Take back control of your GC by switching from helium to hydrogen

Helium shortages have seen labs receiving only 45% of their current supply, while costs have kept rising and delivery disruptions are adding to delays.

A switch to hydrogen carrier gas allows you to take control of your gas supply, reduce costs and improve efficiency.

Carrier run time: He 125 minutes
Carrier run time: H₂ 74 minutes

Achieve faster carrier gas run times with Hydrogen compared to Helium.

www.peakscientific.com

Your local gas generation partner
Why switch carrier gas?

PEAK are able to take your lab gas stress away with their Precision hydrogen generators. Precision comes with zero air and nitrogen modules for all GC carrier and detector gas needs in a single, space-saving stack. With method translation becoming easier, instrument manufacturers taking hydrogen into consideration for new models, and hydrogen offering faster analysis times – it's never been a better time to switch to hydrogen.

Contact us today to discover more!

www.peakscientific.com/contact
Gas Chromatography
Hidden uncertainty in GC methods
Discovery, validation, and development from a single proteomics platform

Take advantage of pioneering proteomics to accelerate research and biomarker discovery with the SomaScan® Assay. Learn how searching 7,000 proteins from a 55-µL sample can help turn raw data into meaningful insight.

Pioneer further at SomaLogic.com/DiscoverMore
ANALYSIS FOCUS
Taking the Red Pill: Alleviating Matrix Effects for Small Molecule Quantitation in Food and Feed

Lewis Botcherby

Rudolf Krska and Michael Sulyok from the University of Natural Resources and Life Science in Vienna, Austria, discuss their recent work developing a multi-analyte approach using liquid chromatography tandem mass spectrometry (LC–MS/MS), and the issues surrounding agrocontaminants in animal feeds.
The Publishers of LCGC Asia Pacific would like to thank the members of the Editorial Advisory Board for their continued support and expert advice. The high standards and editorial quality associated with LCGC Asia Pacific are maintained largely through the tireless efforts of these individuals. LCGC Asia Pacific provides troubleshooting information and application solutions on all aspects of separation science so that laboratory-based analytical chemists can enhance their practical knowledge to gain competitive advantage. Our scientific quality and commercial objectivity provide readers with the tools necessary to deal with real-world analysis issues, thereby increasing their efficiency, productivity and value to their employer.

The doing of an unauthorized act in relation to a copyright work may result in both criminal and civil liability. For further information, please contact the following email: ARockenstein@mjhlifesciences.com.
Welcome to the August/September issue of LCGC Asia Pacific. In this month’s cover story, GC Connections looks at experimental uncertainties that may be present in gas chromatography (GC) methods. It is important to understand that experimental error and uncertainty are inherent in all analytical techniques and plan accordingly.

In the peer review article this month, Amitha K. Hewavitharana and co-authors discuss how complete overlapping of analyte and internal standard peaks is critical for eliminating matrix effects in liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis. They found that a column with reduced resolution achieved coelution of the analyte and the deuterated internal standard and was an effective method in overcoming the problem.

Column Watch takes a look at the overloading of porous layer open tubular (PLOT) columns—the basic types of column overload, volume and mass—and defines the variables that most affect the sample loading capacity in PLOT column chromatography.

In the Analysis Focus feature, Rudolf Krska and Michael Sulyok from the University of Natural Resources and Life Science in Vienna, Austria, discuss how matrix effects continue to be an issue in small molecule quantitation in food analysis, and present their work developing a multi-analyte approach using LC–MS/MS.

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

Mike Hennessy Jr, President and CEO, MJH Life Sciences™
In the process of a liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay development for two antimicrobial drugs using their stable isotope-labelled (SIL) analogues as internal standards to correct for matrix-ion suppression effects, an unusually large scatter of data was observed. A systematic observation revealed that the analytes and their SIL analogues (internal standards) were not completely coeluted; therefore, the analytes were not experiencing matrix effects similarly. When a column with lower resolution ability was used to achieve complete overlapping of the analyte and internal standard peaks, the scatter of LC–MS/MS data was minimized, indicating that the maximum correction of matrix effects by internal standards occurs when they are completely coeluted with the analytes. This study highlights the importance of ensuring complete overlapping of analyte and internal standard peaks in eliminating matrix effects when using SIL analogues as internal standards in LC–MS.

**KEY POINTS**

- Ensuring complete overlapping of analyte and internal standard peaks can be critical for eliminating matrix effects in LC–MS/MS analysis.
- A column with reduced resolution achieved coelution of the analyte and the deuterated internal standard and was an effective method in overcoming the problem.
preventing matrix effects in LC–MS is often unattainable. The only practical option to obtain LC–MS data that are not affected by matrix effects is to perform a correction for those matrix effects.

