outline specification shown in Table 1 is the start of the specification journey, but you can see that it is not a difficult task to develop a meaningful but minimal specification for a chromatograph system with acceptance criteria. Each parameter

**TABLE 2:** A suggested outline for a CDS user requirements specification (1)

| Section | Contents |
|---|---|
| Introduction | • Purpose and scope of the document<br>• Referenced documents |
| Computing platform | • Hardware specification for workstations and servers<br>• Resilience features for continued operation, for example, dual powersupply, redundant disks<br>• Virtual server or physical server?<br>• Operating system(s) defined, for example, name, version, and service pack<br>• Database specification, for example, name and version number<br>• Printing requirements |
| System requirements | • Outline system capacity defined, for example, number of users, chromatographs to be interfaced<br>• Single site or multiple sites supported? |
| Quality or regulatory compliance requirements | • Regulations to comply with<br>• Open or closed system definition for the CDS<br>• Data integrity for electronic records<br>• Electronic signatures<br>• Time and date source<br>• Audit trail requirements<br>• Security and access control |
| CDS functional requirements | • Operation of the system in the laboratory from setup, instrument control, data acquisition, integration and review of the data, SST calculations and acceptance criteria, calibration models used, results calculations and reporting |
| IT support | • Backup and recovery of the system<br>• Database maintenance<br>• User account management<br>• System monitoring |
| Interfaces | • Is the CDS standalone or interfaced with other applications?<br>• If the latter then what, how, and when are data transferred between the two systems?<br>• Care must be taken with the date and time stamps in the two systems |
| Data migration | • Migration requirements from existing version to new version of same software<br>• Migration from old to new application with old system retirement |
| Appendices | • Glossary<br>• Terms and definitions |

can be tested objectively for each module if required, but don't forget that a holistic test to demonstrate that the whole chromatograph system works is also required (14).

## What About Software Specifications for a CDS?

We have looked at how specifications for commercial instruments are expected to be minimal for a liquid chromatograph system. Now we need to ask the same question for software. The answer for any chromatography data system is, quite simply, no. The reason is the difference between a software application vs. an instrument. An instrument, in our case a chromatograph, is relatively simple, with some of the operating parameters outlined in Table 1.

CDS application software is much more complex and its impact is far greater: it can control a single chromatograph system in a single laboratory or multiple systems in multiple sites globally. The URS scope applies for a standalone system as well as a global one. Rather than have a small set of operating parameters, a CDS application has a wide range of functions such as:

- Instrument control
- Sequence file to identify the injections to be made and input of factors such as dilutions, weights, purities, and water content of standards
- Data acquisition
- Automatic and manual integration
- Calculations such as system suitability parameters and the reportable result.

In addition, a CDS also needs to be configured and this must be documented:

- Log on security, for example, password length and complexity
- User roles and access privileges that avoid conflicts of interest
- Unique user identities
- Protection of electronic records, for example, restriction of deletion privileges, audit trail turned on
- Implementation of electronic signatures

- Defining data storage locations
- Custom calculations
- Custom reports.

And there's more requirements:

- Time synchronisation
- Cyber security
- System patching, for example, security patches
- Change control and configuration management
- Backup and recovery
- Archive and restore

If the system is supported by an IT group there also needs to be an agreement of the services provided, and the roles and responsibilities of the laboratory and IT provider as required by EU GMP Chapter 7 and Annex 11 (15,16).

### Scope of a URS for a CDS

The areas listed above need to be arranged into groups of similar requirements. One such way of doing this is presented in Table 2. You can immediately contract this with the minimal requirements for the chromatograph shown in Table 1, the difference is simply the wider scope and complexity needed to adequately define the requirements for a CDS.

### Guidance for Writing User Requirements

The following general guidelines should be followed during the writing of the CDS URS:

- Each requirement statement must be uniquely referenced.
- Do not use the word processor auto-numbering function for requirement numbering. If a new requirement is added all subsequent ones are incremented and traceability will be lost. You have been warned.
- Each requirement should be short (typically no longer than 25 words).
- Each requirement should only contain one requirement otherwise it is difficult to trace.
- The URS should be consistent and requirements should not be duplicated or contradicted.
- Specify requirements and not design solutions. The focus should be on what is required, not how it is to be achieved.
- The exception to the point above is where corporate IT standards become a constraint on the system, for example, when a specific database or operating system must be used and no others are allowed
- Each requirement should be testable or verifiable. Testable is defined as test cases can be derived from the requirement as written. This allows the tests to be designed as soon as the URS is finalised. A requirement can be verified through an activity (for example, IQ of a component or writing of a standard operating procedure [SOP] verifies that the requirement has been met).
- Both the laboratory and the supplier must understand the document. Jargon should be avoided wherever possible and key words are defined in a specific section in the document.
- Requirements should be prioritised. There are various schemes that could be used but I prefer simplicity and typically use mandatory (essential to meet business or regulatory requirements) or desirable (nice to have).
- The URS should be modifiable, but changes should be under a formal control procedure. The easiest is by up-versioning and authorising the new version then archiving the old document.

