

Optimizing Pesticides Protection

Advances in chromatography for pesticides analysis in food

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Ramkumar Dhandapani, Phenomenex

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Kevin A. Schug¹ and Zacariah L. Hildenbrand^{2,3}, ¹The University of Texas at Arlington, USA, ²Medusa Analytical, ³University of Texas at El Paso

The level of uncertainty provided by most forensic laboratories for reported blood alcohol results has been woefully underassessed. Not only is this bad science, but someone's civil liberties may be at stake.



Advances in Chromatography for the Optimal Separation of Pesticides in Food

Ramkumar Dhandapani, Phenomenex, Torrance, USA

Pesticides analysis in food presents various challenges, including meeting regulatory guidelines, critical pair resolution, retention and recovery, matrix interference, and meeting sensitivity requirements. Above all, the ever-growing list of pesticide compounds and the high throughput needed to pass or fail an increasing number of samples each day encourages scientists to explore a combination of sample preparation techniques, separation techniques, and detectors to achieve and exceed goals. This article will review these critical components that can eventually resolve the separation challenges related to pesticides analysis in food.

To establish the safety of our food, toxic and harmful chemicals in food and beverages must be measured before consumption.

To understand the safe levels of these multiresidue compounds, scientists require appropriate sample extraction, separation, and detection techniques to accurately identify and quantify these compounds. Safe levels are set by governmental agencies, and maximum residue levels (MRLs) are commonly referred to as the upper limits of what is

allowable to the general public. This ensures that our food stays safe for consumption.

Sample Preparation for Multiresidue Pesticides Analysis

With diverse limits of detection, the large number of pesticides and other contaminants that need to be analyzed creates a challenge for gas chromatography (GC) analysis. Before analysis can take place, the complex and often dirty matrices in food samples, such



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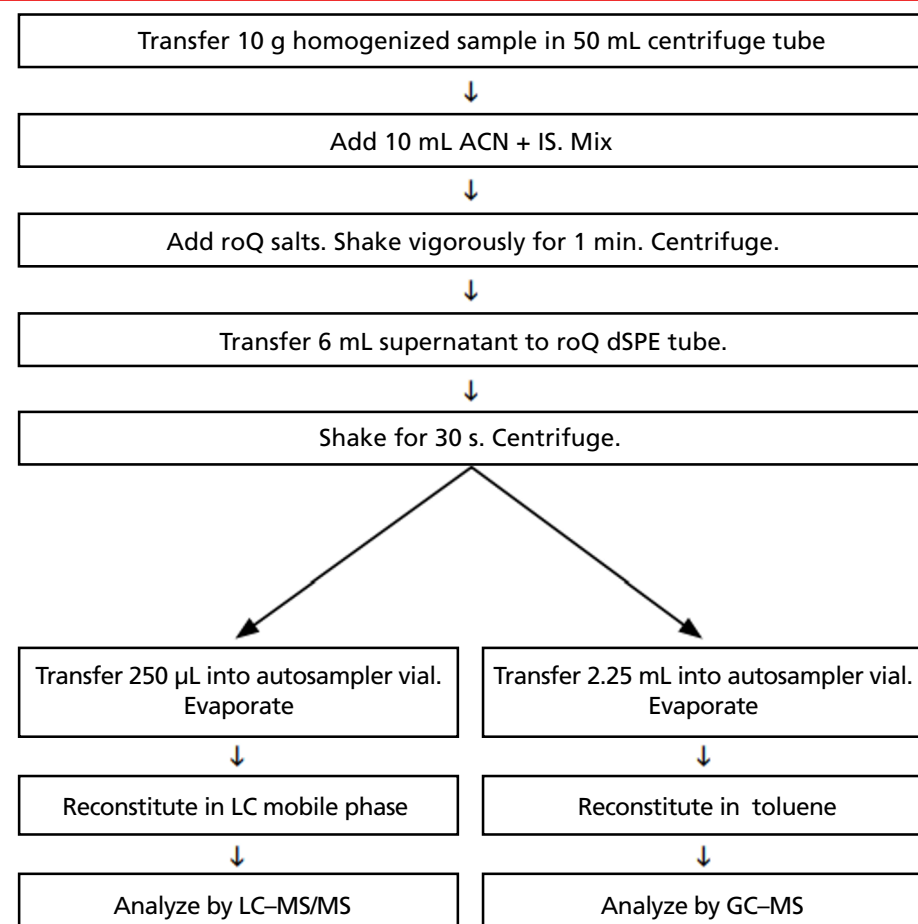
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Figure 1: Flow chart summary for European Summary EN 15662 method for pesticide residues in food. The final extracts were split for LC-MS/MS and GC-MS analyses (4).



as proteins, fats, and pigments, mean that laboratories need to adopt accurate sample preparation methods to remove unwanted matrix components. While there are several sample preparation options available for pesticides extraction from food, a simple sample preparation method can save laboratories time in the downstream and save them from having to re-run data. Even with the additional method development

and time spent on cleanup, the benefits are numerous. In multiresidue analysis of food, QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) is a popular technique and combines extraction and clean-up steps. This technique provides optimal recovery by extracting everything from the matrix and then removing matrix components, including fats and pigments, to provide a cleaner extract and higher recovery for multipesticide

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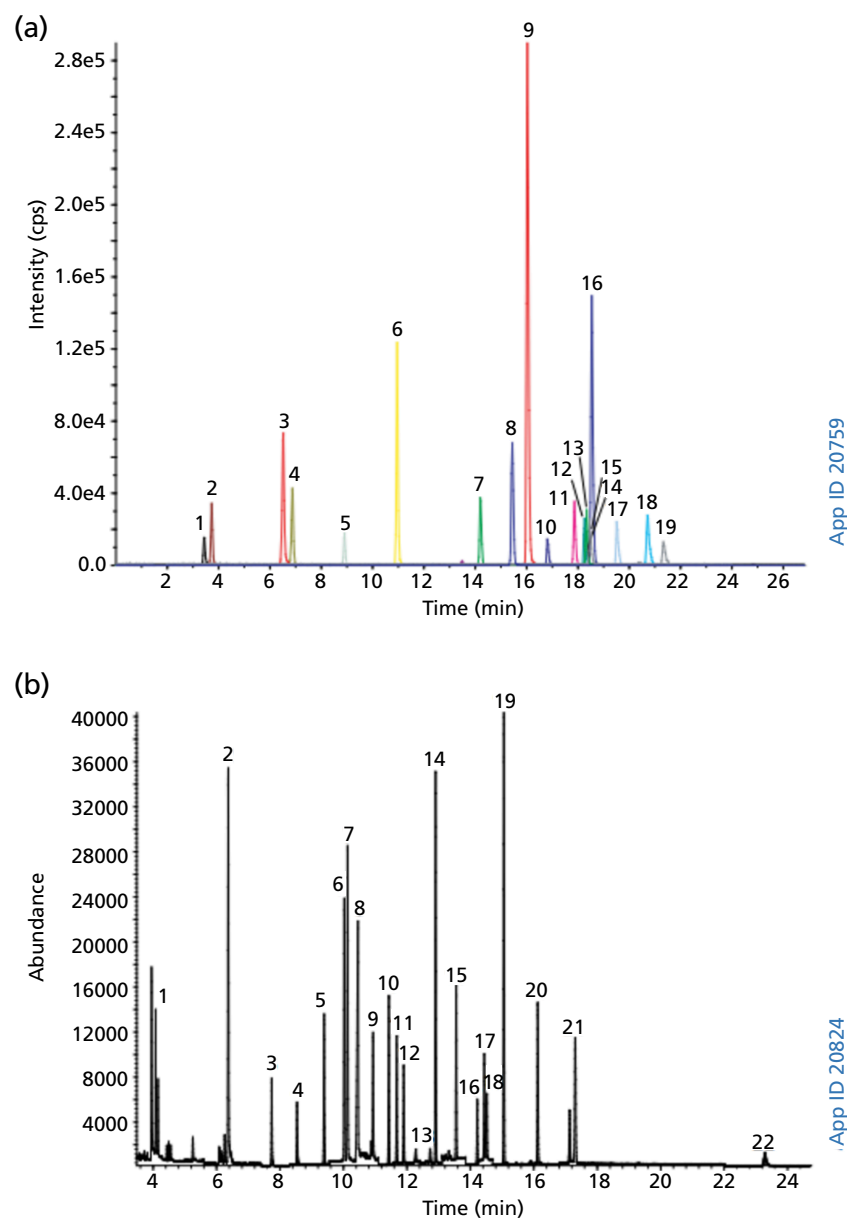
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Figure 2: (a) Pesticides in lettuce extracted by LC–MS. (b) Pesticides in lettuce extracted by GC–MS (4).

(1) classes in a single extract. There are guidelines from official methods, such as AOAC official method 2007.01 and EN 15662 QuEChERS method (2,3), that begin with the extraction of pesticides from food samples

using acetonitrile, which makes the method optimization and selection of QuEChERS easier. In fact, the extract from QuEChERS can be utilized for liquid chromatography (LC) and GC analysis with minimal modification.

A good example explaining the versatility of this extraction is presented in reference 4 (Figures 1 and 2).