Correction for matrix effects is commonly achieved by using a specialized internal standard calibration procedure; the internal standard used must have almost exactly the same physicochemical properties as the analyte so that it will behave the same as the analyte both in the column and in the MS detector. This condition is commonly achieved in LC–MS by using a stable isotope-labelled (SIL) analogue of the analyte as the internal standard. This internal standard is often coeluted with the analyte but can be resolved from the analyte in LC–MS because of the slight difference in its mass from that of the analyte. As the SIL internal standard is expected to be eluted exactly at the retention time of the analyte, the presumption is that it experiences the same matrix/ion-suppression effects as the analyte in its passage through the MS detector. Therefore, by using the internal standard calibration with a coeluting SIL internal standard, the analyte peak response is usually corrected for the ion suppression matrix effects.

FIGURE 1: Chromatograms with scales adjusted to comparable peak sizes, showing the extent of coelution of fluconazole with its deuterated analogue (early peaks), and homoserine lactone with its deuterated analogue (late peaks). (a) Chromatogram is with method-1 and (b) chromatogram is with method-2. (a) Chromatogram shows that the (deuterated) internal standards have slightly longer retention times than the analytes F and H.
This article describes the systematic coeluting SIL internal standard is required coeluting internal standard. Therefore, a}

**Experimental**

**Instrumentation:** Compounds were separated using an Agilent 1100 LC binary pump and an Agilent 1100 autosampler. The columns used were as follows: a 2.1 × 50 mm, 3.5-μm Zorbax Extend-C18, 80 Å high performance liquid chromatography (HPLC) column from Agilent for method 1, and a 2.0 × 20 mm, 2-μm Synergi Fusion RP, 100 Å HPLC column from Phenomenex for method 2. An API 3000 tandem mass spectrometer with a turbo ion spray interface and the software program Analyst 1.5 from Applied Biosystems were used for detection and quantification.

**Materials:** Fluconazole (F) and N-(3-oxododecanoyl)-L-homoserine lactone (H) and fluconazole-d$_4$ (Fd), were purchased from Sigma-Aldrich. The stable isotope analogue of N-(3-oxododecanoyl)-L-homoserine lactone, N$_4$-(12,12,12-d$_4$-3-oxododecanoyl)-L-homoserine lactone (Hd), was synthesized by Dr. S.R. Chhabra at the Center for Biomolecular Sciences at the University of Nottingham in Nottingham, UK. The stable isotope analogue of fluconazole, fluconazole-d$_4$ (Fd), was purchased from BOC Sciences. All solvents used were of HPLC grade.

**Preparation of Samples and Standard Solutions:** Stock solutions of analytes and deuterated analytes were prepared in acetonitrile and stored at -20 °C. Standard solutions for a six-point calibration curve were prepared to match analyte concentrations in 1000 times dilution of samples (as described below), using 100 μL of combined internal standard solution (300 μM N$_4$(12,12,12-d$_4$-3-oxododecanoyl)-L-homoserine lactone and 40 μM fluconazole-d$_4$ in 10% acetonitrile aqueous solution), appropriate volumes of 100 μM fluconazole, appropriate volumes of 100 μM and 3.36 mM N$_4$-(3-oxododecanoyl)-L-homoserine lactone, and 10% acetonitrile to make the final volume up to 1000 μL. The concentrations of calibration standards were 10, 20, 30, 40, 50, and 60 μM N$_4$-(3-oxododecanoyl)-L-homoserine lactone; 1, 2, 4, 6, 8, and 10 μM fluconazole; 30 μM N$_4$(12,12,12-d$_4$-3-o xo dode canoyl)-L-homoserine lactone (in all six standards); and 4 μM fluconazole-d$_4$ (in all six standards) in 10% acetonitrile.

**Preparation of Mobile Phase:** Mobile phase A consisted of deionized water containing 0.1% (v/v) formic acid, and mobile phase B consisted of acetonitrile with 0.1% (v/v) formic acid. Both solutions were filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter (Millipore) before they were used.

**Chromatographic Conditions:** Separation was carried out at an ambient temperature of approximately 25 °C. The flow rate was 200 μL/min with an injection volume of 10 μL. Following injection, analytes were separated using gradient elution: For method 1, mobile phase composition was changed from 10% B to 100% B during the first 15 min, then held at 100% B for 2 min before returning to 10% B from 17 to 20 min. The original composition of 10% B was maintained for the final 8 min prior to the next injection. For method 2, mobile phase composition was kept at 12% B for the first 6 min before increasing it from 12% B to 55% B during the next 3 min. Then, it was held at 55% B for the next 6 min before changing it to 100%
over the next 2 min. Then, the mobile phase composition was held at 100% for 1 min before returning to 12% B over 2 min; the original composition of 12% B was maintained for the final 6 min to equilibrate the column prior to the next injection.

**Mass Spectrometry Conditions:** Multiple reaction monitoring (MRM) was used in positive ion mode. The transitions of 298 m/z ion to 102 m/z ion (for H) and 301 m/z ion to 102 m/z ion (for Hd); and 307 m/z ion to 238 m/z ion (for F) and 311 m/z ion to 242 m/z ion (for Fd) were monitored for each chromatographic run. The MS parameters were optimized for each analyte to obtain the highest sensitivity. The optimized values for H were: orifice–declustering potentials (DP) of 101 V, ring–focusing potentials (FP) of 370 V, collision energy (CE) of 19 V, and collision exit potential (CXP) of 8 V. The optimized values for F were: DP of 56 V, FP of 330 V, CE of 23 V, and CXP of 16 V.