A URS is correct if every stated requirement has only one interpretation and this is met by the system. Unfortunately, this is very rare.

### Who Should be Involved in Writing a URS?

Writing a user requirements specification for a CDS is not difficult, but the process is not a trivial exercise. It requires the involvement of a multidisciplinary team to write a URS consisting of chromatographers, quality, and, if the system is networked, IT. You will notice that there is no role for a supplier. That is because you have not selected the CDS yet and you are writing a generic specification.

After selection you will need to update the document to make it specific for the chosen application (name and version number) and here the supplier can help with training key users and a review of the updated document.

### How to Write Testable or Verifiable User Requirements

This is the heart of a good or bad URS. If you can't test or verify a requirement, it is of zero value. Meaningless requirements may impress management but they don't define the intended use of the instrument or software. To illustrate some of the problems of writing testable user requirements, here are two examples of how not to write requirements for a CDS. Note that both requirements are uniquely numbered, which is good, but these are real examples, which is not.

*Performance requirement: 6.1.8: Operating at normal PC response times with no undue delay in response at low computer utilisation.*

In requirement 6.1.8 there is wording that makes the requirement untestable. These are typically called *weasel words,* which are normal, undue, and low. These render the requirement useless and incapable of being tested. For example, what is a normal PC response time and what is undue delay? These are meaningless and untestable words.

A further example is shown below.

*Reporting 6.2.4: Report production at a rate of at least a page every 10 seconds at modest network and server utilisation.*

Requirement 6.2.4 is marginally better as "report production at a rate of at least one page every 10 seconds" is testable and specific. However, the requirement then snatches defeat from the jaws of victory with the phrase "at modest network speed", rendering it untestable as "modest" cannot be defined.

For those readers that love simplicity when writing requirements there is the ever-popular:

*4.2.1: The system must be 21 CFR 11 compliant.*

When I read such a requirement I do not know if it has been written by a stupid or a lazy person, or both. The writer does not understand that the 21 CFR 11 regulation is divided into technical, procedural, and administrative requirements.

## A URS is a Living Document

It is vital to understand that the contents in a URS are not static. As your chromatographic needs change so too may your CDS and chromatograph requirements. As a simple example, if your UV detector is qualified between 210 nm and 280 nm and a new analyte method has detection at 310 nm, then you need to update the instrument specification and requalify the detector. Similarly, if you change your working practice and implement electronic signatures, then the URS, configuration settings, and testing documents all need to be updated. In regulated laboratories there must be change control that examines the impact of a change on instruments, CDS software, and documentation including specifications and procedures.

## Summary

We have considered what appears to be one of the most difficult tasks in the laboratory: writing effective user requirements for chromatograph systems and chromatography data system software. It is not an arduous task but requires time that management must realise and allow for. One example I saw in an audit consisted of six requirements and 13 words that were only written to keep quality assurance (QA) happy. It may keep QA quiet but it will not impress auditors and inspectors. Improvement of user requirements specifications is a key component of continual improvement in any quality system.

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"Questions of Quality" editor **Bob McDowall** is Director of R.D. McDowall Limited, Bromley, Kent, UK. He is also a member of *LCGC Europe*'s editorial advisory board. Direct correspondence about this column to the editor-in-chief, Alasdair Matheson, amatheson@mjhlifesciences.com

## Sample prep system

Shimadzu's Nexera UC Prep, a semi-preparative fluid chromatography system, provides the pharmaceutical industry with reliable high-performance semi-preparative purification. According to the company, the system resolves a number of issues in preparative tasks, reduces labour, and improves efficiency while fitting into pre-existing workflows.

**www.shimadzu.eu**
**Shimadzu Europa GmbH, Duisburg, Germany.**

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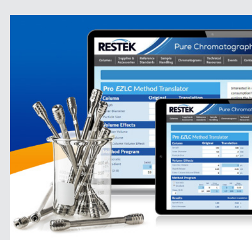
**www.wyatt.com/dawn**
**Wyatt Technology, Santa Barbara, California, USA.**

## Method translator

Pro *EZ*LC method translation software makes it quick and easy to scale down an existing LC method to a smaller column format so that users can speed up run time, increase sample throughput, and reduce solvent use, according to the company. Just input your current column dimensions and method conditions, then specify the dimensions of the new column that users want to try.