GC vs. LC for Multiresidue Pesticides

While both LC and GC have their advantages for pesticides analysis, LC is the “go to” choice for underivatized nonvolatile pesticides, as well as temperature-sensitive semivolatile pesticides. Within the list of pesticides analyzed by LC, polar pesticides (cationic and anionic pesticides) require specialty columns to achieve the desired separation and detection needs. Groups of pesticides analyzed by GC include nonionic and volatile to semivolatile classes; these can extend from a few pesticides to a few hundred pesticides. With GC, the analyte eluting out of the column is in its gaseous form and is easily ionizable in a mass spectrometer. Because of this, and due to the strong ionization of the commonly used electron ionization (EI)-mass spectrometer, almost all GC pesticides achieve good ionization, which eventually translates to peak response. While some pesticides may pose challenges in electrospray ionization (ESI)—a source commonly used in LC–mass spectrometry (MS)—polarity switching and alternative ionization can be used as a mitigation for challenging analytes in LC–MS. Irrespective of LC vs. GC, analytical techniques are complementary and utilize

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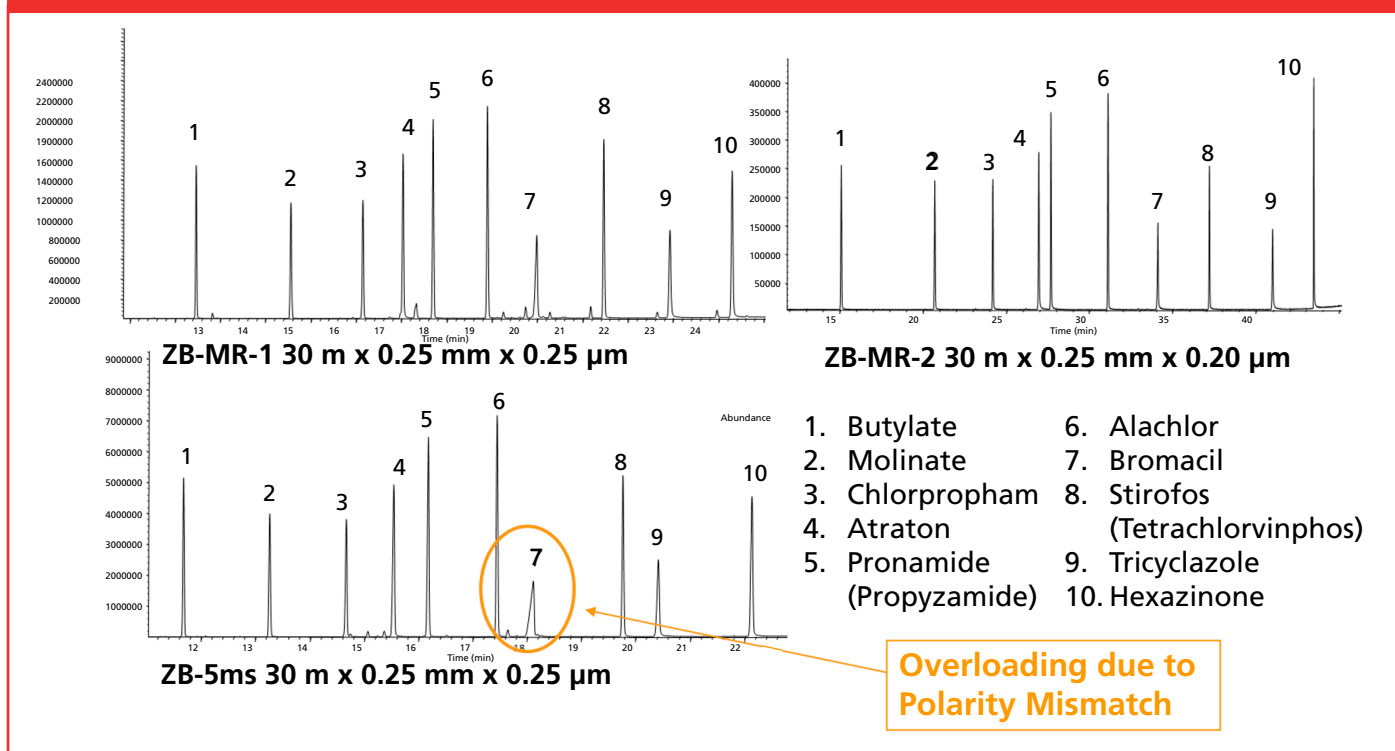
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Figure 3: Nitrogen and phosphorous pesticides on a low- and mid-polar GC column.

orthogonal separation and detection of trace pesticide compounds, which can collectively solve the detection and quantification of pesticides to increase confidence in the safety of food.

GC Column Considerations for Pesticides Analysis

The chromatographic GC column is considered to be the heart of the separation in a GC instrument. The pesticides are partitioned between the mobile phase and stationary phase film to realize chromatographic separation. This is commonly directed by the vapour pressure of

the individual pesticide compound and the selectivity of the GC stationary phase. There are multiple factors to consider before settling on a GC stationary phase for pesticides. This includes identifying the ideal stationary phase selectivity, optimal dimension, and the most inert stationary phase that can reduce breakdown of challenging analytes.

Figure 3 presents a separation of a list of pesticides on three different column selectivities. As is common in multiresidue analysis, certain pesticides may be too polar and may not wet traditional 5MS columns, which are low-polar stationary phase. Mid-polar stationary phases specially

designed for multiresidue analysis can eliminate the dewetting of polar pesticides and can therefore give sharper peaks for both polar and nonpolar pesticides. It is therefore important to screen a few mid-polar selectivities before settling on a 5MS-based column for pesticides analysis. Online column selection tools are useful in making the correct stationary phase selection (5).

While most modern methods in GC use MS-based detection, shorter columns ranging from 10 m to 15 m in length with an inner diameter of 0.1 mm to 0.25 mm are optimal for fast and robust analysis of pesticides. While a 30 m length is still considered for traditional GC-flame ionization detection/electron capture detection (FID/ECD) methods, when considering the mass spec aspect and the throughput that laboratories demand, shorter columns are preferred for multiclass pesticides.

Another important consideration is the selection of an inert stationary phase surface with minimal active spots, as these spots can break down heat-sensitive pesticides such as dichlorodiphenyltrichloroethane (DDT) and endrin.

LPGC for Fast Pesticides Analysis

Low-pressure GC-MS (LPGC-MS) is a technique that uses the MS vacuum system, along with a specially designed column

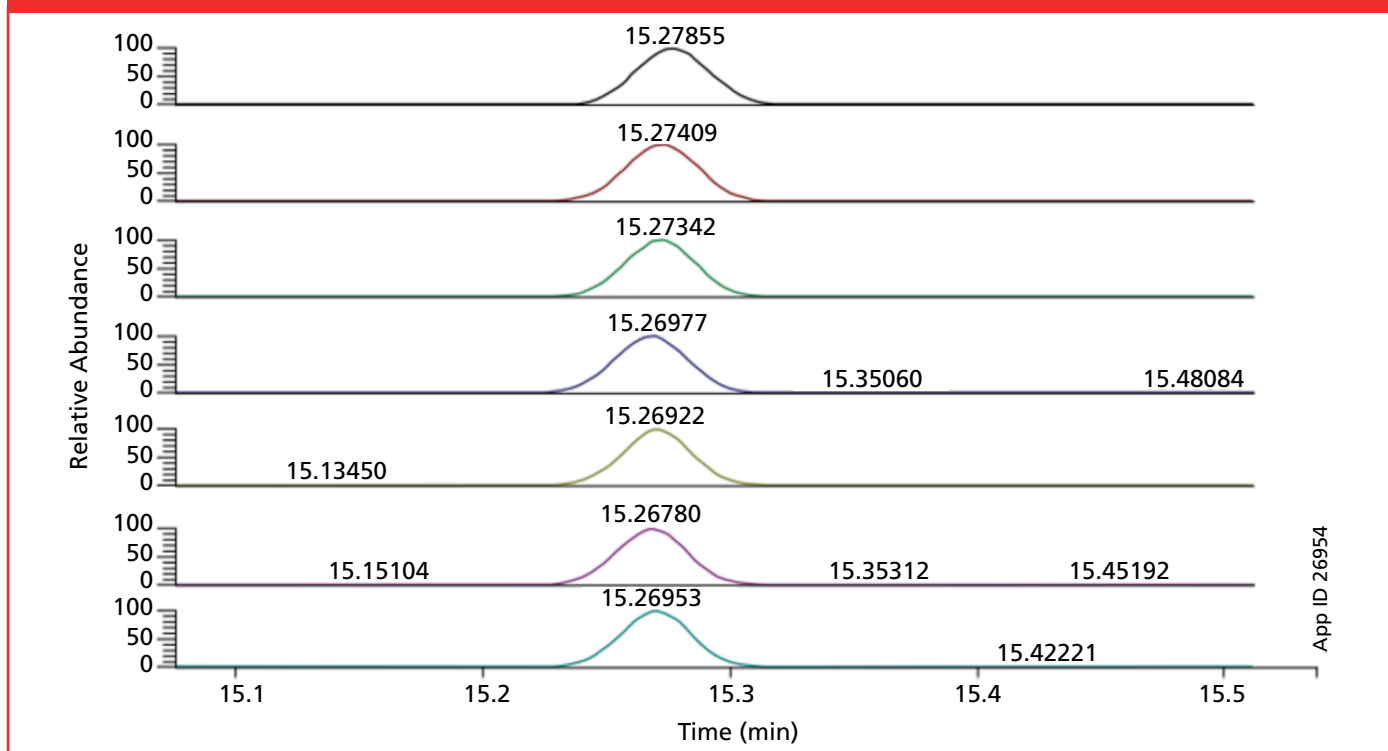
setup with a shorter length and larger inner diameter in combination with a restrictor column for flow maintenance, to lower the pressure inside the GC column. By reducing the pressure inside the column and eventually the viscosity, the analysis is expedited up to threefold with minimal compromise in resolution. This technique has found immense popularity by improving throughput in food testing laboratories (6).