An ion spray voltage (IS) of 5000 V and entrance potential (EP) of 10 V were used. Curtain gas (CUR), nebulizer gas (NEB), and the collision gas (CAD) flows were maintained at 12, 8, and 8 L/min, respectively. The temperature of the ion spray was maintained at 400 °C. A dwell time of 1000 ms was used for all transitions. Resolution of both Q1 and Q3 were 1 amu.

**Results and Discussion**

An unusually large scatter was observed with both H and F data obtained from the developed method (method 1). A systematic study was therefore carried out to identify the reasons behind this phenomenon. A combination standard containing H, Hd, F, and Fd was injected and LC–MS/MS was run six consecutive times using method 1, which was the method in use at the time. The upper chromatogram of Figure 1 shows peaks for H and F along with their coeluted internal standards Hd and Fd. The uppermost row of graphs in Figure 2 shows the individual peak areas of H and F, Hd and Fd, and the peak area ratio of the analyte and internal standard for H and F (all presented as percentage deviation from the third of the six runs). It is clear from Figure 2 that the internal standards did not correct for the differences in elution and detection conditions. The chromatograms, shown in the top of Figure 1, further reveal that there were slight differences in the retention times between analytes and internal standards. Differential retention times between analytes and their deuterated analogues have been previously observed (11,12) and attributed to the small differences in lipophilicity of the internal standard and the analyte because of deuteration. The difference is more pronounced with the F/Fd pair, as Fd has four deuterium atoms whereas Hd has only three. Because of the differences in the retention times, the elution and detection conditions experienced by the analyte and the internal standard differ, and the resultant ion-suppression effects experienced by the two compounds are not
similar. This explains the scatter in the data after correction with the internal standard (the ratio plot on the top right graph in Figure 2). The percent standard deviations for data in ratio plot 1 are 6.67% (H/Hd) and 26.2% (F/Fd), and those for ratio plot 2 are 1.35% (H/Hd) and 1.37% (F/Fd).

To force the two compounds to be coeluted, various gradients were investigated. However, the differences in lipophilicities and the resolution capabilities of the column were too high to achieve coelution simply by changing the elution conditions. Therefore, a column with lower resolution capacity was used to promote the overlap but still to achieve similar retention of both analytes. By using an appropriate gradient with the new column (method 2), we achieved better overlap of peaks (bottom chromatogram in Figure 1). The bottom row of graphs in Figure 2 shows the effect of peak overlapping: Although there is observable scatter in individual peak areas in the first two graphs in the second row, there is insignificant scatter in the third graph (the ratio of analyte to internal standard), indicating the intended function of the internal standard. The extent of overlap in Figure 1 (top vs. bottom) is suggested as the direct cause of the extent of scatter in the last column of Figure 2 (top vs. bottom). Thus, it is clear that despite the seemingly very small difference in peak overlap, its impact on the accuracy and precision of the data is significant.

Therefore, it is advisable to ensure precise coelution by periodic surveillance of chromatograms obtained in routine LC–MS methods as well as in method development because slight changes in the column and in the eluents and solvents may affect the extent of coelution. It is also recommended to examine and ensure the linearity of analyte and the internal standard responses, within the concentration ranges expected, when using the analyte–internal standard response ratio for calibrations (13).

To obtain a significant mass resolution and to prevent cross-talk, the masses between the analyte and the internal standard must differ at least by 3 amu (12). As observed in our study, replacing the three H atoms by deuterium can change the physicochemical properties, likely lipophilicity, of the molecule significantly.

The problem of differential lipophilicity, and its effect on the change in retention, can be minimized by using alternative (to deuterated) stable isotope-labelled analogues such as C-13, N-15, or O-17 as internal standards (11,12). As analysts who develop LC–MS methods are well aware, sourcing a SIL internal standard is a challenge that is often expensive. The most common types of SIL internal standards available are deuterated analogues.

The only other effective alternative to using SIL internal standards, to correct for matrix effects in LC–MS, is to use the standard addition with internal standardization (14).

Conclusion

The effects of incomplete coelution of the analyte and SIL internal standard on the scatter and the accuracy of the LC–MS data were studied. Compound deuteration affects the retention of analytes on reversed-phase chromatography, causing the analyte and its deuterated analogue to separate slightly. This separation leads to incomplete coelution of the analyte and SIL internal standard, and consequently differential matrix effects on the analyte and the internal standard, giving rise to scattered and inaccurate results with internal standard calibration. Using a column with reduced resolution to achieve coelution of the analyte and the deuterated internal standard proved to be an effective method in overcoming the problem.

Acknowledgements

This study was funded by an International Association for Dental Research (IADR)-GlaxoSmithKline Innovation in Oral Care Award 2016 awarded to Dr. H.M.H.N. Bandara.