**www.restek.com/Pages/Pro-EZLC-Method-Translator**
**Restek Corporation, Bellefonte, Pennsylvania, USA.**




## HILIC columns

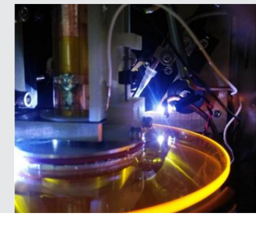
Hilicon offers a broad range of HILIC products to separate polar compounds. Four column chemistries in UHPLC and HPLC, iHILIC-Fusion, iHILIC-Fusion(+), iHILIC-Fusion(P), and iHILIC-(P) Classic, provide customized and complementary selectivity, excellent durability, and ultra-low column bleeding. According to the company, the columns are particularly suitable for LC–MS-based analysis of polar compounds.

**www.hilicon.com**
**Hilicon AB, Umeå, Sweden.**

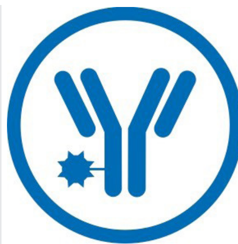
## Solid Phase GC-FTIR

Spectra Analysis' DiscovIR GC-FTIR focuses on real-time connection of infrared spectroscopy to gas chromatography. Temperature-controlled, vacuum deposition of the GC-eluted peaks onto a rotating IR-transparent disc ensures high-resolution IR fingerprinting in an accurate and reproducible way, and opens the gateway for rapid discrimination between regioisomers, a challenging area for MS, according to the company.

**https://spectra-analysis.com**
**Spectra Analysis, Marlborough, Massachusetts, USA.**



## HIC columns



YMC's latest HIC column, BioPro HIC HT, is for biopharmaceuticals such as antibody-drug-conjugates. Higher flow rates are reportedly applicable due to extremely high-pressure stability that allows very short run times and high throughput BioPro HIC HT columns are suitable for DAR determination with high resolution as a result of a novel and optimized surface chemistry.

**https://ymc.de/hydrophic-interaction-columns.html**
**YMC Europe GmbH, Dinslaken, Germany.**

# Direct Analysis of Amino Acids by HILIC–ESI-MS

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Hydrophilic interaction liquid chromatography coupled to electrospray ionization mass spectrometry (HILIC–ESI-MS) has been established as a method to separate and quantify polar and ionic analytes in a direct way for two decades. HILIC separation is based on the polarity of analytes, so the more polar analytes have stronger retention on a HILIC column.

Highly polar amino acids carry great biological weight because they are the basic structural units of proteins or enzymes. Accordingly, they are an essential part of plants, animals, and humans. Quantitative analysis of amino acids is crucial in many fields including clinical diagnostic, biomedical research, and food science (1). Amino acids are classically analyzed by gas chromatography (GC), cation exchange (CEX), or reversed-phase liquid chromatography (LC) with UV absorbance, fluorescence detection (FD), or mass spectrometry (MS) (1). However, pre-column or post-column derivatization is often needed to address either the retention problem or the detection issue. HILIC and ESI-MS is a perfect match for analyzing amino acids in a direct and fast manner (2).

In this application, we separated 14 amino acids with an iHILIC-Fusion(+) column packed with charge modulated hydroxyethyl amide silica. A mixed interaction—for example, hydrophilic partitioning, weak electrostatic interactions, and hydrogen bonding—may be involved in the HILIC separations.

## Experimental

**LC–MS System:** Agilent 1100 LC system and Thermo Fisher LTQ™ equipped with a HESI source, operated in positive ionization mode for analysis of standards. For the dietary supplement, an Orbitrap™ Exactive classic equipped with a HESI source and operated in positive ionization mode.

**Column:** 150 × 2.1 mm, 3.5-µm, iHILIC-Fusion(+) (P/N 100.152.0310, HILICON AB)

**Gradient Elution:** A) acetonitrile–water–1 M ammonium acetate, pH 5.75 (90:5:5); B) water–acetonitrile–1 M ammonium acetate, pH 5.75 (90:5:5); 0–0.5 min (90:10) A–B; 0.5 to 25 min, gradient elution from (90:10) A–B to (60:40) A–B. Total buffer concentration was 50 mM in the gradient elution.

**Flow Rate:** 0.3 mL/min

**Column Temperature:** 40 °C

**Injection Volume:** 5 µL

**Amino Acids:** Arginine, asparagine, aspartic acid, glutamic acid, glutamine, hydroxyl-proline, isoleucine, leucine, lysine, phenylalanine, proline, tryptophan, tyrosine, and valine. 50 µM of each amino acid was dissolved in water–acetonitrile (25:75) solution.