GC-MS for Pesticides

While GC remains the most cost-efficient way to detect pesticides, adding an additional dimension of separation with a quadrupole mass spectrometer increases the authenticity of detection. With appropriate sample clean-up and preconcentration, multiclass pesticides analysis can be reliably performed on a benchtop GC-MS system. The retention profile of over 100 pesticides has been reported on a novel GC column selectivity (7) for accurate identification and quantification of these residues. Sharper peak shape, chromatographic resolution, and low bleed from modern GC columns can help to lower the noise level and improve detection, thereby making complex multiresidue analysis an affordable option.

GC-HRMS for Pesticides

While GC-MS provides separation and

Figure 4: GC–HRMS analysis of the pesticide bromophos-ethyl.

detection across many industries, tandem mass spectrometry (MS/MS) is used to add the extra robustness and sensitivity that is usually required for large panels of pesticide analytes. Another advancement that is recommended for optimization is high-resolution MS; this technique can help in the detection and quantitation of pesticides in complex food matrices better than triple quadrupole (QqQ) mass spectrometry due to high-resolution accurate mass that is utilized for quantification. To understand this method better, 285 pesticides from several food matrices were optimized on a

GC–high-resolution mass spectrometry (HRMS) system (8). HRMS instruments offer great capability for identification of compounds thanks to the measurement of accurate mass. In this sense, the combination of GC with HRMS is a very powerful tool, with nominal spectral libraries for screening and authentication. Figure 4 presents the separation of key pesticides by GC–HRMS on a 5% phenyl-arylene GC column. Sharp peaks, adequate chromatographic separation, and accurate mass for quantification with HRMS helps to achieve low detection limits for a wide range of pesticides.



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GC–MS/MS for Pesticides Analysis

GC–MS/MS, also called GC–QqQ, has an impressive noise level improvement and filtration and improved detection levels for multipesticide residue analysis. Due to the selective nature and advanced engineering design of various triple quadrupole instruments that are available, ultra-trace levels of multicomponent detection are feasible. A detection limit of 5 parts per billion (ppb) was achieved for over 100 pesticides from olive oil (9), which is considered a very challenging matrix with GC–MS/MS analysis. Aside from the detection, modern triple quadrupole instruments are relatively compact and have advanced automated method optimization options, helping to facilitate fast method development and optimization.

Conclusion

Sample preparation offers preconcentration and matrix removal to obtain a cleaner extract for introduction on to the chromatographic system. Following this, choosing a column with an inert surface and optimal selectivity provides chromatographic resolution and high accuracy. Depending on the matrix complexity, instrument availability, and the detection levels, various detector choices including GC–MS, GC–MS/MS, and GC–HRMS are available for quantification of multiresidue pesticides from food matrices. These techniques work in combination to

improve the quality of pesticides analysis and increase the confidence in results.

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Ramkumar Dhandapani has been in the chromatography industry for over 20 years and has hands-on and troubleshooting experience. He has a masters and PhD degree in analytical chemistry from Seton Hall University, with specialization in microextractions, multidimensional chromatography, and tandem MS techniques. He has developed and validated several regulatory compliant methods in the pharmaceutical, food, fuels, and the environmental industry, as well as incorporated method improvement and troubleshooting across a range of separation techniques. He joined Phenomenex in August 2014 and currently works as a senior product manager. In addition to managing the product line, he presents on innovations in separation science at various chromatography conferences.

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From the CEO

Welcome to the August issue of *The Column*! The quality and safety of our food presents challenges for analytical chemists, not least of which includes meeting the strict regulatory guidance issued and the ever-increasing list of pesticide compounds out there. In our cover story for this issue we examine the sample prep techniques, separation techniques, and detectors that can be used for optimal analysis of pesticides in food, with a particular focus on what gas chromatography–mass spectrometry (GC–MS) can offer in this arena.

Our regular column focused on tips in gel permeation chromatography/size-exclusion chromatography (GPC/SEC) asks the question: Can I separate two polymers using GPC/SEC? What factors need to be considered for a successful separation?

With sustainability becoming a critical issue in all industries, new ways of reducing the environmental impact of separation science and promoting the benefits of sustainable analytical methods is essential. Elia Psillakis and co-authors present their views on how greening the analytical methods is directly connected to greening the sample preparation step. Researchers, analysts, and practitioners all need to work together to continue to drive sustainability in separation science forwards.

This month we spoke to “rising star” Selina Tisler from the University of Copenhagen about her research in environmental analysis, using supercritical fluid chromatography (SFC) to detect both very polar compounds and compounds of unknown toxicity in wastewater effluents, and the migrating of compounds from plastic bottles into our drinking water.

Finally, I would like to extend a warm welcome to our new associate editorial director, Caroline Hroncich. We sat her down for a chat to find out more about her and what *LCGC* readers can expect in the future.

Happy reading!

Mike Hennessy Jr.,

President and CEO, MJH Life Sciences

Exploring the Potential of Foodomics in the Fight Against Alzheimer’s Disease

In his keynote lecture at HPLC 2023, Alejandro Cifuentes from the Laboratory of Foodomics, Institute of Food Science Research, CIAL, CSIC in Madrid, Spain, highlighted the promising application of foodomics in addressing Alzheimer’s disease (AD) (1).

Cifuentes emphasized the urgent need for new strategies in combating AD, as the only treatment options are palliative and do not provide a cure. He proposed that diet and food components may hold potential in preventing or delaying the development and progression of AD. With this in mind, he presented natural sources of bioactive compounds and their neuroprotective properties.

To identify potential neuroprotective candidates, Cifuentes and his team employed green extraction processes and *in vitro* methods. They discovered that olive leaves extracts enriched in triterpenoids, a carotenoids-enriched extract from *Dunaliella salina* microalgae, and an extract from orange juice industry by-products enriched in monoterpenes, sesquiterpenes, and triterpenes, demonstrated high neuroprotective potential. Furthermore, these extracts exhibited significant neuroprotective activity in a neuronal cell culture model.

To further validate the findings, a transgenic *Caenorhabditis elegans* was employed as an AD model. *In vivo* experiments demonstrated that the extracts displayed notable neuroprotective effects.

The integration of these diverse approaches under the foodomics umbrella allowed for a holistic investigation of the effects of neuroprotective candidates on the transgenic *Caenorhabditis elegans* model. By examining the lipid profiles, gene expression patterns, and metabolite profiles, the molecular mechanisms underlying the observed neuroprotective activity were elucidated.

This study represents a significant step forward in understanding the potential role of food compounds in the prevention and management of AD. The findings highlight the importance of exploring natural sources of bioactive compounds and demonstrate the power of foodomics in identifying promising candidates for further research and development of potential therapeutic interventions.

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Peaks of the Month



- **Fundamentals of Chromatography: An HPLC Interview with Deirdre Cabooter**—Cabooter spoke with us at HPLC 2023 in Düsseldorf, Germany, where she gave a talk titled, “Detailed Investigation of Intra-particle Diffusion in Liquid Chromatography”. She is a professor at KU Leuven in Belgium. [Watch Here>>](#)



- **The Role of Chromatography in Lipidomics: An HPLC Interview with Michal Holčapek**—Holčapek, a professor at the University of Pardubice in the Czech Republic, spoke on the topic of his talk at the conference, entitled “Potential of Various Chromatographic Modes for Comprehensive Coverage of Human Lipidome”. [Watch Here>>](#)



- **Developments in Multidimensional LC: An HPLC Interview with Andrea Gargano**—An assistant professor in the Analytical Chemistry Group at the Van ‘t Hoff Institute for Molecular Science at the University of Amsterdam in The Netherlands, Gargano presented and moderated on several panels related to research on multidimensional liquid chromatography (LC). [Watch Here>>](#)



- **New Dimensions in Chromatography with AI and Ion Mobility: An HPLC Interview with Gérard Hopfgartner**—Hopfgartner spoke with us at HPLC 2023 in Düsseldorf, Germany, where he expanded on a talk he gave supporting his research presentation, “Separations Sciences Coupled to Mass Spectrometry for Multimodal Analysis: Challenges and Opportunities”. He is the head of Life Sciences/Mass Spectrometry at the University of Geneva in Switzerland. [Watch Here>>](#)



- **Platforms, Technologies, and Standard Reference Samples: An HPLC Interview with Peter Schoenmakers**—Schoenmakers presented several papers and on several panels at the conference. He retired in 2022 from his post as a professor of chemistry at the University of Amsterdam, where he was also the director of the van ‘t Hoff Institute of Molecular Science (HIMS) and a founder and the education director of a public-private-partnership organization on analytical chemistry called “Comprehensive Analytical Science and Technology (COAST)”. [Watch Here>>](#)

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News In Brief

Markes International Enters into Agreement with ExtraTech

Markes International (Bridgend, UK) has recently entered into a partnership with ExtraTech Analytical Solutions (Chania-Crete, Greece) to globally distribute ExtraTech’s vacuum-assisted caps for headspace microextraction. The caps extend the applicability of headspace, solid-phase microextraction (SPME), and SPME Arrow sample extractions by enabling analytes to be extracted under vacuum conditions with milder heating and agitation compared to current nonvacuum methods.

The use of vacuum-assisted caps reduces the risk of sample degradation, resulting in a more representative sample profile and higher extraction sensitivities. Rachael Szafnauer, product marketing manager of Markes’ sample extraction product lines, said: “We are very excited about how the caps enable analysts to benefit from enhanced extraction capabilities. ExtraTech’s technology is a great fit with the Markes’ sampling technologies [...] and we’re looking forward to working closely with our customers.”