References


ABOUT THE AUTHORS

Amitha K. Hewawitharana and P. Nicholas Shaw are with the School of Pharmacy at the University of Queensland in Queensland, Australia. H.D.C. Smyth is with the College of Pharmacy at the University of Texas at Austin in Austin, Texas, USA. L.P. Samaranyake is with the College of Dental Medicine at the University of Sharjah in Sharjah, United Arab Emirates. H.M.H.N. Bandara is with the Bristol Dental School at the University of Bristol in Bristol, United Kingdom.
Is the Solution Dilution?
Hidden Uncertainty in Gas Chromatography Methods

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

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

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

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

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

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

**Dilute-and-Shoot**

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

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

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

As seen in Table 2, the serial dilution procedure, which most analysts would prefer today because it uses far less solvent, has significantly higher experimental uncertainty, and this experimental uncertainty would then be added to any uncertainty in the stock standard. Note that when making extremely low concentration standards, which is a common need with the extremely sensitive methods and instruments of today, the experimental uncertainty involved in using even the most precise available glassware to prepare standards and

---

**FIGURE 1:** Close-up photograph of a 100 mL Class A volumetric flask.
COMPACT, MODULAR AND EFFICIENT
VICI DBS H2, N2 & ZERO AIR 19” RACK GAS GENERATORS

- 19” 3U Rack suitable for all static and mobile cabinets
- H2 Purity 99.99996%, Zero Air Purity <0.1ppm THC
- Primary applications: mud logging, process GCs, THA, stack gas and emissions test analyzers
- No maintenance, high purity gas supply with proprietary cell technology & 2 year warranty
- RS232, RS485 and USB connections for remote monitoring

For more information scan the code
samples in multiple dilution steps can lead to relatively large uncertainties that may reduce the number of significant figures in the experimental results. As a result, it is likely that any determinations at sample concentrations lower than parts per million (ppm) level should be reported with no more than one or two significant figures.

**Uncertainty In Sample Injection**

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

With the use of an auto-injector and proper maintenance, a syringe can remain highly reproducible for hundreds or possibly thousands of injections. Syringe manufacturers provide quick guides on how to take care of and perform maintenance on syringes (6) that include suggestions for extending syringe lifetime. With the high precision of auto-injectors, users can typically expect injections with less than 1% RSD in the resulting peak areas from injection to injection. Difficulty can arise when the syringe is changed; you can expect peak areas to vary by several percentage points up or down from the original syringe. With effective calibration, and either internal or external standard, this change may not be noticed unless working at or near the limit of detection (LOD) or limit of quantitation (LOQ), where a few percent of reduced injected sample volume might move results lower than the LOD or LOQ.

**Uncertainty In Detection and Quantitation**

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

In general, greater increases in experimental uncertainty from calculations occur when subtraction and division are used in equations and formulas. In both cases, the calculation result becomes smaller while the uncertainty becomes larger. Table 3 illustrates this principle with some simple calculations, in which the values $1.100 \times 10^2 \pm 0.2$ and $1.000 \times 10^2 \pm 0.2$ are combined by addition, subtraction, multiplication, and division. Note that both initial values would be seen as highly precise. As seen in Table 3, the original data have a relative uncertainty of 0.2%. When the data points are multiplied or divided, the uncertainty is determined from the relative uncertainties, so the final uncertainty is the seam on a relative basis for both. Since experimental uncertainties are additive for addition and subtraction, adding the two data points results in a decreased relative

### Table 2: Calculation of experimental uncertainty from the glassware for two 1:100 dilution schemes. Assumes ±0.1% relative uncertainty in the concentration of the original stock solution, use of Class A glassware, and use of the classical dilution expression: $M_2 = M_1 \times \frac{V_2}{V_1}$.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Glassware Used</th>
<th>Glassware Percent Uncertainties (%)</th>
<th>Uncertainty in Final Dilution (%)</th>
</tr>
</thead>
</table>
| 1         | 1 × 100 mL volumetric flask
 1 × 1 mL volumetric pipette | 0.08% (flask)
 0.60% (pipette) | 0.6% |
| 2         | 2 × 10 mL volumetric lask
 2 × 1 mL volumetric pipette | 0.20% (flask)
 0.60% (pipette) | 0.9% |

### Table 3: Propagation of uncertainty for simple operations with impact on relative uncertainty and significant digits of the result using two artificial data points: $110.0 ± 0.2$ and $100.0 ± 0.2$.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Raw Result</th>
<th>Uncertainty</th>
<th>Final Result</th>
<th>Final Uncertainty (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original data</td>
<td>100.0</td>
<td>0.2</td>
<td>100.0 ± 0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Addition</td>
<td>110.0</td>
<td>0.2</td>
<td>110.0 ± 0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Multiplication</td>
<td>$1.100 \times 10^1$</td>
<td>30</td>
<td>$1.100 \times 10^1 ± 3 \times 10^1$</td>
<td>0.3</td>
</tr>
<tr>
<td>Subtraction</td>
<td>10.0</td>
<td>0.3</td>
<td>10.0 ± 0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Division</td>
<td>1.100</td>
<td>0.003</td>
<td>$1.100 ± 0.003$</td>
<td>0.3</td>
</tr>
</tbody>
</table>
uncertainty as the result increases faster than the uncertainty. However, subtracting them results in a much greater relative uncertainty, since the result is now smaller and the uncertainty larger. In this case, the four significant figure original data are reduced to three in the result. The relative experimental uncertainty increases from a fraction of a percent to nearly 3% in the subtraction case. When I examine GC methods with unsatisfactory reproducibility, the first places to look for the source of the problem are calculations and glassware choices, followed by injection and detection.