**Dietary Supplement:** Whey Prime (Prozis). A 20 mg/mL measure was dissolved in water and then filtered. The sample was further diluted to 2 mg/mL using water-acetonitrile (25:75) solution for injection.

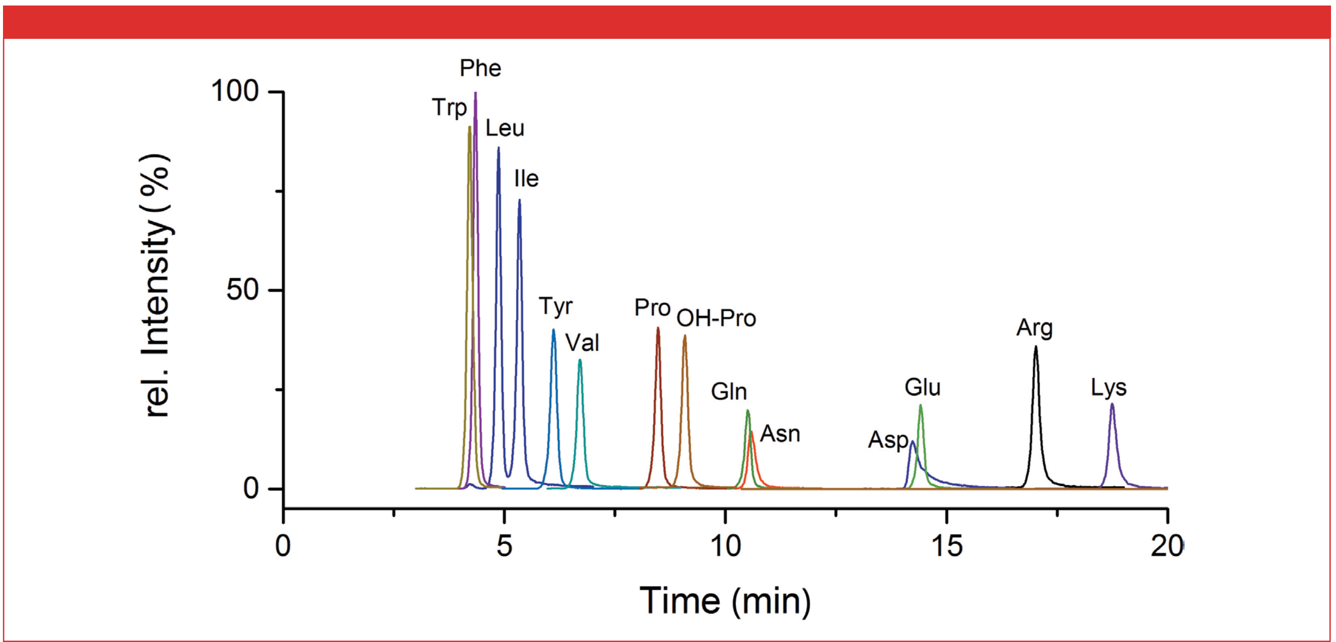


**Figure 1:** Extracted ion chromatograms of amino acid standards in HILIC separation with iHILIC-Fusion(+).

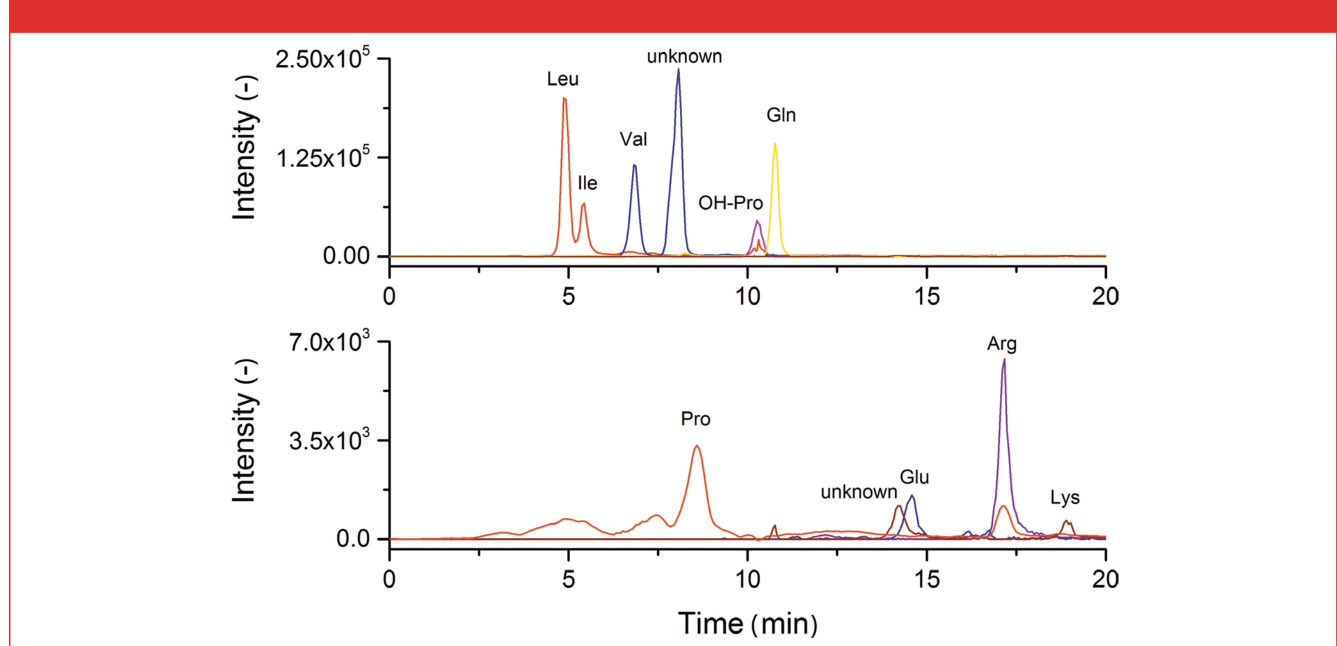


**Figure 2:** Extracted ion chromatograms of amino acids in dietary supplement.

**Table 1: Retention times, classification of amino acids by their side-chain, and pI values**

| Amino acid | Abbr. | Classification | $t_R$ (min) | pI[a] |
|---|---|---|---|---|
| Tryptophan | Trp | nonpolar | 4.2 | 6.0 |
| Phenylalanine | Phe | nonpolar | 4.3 | 6.0 |
| Leucine | Leu | nonpolar | 4.9 | 6.2 |
| Isoleucine | Ile | nonpolar | 5.3 | 6.2 |
| Tyrosine | Tyr | polar | 6.1 | 5.5 |
| Valine | Val | nonpolar | 6.7 | 6.2 |
| Proline | Pro | polar | 8.5 | 7.1 |
| Hydroxy-proline | OH-Pro | polar | 9.1 | 6.7 |
| Glutamine | Gln | polar | 10.5 | 5.7 |
| Asparagine | Asn | polar | 10.6 | 5.2 |
| Aspartic acid | Asp | acidic | 14.2 | 3.4 |
| Glutamic acid | Glu | acidic | 14.4 | 2.8 |
| Arginine | Lys | basic | 17.0 | 10.8 |
| Lysine | Arg | basic | 18.7 | 9.8 |

[a]pI values are based on www.chemicalize.com

## Results and Conclusion

As shown in Figure 1, 14 standards of proteinogenic amino acids can be simultaneously determined using a iHILIC-Fusion(+) column coupled with ESI-MS detection. The retention times, classification of amino acids, and pI values are summarized