ExtraTech’s founder, Elia Psillakis, commented: “Our long-standing collaboration with Markes has been driven by our shared passion, determination, and commitment to innovation. Now we have brought vacuum-assisted extraction technology to market, I am convinced that our products and methods will benefit laboratories worldwide.”

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Can I Separate These Two Polymers by Gel Permeation Chromatography/Size-Exclusion Chromatography?

Wolfgang Radke, PSS - Part of Agilent, Mainz, Germany

To tailor the properties of polymeric materials, different polymers are often mixed. To identify and quantify this blend ratio, the individual components must be separated. As gel permeation chromatography/size-exclusion chromatography (GPC/SEC) is the main technique for the separation of macromolecules, the question often arises if two polymeric materials differing in molar mass can be sufficiently separated by GPC/SEC. This article will offer tips on how to judge the potential success of separating two polymers using GPC/SEC.

Polymeric components are often blended together to optimize application properties. While the mixing ratio of chemically differing components can be obtained using spectroscopic techniques, information on the molar mass distributions of the

blend components cannot be assessed spectroscopically. Even more challenging is obtaining the blend ratio of two components that differ in molar mass but consist of the same monomer units. Such blends can be used to tailor a product's molar mass distribution if the



molar mass distribution of a single component does not provide the desired properties.

When separating the peaks of monodisperse substances, a chromatographic resolution of at least $R = 1.5$ is usually required for reliable quantitation of the components (1). The resolution is defined by the ratio of difference in peak retention time to the average peak width (1).

However, macromolecular samples are usually heterogeneous with respect to molar mass. This means there exists a very large number of species differing in molar mass. Each of these species should in principle provide a peak. However, for high molar mass samples, the small differences in the peaks' retention times does not allow for their separation. Therefore, a broad peak rather than a series of separated peaks is obtained.

As a consequence, the information that the two samples are of different molar mass is insufficient to predict whether these samples can be separated by gel permeation chromatography/size-exclusion chromatography (GPC/SEC). This is due to the fact that the low molar mass tail of the higher molar mass component might contain identical molar masses to the high molar mass end of the component of lower molar mass.

Therefore, peak width in GPC/SEC—which is still the main separation technique for polymers and macromolecules—is determined

by two elements: the width of the sample molar mass distribution, which is related to sample molar mass dispersity, D , and the band broadening contribution of the column or column combination.

If two macromolecular species are to be separated by GPC/SEC, their molar mass difference and the widths of their molar mass distributions dictate whether a separation of the components is possible at all. The peak broadening contribution then impacts on whether the components can be separated in practice.

Therefore, when separating macromolecular mixtures, one should first estimate whether the sample components can be separated in principle. To do so, the difference in molar mass, as well as the width of the molar mass distributions, need to be considered.

How Do Dispersity and Molar Mass Affect Resolution of Macromolecular Blends in GPC/SEC?

In GPC/SEC, the hydrodynamic volume defines the elution volume. If two heterogeneous sample components are to be separated by GPC, the first question that needs to be asked is whether chains of identical hydrodynamic size exist? Information on hydrodynamic size distributions is usually rare. Therefore, we will investigate under what conditions molar mass distributions differ sufficiently



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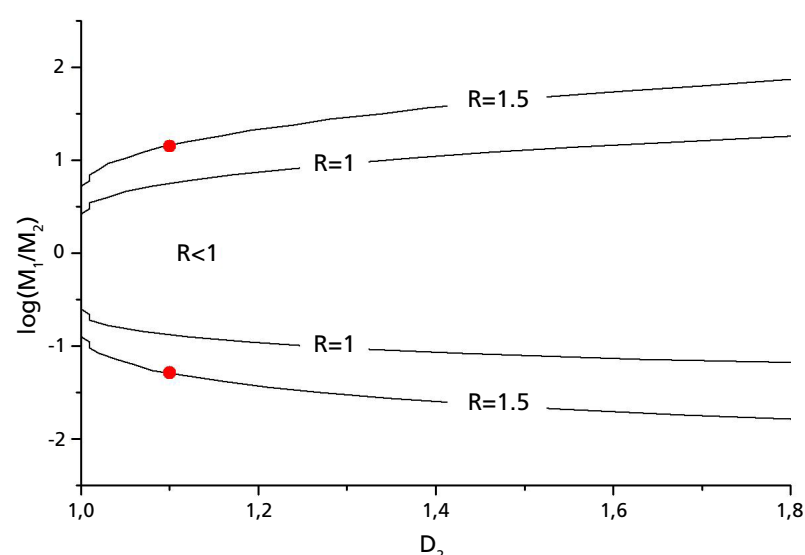
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Figure 1: Resolution as a function of ratio of weight average molar masses and 2nd component dispersity, for a 1st component dispersity of $D_1 = 1.5$.



enough to allow for their separation. This is justified when comparing samples of identical chemistry and topology. However, the assumption that identical molar masses correspond to identical hydrodynamic sizes is not justified when the components differ in chemical structure or topology. However, as the purpose of this article is to provide reasonable estimates, rather than strict scientific limits, the approximation seems to be justified. Furthermore, it is assumed that the molar mass distributions follow log normal distributions, as characterized by weight average molar masses of $M_{w,1}$ and $M_{w,2}$ and dispersities D_1 and D_2 , respectively. Again, the assumption of a log normal distribution will suffice for the purpose.

For a log normal distribution, the peak molar mass, M_p , and variance, σ_{\ln} , are related to the weight average molar mass, M_w , and sample dispersity, D , by (2):

$$M_p = M_w \exp \left\{ \frac{\sigma_{\ln}^2}{2} \right\} \quad [1]$$

$$\ln D = \sigma_{\ln}^2 \quad [2]$$

where once again the index \ln refers to a log normal distribution.

Similar to peak resolution in chromatography, the resolution between two molecular weight distributions (MWDs) can be defined as:

$$R = \frac{|\ln M_{p,1}/M_{p,2}|}{2(\sigma_{\ln,1} + \sigma_{\ln,2})} \quad [3]$$

which can relate the weight average molar masses and dispersities to yield:

$$R = \frac{|\ln M_{w,1}/M_{w,2} + \ln \sqrt{D_1/D_2}|}{2(\sqrt{\ln D_1} + \sqrt{\ln D_2})} \quad [4]$$

Figure 1 shows the resolution as a function of the ratio of the weight average molar masses and the dispersity of the second component for a fixed dispersity $D_1 = 1.5$ of the first component. Quantitation in chromatography is usually assumed to be possible for $R > 1.5$, while for $R = 1$ there is still a significant mutual overlap of the peaks. As can be seen, baseline separation can only be achieved in a very small region. If the dispersity (D_2) of the second component exceeds 1.1 (see red points in Figure 1), a full separation can only be realized for $M_{w,1}/M_{w,2} > 14.1$ ($\log[M_1/M_2] > 1.149$) or $M_{w,1}/M_{w,2} < 0.0514$ ($\log[M_1/M_2] < -1.289$), thus the weight average molar mass of the second component must be at least 14 times higher (or 19 times lower) than that of the first component. To visualize the distribution of such mixtures, Figure 2 shows the distribution of a polymer with $M_{w,2} = 10^5$ g/mol and $D = 1.1$ and compares it to the distributions of $M_{w,1} = 1.4 \times 10^6$ or $M_{w,1} = 5250$ g/mol and $D = 1.5$.

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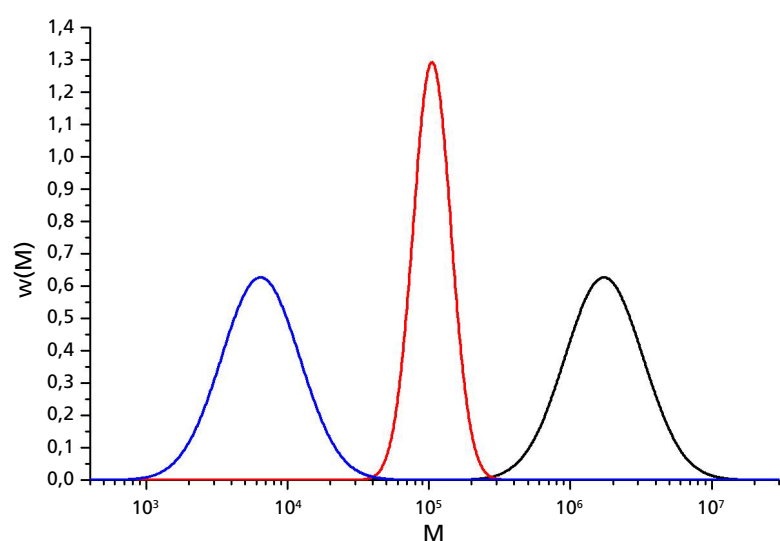
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Aging and Stability: Degradation and Deterioration

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 Dr. Michael Soll, michael@frontier-lab.com, +49 171 64881 48



Figure 2: Molar mass distributions for $M_w = 10^5$, $D = 1.1$ (red); $M_w = 1.41 \times 10^6$, $D = 1.5$ (black), $M_w = 5250$, $D = 1.5$ (blue) achieving a resolution of $R = 1.5$.



Despite the fact that there is a large difference in weight average molar masses between the broadly and the narrowly distributed samples, any increase in dispersity of the sample with $M_w = 10^5$ g/mol would result in insufficient separation.

In Figure 3, the ratio of weight average molar masses required to achieve a baseline resolution ($R = 1.5$) is plotted as a function of the dispersities of both components.