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

References

ABOUT THE AUTHOR
Nicholas H. Snow is Founding Endowed Professor in the Department of Chemistry and Biochemistry at Seton Hall University, USA, and Adjunct Professor of Medical Science. Direct correspondence to: amatheson@mjhlifesciences.com

---

Dextran
PEG
PEO
PMMA
Polystyrene
Polystyrene (High Temp)
Pullulan

When Standard means Superior GPC/SEC ReadyCal Calibration Kits

---

www.chromatographyonline.com 15
Injecting too much sample onto a chromatographic system will eventually overload the column. Analytical chemists are concerned about column overloading because it reduces the column efficiency, and, as a result, the resolution is sacrificed. Surprisingly, there is little data in the literature about the overloading of the porous layer open tubular (PLOT) columns. This month’s article looks at the basic types of column overload, volume and mass, and defines the variables that most affect the sample loading capacity in PLOT column chromatography.

Column overloading in chromatography occurs when we inject too much sample onto the column. Injecting too much sample volume corresponds to volume overload, and will result in peak broadening. Peaks can either stay symmetrical or exhibit tailing. Volume overload is hard to demonstrate, and it is fairly rare. Peak broadening often happens due to the slow sample transfer onto the column or dead volume in the system. As an illustration, in the chromatograms presented in Figure 1, the same sample volume and concentration were injected on-column. In the chromatogram labelled Figure 1(a), the lower total flow through the injection port contributes to slow sample transfer, a source of peak broadening and tailing. However, the resolution can be significantly improved by accelerating the sample transfer using a split injection. A narrower initial sample band will allow for narrower peaks with less tailing, increasing resolution.

A completely different overloading case results when injecting too high of a concentration of analyte onto the column. That is considered a mass overload. Overloaded peaks are much broader, and their shape is distorted and non-Gaussian. As a result, we notice considerable changes in the retention times.

There are two basic types of capillary columns in gas chromatography (GC): the standard, wall-coated open tubular (WCOT) column with a thin layer of a liquid stationary phase coated to the deactivated wall of the column, and the porous layer open tubular (PLOT) column, where the stationary phase is a solid adsorbent that is coated onto the column wall (1). Separations on a liquid stationary phase are based on partitioning and principles of solubility as opposed to PLOT columns, where separation is based on adsorption, which is a surface process (2). Due to the fundamental separation disparities between both types of column, the main difference in how overloading will be observed is by their opposite peak shapes (Figure 2).

Compared to the liquid stationary phase, where an overloaded peak has a typical shark fin shape (slowly increasing in response then sharply falling back on the baseline), the overloaded peak on a PLOT column has the opposite shape (Figure 2) (3). When the adsorbent sites are occupied with the analyte, a portion of the sample travels with the mobile phase, and is eluted from the column faster. The consequences are that the retention times move towards lower values and the peak exhibits tailing. This is not a classical tailing usually caused by active sites present in the column. The peaks are more similar to a right triangle—the signal rapidly raises when the component is eluted, followed by a slow decrease, which results in a tail observed in the chromatograms in Figures 2 and 3. Note that the peak-end remains almost at the same retention time. At the signs of overloading, we say we have exceeded the sample loading capacity of the column.

Broader peaks show a loss in resolution, and changes in retention times could potentially be a source of misidentification. For these reasons, we like to avoid overloaded peaks. Nevertheless, overloaded peaks do not necessarily impact linearity. Figure 3(a) shows an overlay of the calibration chromatograms. The gradual changes in peak shape indicate we are overloading the column with the sample. However, the calibration curves show the linearity was not compromised, and r^2 values greater than 0.998 were achieved for all three analytes (Figure 3(b)).
that can be put on a GC column at some set of conditions where the peak shape of the chromatogram is symmetrical (4). However, perfectly symmetric peaks are rare in PLOT column chromatography, with most peaks showing some degree of tailing. To estimate the overloading point, we used a "capacity cup-full point" Dean Rood's approach, which is the amount of solute where broadening of the peak width by 10% at the half-height occurs (5). At that point, the increase of peak width is so severe that the loss of resolution is approximately 10% (2).

An experimental evaluation of the effects column internal diameter (i.d.) has on the loading capacity of PLOT columns was performed using two porous polymer Q-Bond PLOT columns. The increase in loading capacity for ethyl acetate was measured when switching from a 0.25 mm i.d. column to a 0.53 mm i.d. column with the same phase ratio and translated analysis conditions. The capacity point was determined by injecting samples with a gradual increase of analyte concentration from 3–140 ng for a 0.25 mm i.d. column, and 10–550 ng for a 0.53 mm i.d. column (Figure 4). At the overload point, where the peak width increased by 10% of the initial value, loading capacity decreased from approximately 350 ng on a 0.53 mm i.d. column to the 50 ng for a 0.25 mm column i.d. As we move from a 0.25 mm i.d. column to a 0.53 mm i.d. column, we increase the sample capacity for our system nearly sixfold.