in Table 1. As expected, the amino acids with a nonpolar side chain like tryptophan, phenylalanine, leucine, and isoleucine eluted first with the lowest interactions with the HILIC stationary phase. The polar amino acids like proline, glutamine, or asparagine had more retention and were mainly retained by hydrophilic partitioning. The acidic and basic amino acids are not neutral and had extra electrostatic interactions with the stationary phase. Interestingly, it was found that the constitutional isomers leucine and isoleucine were almost baseline separated in a generic linear gradient elution.

In the second step, we verified the applicability of the newly developed method using a dietary supplement containing various numbers of amino acids. As can be seen in Figure 2, leucine and isoleucine are well separated. Valine, glutamine, and hydroxyproline present intense signals. In addition, proline, glutamic acid, arginine, and lysine were also detectable at lower intensities.

This work demonstrates that amino acids can be easily identified through a HILIC–ESI-MS method. It offers the possibilities to quantify this group of polar analytes in many application areas such as medicine, biology, or nutritional science.

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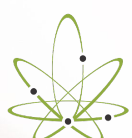

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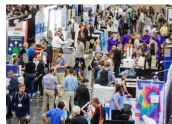

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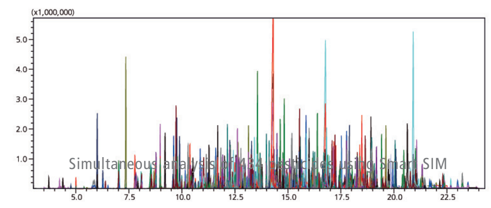

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