If one component has a dispersity of 1.2, while the other has a dispersity of 1.5, the higher molar mass component must exceed the lower molar mass by at least a factor of 27, if the higher molar mass component is the one of higher dispersity (point 1 in Figure 3). If, however, the higher molar mass component has the lower dispersity (point 2 in Figure 3), the

higher molar mass component must exceed the molar mass by a factor of approximately 22. If both samples have a dispersity of $D = 1.6$ (point 3 in Figure 3), the weight average molar masses need to differ by more than a factor of 60.

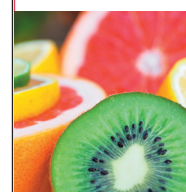
The Impact of Band Broadening on the Separation of Polymer Mixtures

Once it has been established that the two components differ sufficiently enough so that their molar mass distributions do not overlap, it now needs to be determined what GPC/SEC column or column combination is needed to achieve this separation. Of course, the column combination must provide the required separation range. However, all chromatographic systems suffer from band broadening. Even if the

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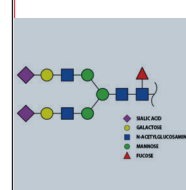
Prebiotics



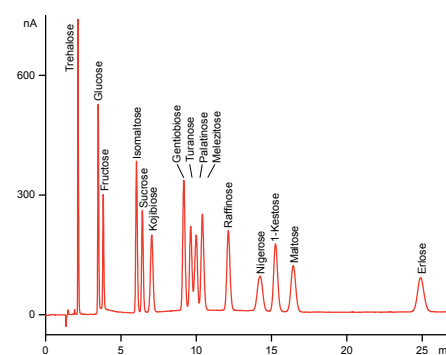
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Analysis of 15 sugar standards found in honey on a SweetSep AEX200 column using HPAEC-PAD

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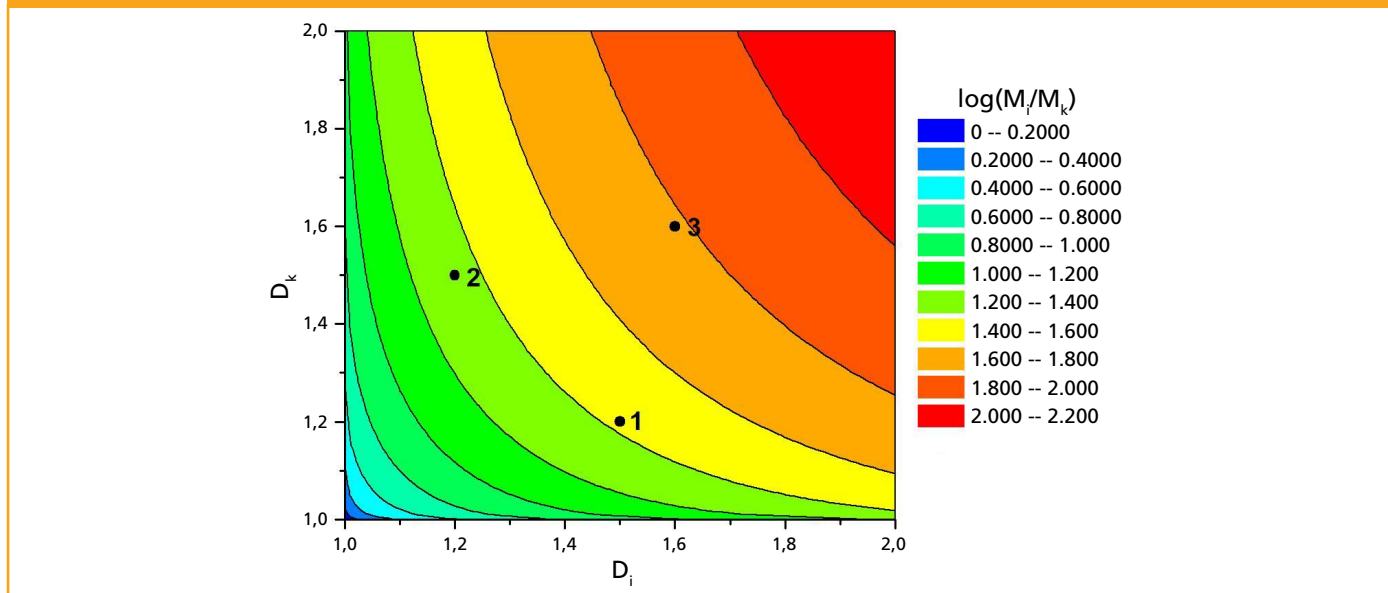
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Figure 3: Required logarithms of ratio of weight average molar masses ($\log[M_w(D_i)/M_w(D_k)]$) required to achieve baseline resolution ($R = 1.5$) as a function of dispersities of the components.



molar mass distributions provide a sufficient mass difference for the separation, band broadening effects diminish resolution, meaning that the separation might not be attainable in a real-life analysis.

A comprehensive analysis of the effect of dispersity and band broadening is beyond the scope of this article. For mixtures of broadly distributed samples, the above estimate on whether a separation is possible at all helps to reduce unnecessary attempts by GPC/SEC.

However, in the case of oligomer separations, some thoughts on the resolution and its dependence on plate count, slope of calibration curve, and molar mass difference between the oligomers may help in the selection of appropriate chromatographic

conditions. The resolution, R , of two molar masses, M_1 and M_2 , can be estimated as:

$$R = \frac{\sqrt{L \times N_{th}} \log M_1/M_2}{40 \times B \times V} \quad [5]$$

where $B = |(\log M_1 - \log M_2)/\Delta V|$ is the slope of the calibration curve, L is the column length, and N_{th} is the number of theoretical plates per metre (m^{-1}). The slope of the calibration curve for a column or column combination can be easily obtained from either the column manufacturer's catalogue or from measurements of the calibration curve (see Figure 4). The number of theoretical plates on the column of interest is easily determined by injecting a monodisperse





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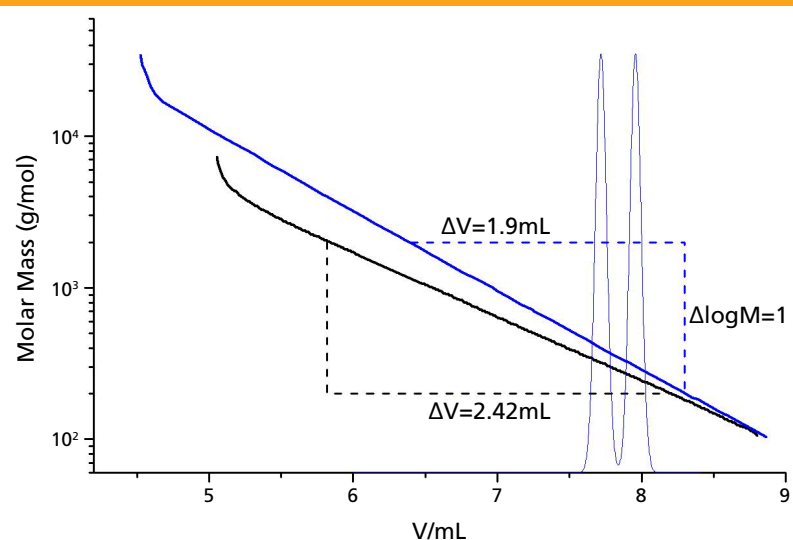




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Figure 4: Calibration curves for a typical 100 Å column (black) compared to a linear column (blue). Determination of the slopes of the calibration curves are indicated. The simulated chromatogram corresponds to the separation of the molar masses $M = 300$ and $M = 400$ g/mol for a 30-cm linear column with $N_{th} = 131,000$ m⁻¹.



substance and determining the peak width and elution volume.

To apply equation 5 for a given problem, let us assume that the task involves quantifying the amount of oligomers of three (trimer) or less monomer units in a polymer mixture. The quantification requires a resolution between trimer and tetramer of $R > 1.5$. For simplicity it is assumed that the repeating unit has a molar mass of 100 g/mol.

The calibration curves in Figure 4 represent the calibration curves of a typical linear, also called a *mixed-bed column*, in comparison to a single-pore size column with a nominal pore size of 100 Å.

From Figure 4 the slopes of the calibration curves of the mixed-bed and 100 Å column were estimated as 0.53 and 0.41 mL⁻¹, respectively. Trimer and tetramer elute at approximately 7.8 mL, as can be read from the calibration curves. To achieve a resolution of $R = 1.5$ using a single linear or a single 100 Å column of 30 cm in length requires at least 131,000 m or 78,600 m⁻¹, respectively, as calculated from equation 5. The steeper slope of the linear column requires less band broadening (higher plate counts) due to the smaller volume difference on the mixed-bed column. A larger number of theoretical plates can be obtained when using smaller particles;

however, this has an impact on column back pressure.

The very high number of theoretical plates required are rarely provided by typical GPC/SEC columns. If the column does not provide the required number of theoretical plates, a longer column could be applied. Equation 5 helps to estimate the required number of theoretical plates. When doubling the column length (thereby also increasing V_p and reducing B by a factor of 2, respectively) to achieve the desired separation, the required numbers of theoretical plates reduced to 65,700 m and 39,300 m⁻¹ for the linear and 100 Å columns, respectively.

While the required number of theoretical plates might not be easily achieved by two linear columns, the targeted separation is easily achievable on a column set of two 100 Å columns. These are better suited for low molar mass separations, due to the lower slope of the calibration curve.

Knowing plate counts and calibration curves of the column under consideration can help to identify whether a separation can be achieved on the column set. This avoids needless experiments, thereby reducing workload, solvent consumption, and instrument wear.

Summary

- Separating two polymers using GPC/SEC does not depend on the column performance alone.