**Evaluation of Increase in Stationary Phase Layer Film Thickness on the Loading Capacity**

A similar approach was used to evaluate the impact of film thickness on capacity. Two columns with the same dimensions, but different thicknesses of the porous polymer layers, were compared in the analysis of ethyl acetate. Overloaded peaks show signs of tailing, which is measured as symmetry. In Figure 5, we plotted the loss of symmetry for ethyl acetate—an increase in tailing from the initial value as measured by the Agilent ChemStation (6)—with the increasing
concentration of the analyte on-column. The column loading capacity somewhat increases as we increase the stationary phase film thickness, as observed from a less drastic loss of peak symmetry on a 12 µm film thickness stationary phase. As we moved to a thicker layer column, the capacity increased from 50 ng to 75 ng of ethyl acetate on the column measured at the point when the peak width exceeded 10% of the initial value. A 33% increase in the thickness of the stationary phase resulted in a 0.5 times increase in loading capacity in our study.

**Conclusion**

Due to surface interactions on a PLOT column, the loading capacity is inherently limited, and signs of overloading are easily observed. Similar to a WCOT column, higher capacity can be achieved by considering larger column internal diameters and the thickest possible layer of stationary phase. Overloading with too high a concentration of analyte results in broader, distorted peaks, and can be avoided by injecting less, diluting the sample, increasing the split ratios, or even evaluating different phases. Sometimes overloading cannot be prevented. For example, overloaded peaks are common in the analysis of impurities due to the significant difference in concentrations between the compounds.

**References**

4. J. Cochran, “Sample loading capacity for PAHs on a 30m x 0.25mm x 0.25µm Rxi-5ms GC column,” Restek Chromablography: https://www.restek.com/en/ chromatography/chromablography/sample-loading-capacity-for-pahs-on-a-30m-x-0.25mm-x-0.25mu-5ms-gc-column/

**ABOUT THE CO-AUTHORS**

Katarina Oden is an application chemist at Restek Corporation in Bellefonte, Pennsylvania, USA.

Jaap de Zeeuw is CEO of CreaVisions in Boxtel, The Netherlands.

**ABOUT THE COLUMN EDITOR**

David S. Bell is a Research Fellow in Research and Development at Restek. Direct correspondence to: amatheson@mjlifesciences.com
**Taking the Red Pill: Alleviating Matrix Effects for Small Molecule Quantitation in Food and Feed**

Matrix effects continue to be an issue in small molecule quantitation in food analysis, with researchers exploring different approaches to circumvent the issue. LCGC Asia Pacific talked to Rudolf Krska and Michael Sulyok from the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria, about their work to develop a multi-analyte approach using liquid chromatography tandem mass spectrometry (LC–MS/MS), and the issues surrounding agrocontaminants in animal feeds.

**Q.** Liquid chromatography–tandem mass spectrometry (LC–MS/MS) has become ubiquitous for the quantitative determination of small molecules in food and feed samples. However, according to your 2020 paper (1), matrix effects continue to be an issue for those using an LC–MS/MS method—in particular for multi-analyte approaches. What are the issues surrounding matrix effects in this area?  

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

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

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

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

Q. A further paper published in 2020 presents a comprehensive quantitative LC–MS/MS approach for the analysis of agrocontaminants in animal feeds. What challenges exist in the analysis of animal feeds and why are they important? MS: Animal feed is particularly challenging...
because the fractions of the main ingredients vary to a large extent, thus emphasizing the issue of relative matrix effects. In addition, finding samples that are true blanks for all analytes of interest is close to impossible, which makes it difficult to find a suitable sample set for method validation. We have proposed to prepare model samples from the related single feed ingredients mirroring the common ranges of the exact composition.

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

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

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

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

References
2) B. Greer et al., TrAC 141, 116284 (2021).
4) www.romerlabs.com/shop/inter_env/reference-materials/biopure-standards/mycotoxins

This interview has been edited for length. The full version can be viewed at www.chromatographyonline.com
**Solid-Phase Extraction**
The compact ASPEC 241 system combines positive pressure solid-phase extraction (SPE) with liquid handling for automated SPE applications. With its minimized footprint, it can be used for SPE automation on 1, 3, or 6 mL cartridges for labs with limited throughput. The system generates reproducible sample clean-up for forensic, food, and environmental analysis.

www.gilson.com
Gilson, Wisconsin, USA.

**Protein Kit**
This kit contains 10 different proteins preweighted into autosampler vials for the convenient molecular weight and radius calibration of aqueous GPC/SEC systems. The concentrations are optimized for UV detection @ 280 nm.

www.pss-polymers.com
PSS Polymer Standards Service GmbH,
Mainz, Germany.

**TOC Vials**
Ultra-low TOC certified vials <10 ppb, suitable for air, water, and soil analysis as well as pesticide residues, pharmaceutical manufacturing, and forensic tests. TOC vials are supplied with white open-top PP screw cap with 22 mm natural PTFE/white silicone 3.0 mm thick septa (EPA-quality) and dust cover. 72 pcs/pack.

Teknikroma Analítica S.A.,
Barcelona, Spain.

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

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

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

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

**Prep System**
Prepbox is a compact alternative to Ecom’s modular preparative systems. It is used for high-capacity systems or with centrifugal chromatography systems, such as in pharmaceutical purification of cannabis extracts. One basic gradient pump, optionally a secondary pump for repetitive sampling or with injection valve, switching valve for CCC applications, PDA 4-channel detector up to 800 nm.

www.ecomsro.com/systems/compact-preparative
ECOM spol. s r.o., Chrastany u Prahy, Czech Republic.
Exosome Characterization in Biological Matrices Using Asymmetrical Flow Field-Flow Fractionation and Nanoparticle Tracking Analysis

Postnova Analytics GmbH

For therapeutics development, a comprehensive investigation of formulations during the optimization process is essential for later clinical trials. Often the in vivo behaviour differs significantly from the previous in vitro activity. Therefore a characterization under conditions mimicking the in vivo environment is gaining in interest (1).

Both field-flow fractionation (FFF) and nanoparticle tracking analysis (NTA) have gained in increasing popularity for the analysis of complex biological samples, including extracellular vesicles such as exosomes, over recent years (2–4).

In this application note, we present the online hyphenation of asymmetrical flow field-flow fractionation (AF4), multi-angle light scattering (MALS), and NTA to analyze exosomes spiked into rabbit serum.

Experimental
The exosome pellet (exosome standard, which was extracted from human urine) was recovered according to the manufacturer’s guidelines. Prior to injection, the exosomes were diluted to the final concentration of $1.64 \times 10^{10}$ particles mL$^{-1}$ in phosphate-buffered saline in a 1:10 dilution of rabbit serum in ultrapure water (UPW).

The experimental setup is illustrated in Figure 1. The samples were fractionated in the AF4 channel according to their hydrodynamic size. To reduce the flow rate for NTA measurement and to increase the sample concentration after the dilution in the channel, the Slot Outlet technique (also known as Smart Stream Splitting) was used: the upper sample-free solvent stream was split away at the end of the fractionation channel. The AF4 system was also coupled with a MALS detector to derive the size (radius of gyration, $R_g$) of the fractionated sample constituents. To deliver the sample at an appropriate flow rate (here approximately 12 μL min$^{-1}$) for the NTA measurement, an additional flow splitter was used for coupling both systems. The NTA determined the hydrodynamic diameter, $D_h$, and the concentration of the separated sample constituents.

Results
The rabbit serum represents a highly complex mixture containing a variety of proteins and electrolytes. To exclude matrix-induced interferences, a comprehensive fractionation step is advised prior to the analysis. Otherwise, various interactions between the matrix components and the incident laser light, such as fluorescence, would overlap with the scattered light and may render an accurate size determination by MALS and NTA virtually impossible. In addition, in NTA, the significantly lower intensity of scattered light from small particles may also result in a size distribution that is skewed towards the bigger particles.

Figure 1: Schematic principle of the AF4-MALS-NTA setup (1).

Figure 2: AF4-MALS fractograms overlaying different measurements comparing MALS signals and $R_g$’s (dots) from the exosome-serum sample (green line) with the exosome standard (blue line) and the serum blank (orange line) (1).

Figure 2 shows the obtained AF4-MALS fractograms overlaying the exosome standard, the serum blank, and the exosomes in serum, including the obtained $R_g$ distributions. The data confirm the successful separation of rabbit serum constituents from the exosomes. However, the serum blank also shows particles in the same size range as the exosomes, indicating that there may also be exosomes already present in the serum itself.
The $R_g$ distribution of the exosomes ranged from 23 nm to 100 nm and was not affected by the spiking into rabbit serum. In Figure 3, AF4-NTA fractograms with an overlay of $D_h$ and the particle number concentration are displayed for (a) the exosome standard and (b) the exosomes in serum. The $D_h$ distribution of the exosomes ranged from around 43 nm up to a maximum of 150 nm. The $D_h$ distribution of the exosome-serum sample ranged from roughly 35 nm up to 90 nm, with increasing variation to the exosome standard. The particle concentration and the relationship of $R_g$ and $D_h$ in a blank medium and in serum provide information on the morphology of the exosomes and indications of a corona formation. The hyphenation of AF4-MALS-NTA delivers this information within one single measurement, which reduces time, costs, and sample material.

Conclusions
Asymmetrical flow field-flow fractionation hyphenated to multi-angle light scattering detection and nanoparticle tracking analysis represents a powerful analytical platform to study the behaviour of promising drug delivery vehicles under in vivo-like conditions. Both techniques complement each other perfectly. The online matrix removal and sample purification capability of AF4 support the NTA to overcome its limitations, while NTA acts as a true particle-counting detector for AF4. Together with MALS, AF4-NTA may also enable particle shape analysis.