- The equation provided can help to estimate the achievable resolution based on the weight average molar masses and the corresponding dispersities.
- To achieve a resolution of $R = 1.5$ and to allow for quantitation of two polymer components with a dispersity of 1.5, the weight average molar masses need to differ by approximately a factor of 45.
- The resolution of two monodisperse peaks of a given molar mass depends on a combination of plate count, slope of calibration curve, and column length.

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Maximizing Your Lab Productivity Anytime, from Anywhere, with waters_connect System Monitoring

How having a holistic view of all chromatography systems allows labs to run day-to-day operations more efficiently

LCGC: Can you tell us about waters_connect System Monitoring?

KATE WEARDEN: The waters_connect System Monitoring software is a new cloud product from Waters. It allows you to monitor your chromatography fleet, enabling you to optimize your lab operations from anywhere at any time. Users can find systems that are available for use in their lab much easier and quicker, and it provides a really detailed information, so decisions can be made about how to run the day-to-day operations of the lab. The software also provides real-time notifications when the status of an instrument changes, such as when a system is in an error state, and that allows users to react to and deal with those errors much more quickly. Information can also be provided about the type of error, making troubleshooting much easier and increasing system uptime. Detailed asset history information can also be gathered from System Monitoring that allows users and anyone interested in the operations of the lab to understand more about how and when the systems are being used. The views of the software can be customized, by the team or lab depending on the information pertinent to that particular group.

From a hardware perspective, the System Monitoring is vendor agnostic. That means you don't just see information from your Waters instruments on Empower, but other vendors' chromatography instruments, too. It really does provide much more of a holistic view of the lab. Multiple versions of Empower are supported, so all your systems can be viewed within System Monitoring provided you are running Empower Feature Release 4 and onwards. It can sit above multiple Empower instances, too, as long as they're all compatible with Windows 10. Importantly, System Monitoring sits outside of your validated Empower environment. We work with our cloud provider to make sure that the cloud infrastructure and the platform is fully secure and completely in line with best-in-class industry standards. There's no risk to the integrity of the data.

LCGC: What was the reasoning behind developing the software?

KATE WEARDEN: We found that labs are increasingly looking to find ways to improve uptime and efficiency. Labs are currently facing many challenges: rising consumer demand, limited resources, and a very rapidly evolving business landscape where things like compliance and data requirements for products must be met within much tighter timelines and smaller budgets. We have heard at Waters from many of our customers that it is a real challenge to get a universal, real-time view of their lab operations. Many labs have instrument management and scheduling tools, but they are often homemade. They can be a little bit fragmented, and they are not particularly dynamic or flexible enough to meet the changing needs of the lab. Things like troubleshooting can be slow and arduous. This translates to increased system downtime, which causes labs to start to become inefficient. As a result, samples often must be rerun, and we all know that wastes valuable resource and can delay getting product to market. To make sure that the lab is running optimally, scientists and lab managers often have to be physically on site as well, spending a significant amount of time to check individual systems and address issues. These are the challenges that were the core drivers in developing the new waters_connect System Monitoring software.



Kate Wearden
Principal Product Marketing Manager
Waters Corporation

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LCGC: The software is hosted on the waters_connect Cloud. Could you tell us what that means?

KATE WEARDEN: System Monitoring is deployed via the waters_connect Cloud platform. There are five key benefits of a cloud, or software-as-a-service (SaaS), model when it comes to lab software. First, it delivers connectivity between data and systems. As I mentioned earlier, it provides a holistic view of all chromatography systems on Empower, and that really gives you a complete picture of the lab operations and efficiency. The waters_connect Cloud also enables insights from different data sources, reducing silos and redundancy. Second, the system is connected to the cloud, and that ensures that you have instant access to new and incremental features and improvements as well as important security updates. All these are delivered automatically and seamlessly to users.

Thirdly, the cloud infrastructure itself is easily scalable and can flexibly respond to the changing needs of the lab. Our cloud approach allows us to remove that traditional infrastructure burden and cost from customers so they can focus on business objectives. The fourth benefit is that a SaaS model supports a shift from capital expenditure to operational expenditure. That allows increased flexibility, and it is typically an easier route to benefit realization, as well. Finally, the cloud really encourages collaboration amongst and across teams, departments, and other scientific partners, because they are more easily able to work together across the same platform and gain a deeper understanding of the day-to-day lab operations to make decisions faster.

LCGC: Can you give some examples of the new functionality you've been able to release as a result of customer feedback?

KATE WEARDEN: One feature we are really excited about based on feedback from customers is the addition of the new system scheduling functionality. I am sure there are many people reading this who have reserved systems with Post-it Notes or via a central whiteboard in the lab. That does the job, but it does not provide a real-time view. Even a lot of digital solutions that are available do not provide that either, and particularly not alongside a real-time view of the instrument status. With System Monitoring, the scheduling functionality will allow users to reserve systems for multiple reasons, such as for things like regular sample runs or calibration maintenance. Analysts, lab managers, and all those with permissions to view specific systems will be able to create reservations by searching for the appropriate systems for their analysis. We are hopeful that this and future developments to the software can help to overcome significant inefficiencies in the day-to-day activities of the lab. As we get more feedback from our users, we can continue to add more functionality to the software.

LCGC: What is the connected lab of the future?

KATE WEARDEN: Connectivity plays a key role in building the lab of the future. Many of us regularly use cloud software to manage our day-to-day

When viewed objectively, with the core aim of building a lab of the future that really sustains efficiency and productivity, there are quite a few benefits to considering connectivity as part of that goal.

lives, but the implementation of the cloud and cloud software and the practice of managing data via the cloud has historically been a bit more challenging for labs to adopt. Many labs are still quite hesitant to use the cloud and adopt the SaaS model as part of their digital transformation. There can be many, many reasons for that, such as a perceived financial commitment, concerns over security and additional IT infrastructure labs think might be required, or simply an aversion to change. When viewed objectively, with the core aim of building a lab of the future that really sustains efficiency and productivity, there are quite a few benefits to considering connectivity as part of that goal.

As mentioned earlier, shifting capital expenditure to operating expenditure places less financial burden on the lab. A SaaS model provides increased flexibility to scale more easily as the needs of the lab change. It is easier to install upgrades, so labs always have access to the latest version of the software. Breaking down of internal silos within an organization and with partners really allows labs to collaborate more effectively. All that is part of becoming a lab of the future. Ultimately, the cloud and system connectivity give labs the ability to share much richer data more easily and quickly, which expedites decision making and increases productivity.

LCGC: How is Waters supporting labs in their journey to becoming that lab of the future?

KATE WEARDEN: At Waters, we are committed to providing software and hardware solutions that allow labs to adapt easily as the laboratory environment changes, with the ultimate goal of increasing efficiency and productivity. Focusing on solving the problems that matter to labs allows us to support the digital transformation of labs with solutions like automation, connectivity, analytics, data security, and more. We really listen to our customers, and we deliver what they need to do their valuable work. The customer is essentially at the center of everything that we do, and it is paramount in our efforts to support the lab of the future.





Rising Stars of Separation Science: Selina Tisler

Publication of *Supercritical Fluid Chromatography Coupled to High-Resolution Mass Spectrometry Reveals Persistent Mobile Organic Compounds with Unknown Toxicity in Wastewater Effluents*

Named Assistant Professor in Analytical Chemistry at the University of Copenhagen

Publication of *Non-Target Screening for the Identification of Migrating Compounds from Reusable Plastic Bottles into Drinking Water*

2023

2022

2019

2015

Received master's degree

Received PhD degree

This month we interview Selina Tisler from the University of Copenhagen, Denmark, about her work using supercritical fluid chromatography (SFC) to detect very polar compounds and compounds of unknown toxicity in wastewater effluents, and her recent investigation into the migration of compounds from reusable plastic bottles into drinking water using a nontargeted screening approach.

—Interview by *Kate Jones*



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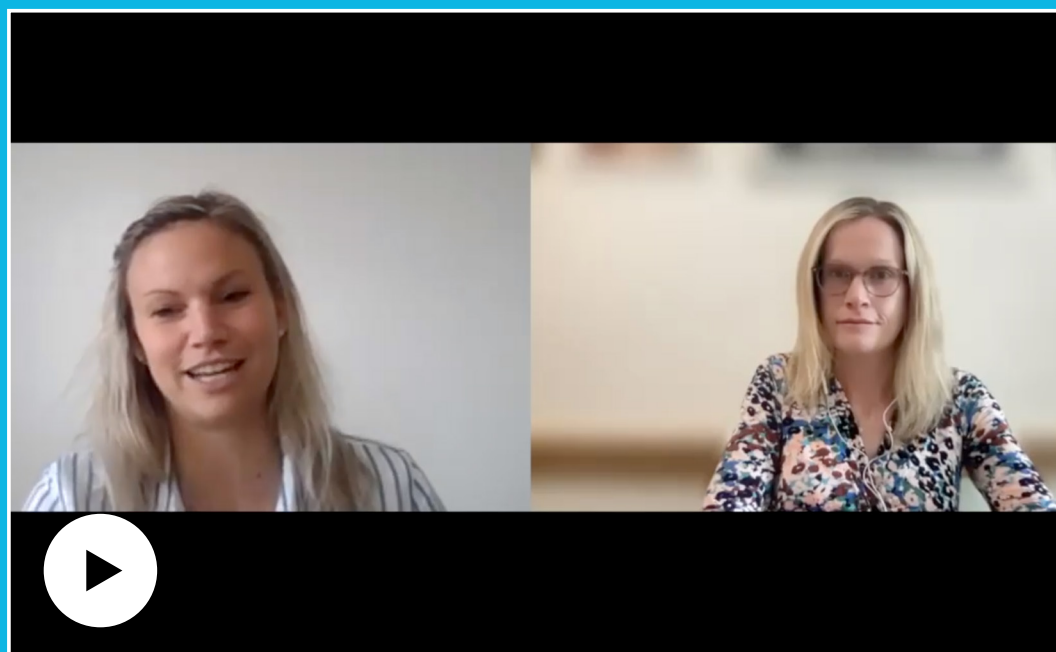
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SELINA TISLER is an assistant professor in the Analytical Chemistry Group in the Department of Plant and Environmental Science at the University of Copenhagen, Denmark. She received her PhD in environmental analytical chemistry in 2019 from the University of Tübingen in Germany. She was a postdoc at Aarhus and Copenhagen University in Denmark from 2019–2022. Her research focus lies in the advanced analysis of water, including nontarget screening of compounds of emerging concerns (CECs) and the identification of their transformation products in the aquatic environment. She has published 32 papers and book chapters, including 16 as first author. She has presented her work at more than 20 national and international scientific meetings, and she has been an invited speaker at more than 10 conferences.



To view our interview with our August Rising Star, Selina Tisler, please **SCAN HERE.**



RISING STARS OF SEPARATION SCIENCE

LCGC's sister digital publication, *The Column*, will be running a series of interviews in 2023, featuring the next generation of separation scientists. Information for nominating a "rising star" can be found here: bit.ly/3UFaS61.

Any questions about the submission process should be directed to Kate Jones, managing editor of *The Column*, at kjones@mjhlifesciences.com

Papers Mentioned in Interview

1. Tisler, S.; Savvidou, P.; Jørgensen, M. B.; Castro, M.; Christensen, J. H. Supercritical Fluid Chromatography Coupled to High-Resolution Mass Spectrometry Reveals Persistent Mobile Organic Compounds with Unknown Toxicity in Wastewater Effluents. *Environ. Sci. Technol.* **2023**, *57*, 9287–9297. DOI: 10.1021/acs.est.3c00120
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Analytical Chemistry: There is No Green Like More Green

Elia Psillakis¹, Stig Pedersen-Bjergaard^{2,3}, and Sibel Ozkan⁴, ¹School of Chemical and Environmental Engineering, Technical University of Crete, Greece, ²Department of Pharmacy, University of Oslo, Norway, ³Department of Pharmacy, University of Copenhagen, Denmark, ⁴Faculty of Pharmacy, Department of Analytical Chemistry, Ankara University, Turkey

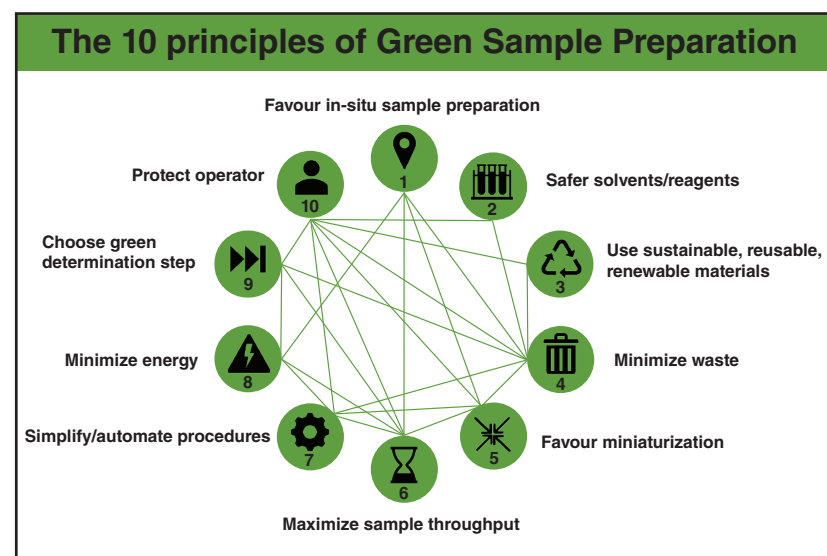
An examination of how greening analytical methods is directly connected to greening the sample preparation step

Analytical chemistry is an important tier of environmental protection and has been traditionally linked to the assessment of the environmental quality status of systems. Although essential, analytical chemistry may also contribute to further environmental problems mainly due to the high energy demands and large quantities of hazardous substances that may be used or generated throughout an analytical procedure. These two distinct and contradictory roles of analytical chemistry were highlighted by Paul Anastas a year after the introduction of green chemistry (1), when the concept of green analytical chemistry (GAC) was defined as an emerging area, relevant to the research arena and

commercial sector. At that time, traditional sample preparation methods were identified as a major source of the total negative impact of analytical methodologies on the environment and their replacement with contemporary ones was considered central in settling greener analytical methods. Indeed, early sample preparation methodologies were tedious, time-consuming, and, more importantly, expended large quantities of resources that resulted in the generation of hazardous laboratory waste.

In 2013, the concept of GAC was formulated in the form of 12 principles that expressed the willingness to care for the environment and human safety as part



Figure 1: The 10 principles of GSP and their interconnections.

of the development and application of analytical procedures (2). The introduction of GAC aimed to redefine and reevaluate analytical methods by addressing safety of solvents/reagents, toxic laboratory waste generation, workers' safety, and energy efficiency. In the formulation of GAC, the first principle suggested applying direct analytical techniques to avoid sample preparation and also concluded that any "green" action taken during the sample preparation step (for example, minimal use of energy, safety for operator, use of non-toxic reagents or reagents from renewable source) would have a negative impact on accuracy, precision, selectivity, sensitivity, and detectability of the analytical process. This was not a

well-reflected assumption, especially when considering the analytical performance of mature and green sample preparation technologies available at that time (for example, the solventless and reagentless solid-phase microextraction [SPME]). The use of direct analysis may be a straightforward approach to address problems related to sample preparation. However, to meet sensitivity needs and overcome matrix-related problems, the use of sophisticated, expensive, and energy-consuming instrumentation is required that generally shifts the environmental impact from sample preparation to the determination step. More importantly, direct analysis is not always an option and a step of sample cleanup, analyte

enrichment, or analyte conversion into a form suitable for analysis is commonly needed. In other words, sample preparation remains a key step in analytical procedures.

The first principle of GAC was commonly misinterpreted and created the false impression that omitting the sample preparation step is a green approach, fully neglecting the "green" technological advances in the field. The "exclusion" of sample preparation from GAC also created a gap by not considering cases where direct analysis was not an option. Instead of neglecting this step, efforts should have been devoted to fully defining sample preparation within the context of green chemistry and GAC. Over the years, redefining sample preparation to address sustainability issues and promote the practice of green sample preparation (GSP) has become a necessity. After all, green chemistry was never about what to stop doing, but was always about invention and the things one can do better.

Earlier this year, the concept of GSP was proposed (3). It was formulated in the form of 10 principles that represented a road map towards the development of overall greener analytical methodologies (Figure 1). This set of principles represented the optimum number of characteristics needed to describe the inner structure of the GSP concept, its properties, and function mechanism. The

10 principles were not isolated but formed an integrated system of design (Figure 1), where improvements achieved by aligning to the fulfilment of a given principle could synergistically help to reduce the deficiencies associated with other interconnected principles. GSP set goals that were common to GAC but also had several distinctive and innovative features. In every case, the GSP approach put sample preparation at centre stage and translated greenness based on the needs and requirements of sample preparation.

The aspects considered by GSP included the use of safe solvents/reagents; materials being reusable and from renewable, recycled sources, minimizing waste generation and energy demand; minimization of samples, chemicals, and materials; procedure simplification and automation; operator's safety; and preparing a high number of samples per unit time. Based on the advances in sample preparation, several contemporary and mature sample preparation technologies align with the principles of GSP and fulfil the requirements for greening this key step in analysis. It is equally important to note that the adoption of these methods in the laboratory not only aligns with the principles of GSP but also assists in improving the analytical characteristics of the overall method. The latter contrasts with the GAC approach that faces the challenge of reducing



the environmental impact of methods without negatively impacting the analytical efficiency of the method, that is, sensitivity, selectivity, accuracy, precision, robustness, and, in turn, the quality of the analytical information obtained.

Current environmental challenges are of a global scale, highlighting the urgent need to align with pollution abatement and the principles of sustainable development. Analytical chemists face increasingly complex interrelated problems both on-site and at the laboratory and the application of environmentally benign analytical practices has become a critical factor to consider. The adoption of contemporary sample preparation practices with a low environmental impact should therefore be a priority for all researchers, practitioners, and routine analysts. Greening analytical methods is directly connected to greening the sample preparation step. In other words, there is no green like more green.

Acknowledgements

This article is based upon work from the IUPAC project No 2021-015-2-500 "Greenness of official standard sample preparation methods" and work from the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society.

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Introducing Caroline Hroncich, New *LCGC* and *Spectroscopy* Associate Editorial Director



LCGC and *Spectroscopy* magazines recently welcomed Caroline Hroncich to lead their joint editorial staff in print and online. In this interview, Hroncich talks with editor Patrick Lavery about her experience and what readers can expect for the future.

—Interview by Patrick Lavery

Q. Tell us where you're from, and a little about your background.

A: I'm from New Jersey, I grew up in Bergen County and now I live in Hudson County, not too far from the MJH offices. I've been working in media for nearly a decade. I've written and edited for several consumer and trade publications including Reuters, Business Insider, MarketWatch, CannabisWire, and more. I have degrees from Villanova University and Columbia University.

Q. You're actually returning to the MJH family, correct? What did you do in your previous time here?

A: I started my career with *BioPharm International* and *Pharmaceutical Technology* magazines, which are both MJH brands. I was an associate editor on the team, and I managed both daily news content and more long-form print pieces. Working at *PharmTech* and *BioPharm* got me really excited about science journalism and



it's one of the reasons I've decided to come back.

Q. What drew you to this position, and were you familiar with our publications previously?

A: *LCGC* and *Spectroscopy* are incredibly well-respected publications in their industries. I've always been a fan of the work that you are doing and I'm glad that I now get to be a part of it. *LCGC* and *Spectroscopy* are on the cutting edge of the science and the topics we are covering will have impacts in research for years to come.

Q. Even in your short time on the job, what have you learned about separation science? Did you do a lot of background research before starting here, and what challenges do you expect?

A: I am still learning about separation science but as a journalist, I see it as a

new and interesting challenge. I'm a very curious person and I'm not afraid to ask questions. Right now, my goal is to meet with as many industry experts as possible to understand their work and how we can continue to cover the topics they are interested in.

Q. In addition to being legacy print brands, *LCGC* and *Spectroscopy* also have websites and social media footprints. As we know, people continue to get news and information on their devices more and more every day. Talk about how you envision the continued integration of our print and online products and what readers can expect to see on both sides of that equation.

A: I think it is all about balance. We have an incredibly loyal readership, and we'll continue to publish informative news and

peer-reviewed content in print and online. I want our readers to be able to engage with us wherever they are—whether that's on LinkedIn, by reading the magazine, or at a conference.

Q. What's it been like meeting and working with our team so far? What are the strengths that you see and how are you planning to use your experience to mold our product?

A: We have a great team at MJH. Even in my short time here, I've been impressed with how innately curious and passionate everyone is about the topics they cover. We have a team with a variety of talents, and I plan on tapping into that to make our content even better.

Q. Enough about work. Tell us more about yourself. What are your interests,

hobbies, fun facts, anything else you'd like to share?

A: I spend a lot of my free time with my friends and family. I enjoy cooking, reading, and traveling. I'm a very creative person. I like to paint and have recently gotten into ceramics. I also have two cats that I recently adopted and I'm always sharing photos of them on Twitter (@chroncich1)!

Q. Anything else you'd like our readers to know as we introduce you to them?

A: Just that I'm very excited to be working with our staff editors and the editorial board. I am always available to chat and you can reach out to me on the email listed below.

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The *LCGC* Blog: Forensics Laboratories Underassess Uncertainty in Blood Alcohol Determinations

Kevin A. Schug¹ and Zacariah L. Hildenbrand^{2,3}, ¹The University of Texas at Arlington, USA, ²Medusa Analytical, ³University of Texas at El Paso, USA

The level of uncertainty provided by most forensic laboratories for reported blood alcohol results has been woefully underassessed. Not only is this bad science, but someone's civil liberties may be at stake.

Over the last two years, our consulting firm has had the opportunity to review and assess more than 150 litigation discovery packets from a multitude of forensic testing laboratories. We have written previously about the overall lack of sufficient method validation and quality control in the cases that we have reviewed, the majority of which have been for blood alcohol determinations (1–3).

We have argued these deficiencies and others in the courtroom in several instances. It is disheartening to see forensics analysts from crime laboratories cling to outdated standard operating

procedures that do not conform to consensus standards propagated by nationally recognized organizations, such as the American Academy of Forensic Science (4,5). As analytical chemists who are regularly involved in the development of new methods, be it for environmental, pharmaceutical, or forensic science, we rely on consensus standards to define the steps and procedures needed to prove that a method and the measurements made are reliable. When these steps are not followed, the method and measurements may be subject to uncertainties and inaccuracies that have not been properly assessed.

In the scientific publication process, studies lacking appropriate validation and quality control are regularly rejected during peer review. Similarly, in forensics, measurements that have not been supported by widely accepted criteria for validation and quality control should not be relied upon in litigation, especially considering that someone's civil liberties may be at stake.

The uncertainty (or error) associated with a reported value is an important criterion to assess the reliability of a measurement. Accuracy can quickly be called into judgement when uncertainty becomes elevated. Uncertainty should also be assessed regularly, during the course of routine measurement of samples, because instrument performance does not remain constant over time. Instruments have to be regularly maintained and repaired because their performance will eventually deteriorate with use.

Uncertainty should also be comprehensively assessed on the instrument in question. Performance results obtained from one instrument should not be used to indicate the performance of a different instrument. This statement is obvious to the readership of *LCGC*, but such assessments—using results from one instrument to validate the performance of

another—has been commonly encountered in our review of forensic laboratory documentation.

When a blood alcohol concentration is reported, it is usually accompanied by a value for uncertainty at the 99.7% confidence interval. In a large collection of cases we have reviewed, this level of uncertainty has been declared to be 4.3% (for example, 0.188 ± 0.008 g/dL). This assertion claims that the "true" result for this blood alcohol determination has a 99.7% chance of being between 0.180 and 0.196 g/dL, and only a 0.3% chance of being outside that range.

When you look at these cases to see from where the 4.3% uncertainty value is derived, you find that it has been assessed solely based on a) the repeated analysis of calibration and control standards in neat aqueous solution with internal standardization, and b) the manufacturer's indicated uncertainty in the certified reference ethanol standards that they provided. In the end, they ascribe more than 70% of the assessed total variability in a reported blood alcohol determination to the variability coming from the repeat analysis of pure standards, with the remainder being attributable to the variability in the concentration of the certified reference materials, as assessed by the manufacturer.

In our opinion, this is a gross underassessment of uncertainty, especially for a method that is intended to measure a chemical substance from a biological fluid. Additionally, this uncertainty evaluation is only performed semi-annually, and the assessed uncertainty determined (4.3% at the 99.7% confidence interval) is applied across all instruments in, and results from, laboratories in the forensic laboratory system for blood alcohol determinations. Such a level of uncertainty can hardly be expected to be consistent for every instrument and operator in a large system of forensics laboratories, nor does it contain an assessment of uncertainty arising from biological matrices.

In the documentation for uncertainty evaluation for this collection of cases, the laboratories claim that blood matrix effects are negligible and do not need to be assessed because they were evaluated on a couple of instruments in one of the crime laboratories in 2016. To be clear, they contend that blood matrix interferences are absent in all the instruments across the forensic laboratory system because a set of tests were performed on one set of instruments at a single crime laboratory, seven years ago. Additionally, not all the headspace gas chromatography (GC) instruments across the system are from

the same manufacturer. The instruments used to perform the blood matrix interference studies in 2016 were from PerkinElmer, whereas many of the other laboratories in the system use Shimadzu gas chromatographs. Some laboratories use pressure-loop headspace systems and some use rail-based syringe autosamplers. They assume that all the instruments behave identically, which cannot be true.

Total error in an analysis method can be determined by assessing error propagation. Total error propagates as the square root of the sum of the squares of the errors from different error sources. Detector noise is a source of error, but this is usually very minor compared to other sources of error. Gas chromatographs are high precision instruments, and with internal standardization, they can provide very precise data, especially for pure standards. When the samples become more complex, such as moving from analysis of ethanol in water to analysis of ethanol in whole blood, greater variability will be imparted and must be assessed. Most analytical chemists will agree that the primary source of error in an overall method is sample preparation. Though sample preparation for blood alcohol determination is straightforward and generally involves a series of pipetting steps, it is not unreasonable to point out



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that pipettes can perform differently when transferring water vs. whole blood, just based on viscosity alone. This variability can also depend heavily on the pipetting technique used by the analyst.

There are other sources of uncertainty that are often unaccounted for. As mentioned, matrix effects can develop over time as instruments are used. If blank and ethanol-fortified whole blood controls are not regularly analyzed as part of quality control in a batch sequence, to verify absence of matrix effects and maintenance of accuracy, respectively, the forensic laboratory has no way to know whether their data are subject to additional uncertainties. The magnitude of the effects that these can exert on results is also difficult to conjecture. Besides neglecting matrix effects, enormous variability can be introduced through improper sample handling and storage. This particular issue is a topic that deserves its own subsequent blog post.

Overall, the level of uncertainty provided by most forensic laboratories for reported blood alcohol results has been woefully underassessed. The methodology that has been used to estimate uncertainty does not capture changing variability amongst different instruments and instrument types as they are used over time. It does

not capture variability associated with the preparation and measurement of complex biological samples, and it definitely does not capture variability in sample handling and storage. When these sources of error are not adequately assessed, they can only be accounted for by assuming reasonable levels of the variability possible for each. When those errors are propagated together with the limited assessment of variability from the forensic laboratory, then the window of “true” values represented by a reported measurement becomes much wider, such that the accuracy of the measurement, especially relative to some threshold (for example, 0.08 g/dL) becomes very debatable. Without proper assessment of the uncertainty of a method, the accuracy of the result it provides cannot be reliably established. In many of the cases we reviewed, forensic laboratories need to revise their procedures for uncertainty assessment, to be more realistic.

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The GC and GC–MS Clinic 5–6 October 2023

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Website: www.anthias.co.uk/content/2-day-gc-gc-ms-clinic-1

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Website: www.chromacademy.com/channels/infrared/principles/introduction-to-infrared-spectroscopy

Hands-On ICP-MS 13–14 November 2023

Location TBC

Website: www.anthias.co.uk/training-courses/hands-on-icp-ms

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Website: <https://2023.balatonsymposium.hu>

17–22 September 2023

6th International Mass Spectrometry School

Cagliari, Sardinia (Italy)

Email: gianluca.giorgi@unisi.it

Website: www.spettrometriadimassa.it/imss2023

24–27 September 2023

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