References
• 3× faster analysis of multiresidue pesticides in foods.
• Simple setup using a factory-coupled, leak-free kit.
• Ideal for fast GC–MS and GC–MS/MS methods.

Using a mass spectrometer as a GC detector has many advantages for compound identification and quantification, but there is another less known benefit: speeding up analyses by using the MS vacuum to lower pressure within the column. This technique, also known as “vacuum-outlet GC” or “low-pressure GC–MS” (LPGC–MS), can provide significant gains for fast GC–MS (1). For example, Figure 1 demonstrates that LPGC–MS using an LPGC column kit is three times faster than a conventional GC–MS analysis of multiresidue pesticides in food. Historically, the difficulty of making a manual leak-free connection has been a barrier to LPGC adoption but using a factory-connected kit makes leak-free installation simple and allows the speed gains of LPGC to be reliably achieved.

Why Use LPGC–MS for Fast GC–MS?
There are several options for fast GC–MS, so what makes LPGC–MS advantageous? For MS work, a 30 m × 0.25 mm column is usually used. This format generates about 120,000 theoretical plates; has optimum carrier gas flow rates within the MS vacuum pump capabilities; and can maintain positive inlet pressure, despite the vacuum at the end of the column. Here is a comparison of several common fast GC strategies to the LPGC–MS approach used in Figure 1.
1. A shorter, narrower column
A 10 m × 0.10 mm column has similar efficiency (plate number) and resolving power to a 30 m × 0.25 mm column. But this format has very low column capacity, so low concentrations or injection volumes must be used to avoid peak distortion.
2. A 30 m × 0.25 mm column run at a higher flow
Increasing the flow rate is the easiest way to reduce analysis time. But, to get the 3× faster analysis shown in Figure 1, a flow of approximately 12 mL/min is needed. This requires an inlet pressure of approximately 63 psi, which is problematic for injection, MS data acquisition rate, and MS pump capacity.
3. A 10 m × 0.25 mm column run at optimal carrier gas flow rate
A 3× shorter column has about 40,000 theoretical plates and should speed up analysis by 3–4×, but the inlet pressure required for this scenario is about 0.35 psi, which is very difficult to control. At such pressures, split injection is a challenge, column trimming is barely possible

Figure 1: Compared to a conventional setup, this LPGC–MS analysis of pesticides in food is 3× faster, even though a lower efficiency column is used. Sensitivity may be higher because the increased linear velocity creates taller, narrower peaks. Note that even closely eluting peaks can still usually be resolved by MS.
because it affects pressure, and extremely narrow peak widths make MS data acquisition challenging.

4. An LPGC column kit

An LPGC column kit consists of a 15 m × 0.53 mm analytical column that is factory coupled to a 5 m × 0.18 mm restrictor column. This configuration produces about 30,000 theoretical plates and can be operated at standard flow rates of about 2 mL/min. Optimal carrier gas linear velocities are very high because of the vacuum inside the 0.53 mm i.d. analytical column. This results in very short analysis times (typically 3× faster compared to a 30 m × 0.25 mm column). Peak widths are 1.5–2 s, which is broad enough for MS data acquisition. In addition, the 0.53 mm column has high capacity due to the 1 µm Rtx-5ms film.

How It Works

LPGC–MS speeds up analysis by using the MS vacuum, along with a specially designed column setup, to lower pressure inside the entire column. As Figure 2 illustrates, a 0.53 mm analytical column is inserted directly into the MS, and a flow restrictor is used on the GC inlet side; this allows low pressure to be maintained throughout the 0.53 mm analytical column. The reason an LPGC column kit makes this technique easier is because it provides a robust, zero-dead-volume, factory coupling of the necessary restrictor column and the recommended analytical column (which also includes an integrated transfer line). The LPGC column kit has been specifically designed to install easily, and each one is tested to ensure leak-free performance, meaning the setup for LPGC–MS is now essentially as easy as changing a column.

Implementation Considerations

Although the LPGC column kit makes the technique’s physical setup much simpler and more reliable than manual connections, some method development time is still required when translating a conventional method. This includes setting the column dimensions (restrictor column only) in the GC software and adjusting the oven ramp and flow rate as necessary. Online method translation calculators should not be used because they do not allow you to directly calculate new method conditions for low-pressure GC. Although some method development work is necessary when implementing LPGC, the significant increase in sample throughput makes it a worthwhile investment.

References

The Gold Standard in Field-Flow Fractionation

FROM THE COMPANY THAT INVENTED FFF

The Postnova FFF-MALS-DLS analytical characterization platform is the premier solution for the advanced analysis of nanoparticles, vesicles, proteins and macromolecules.

Direct access to molar mass, size, charge, structure, conjugation and elemental speciation are provided by hyphenation of our unique Field-Flow Fractionation platform technologies with:

- Multi-Angle Light Scattering
- Dynamic Light Scattering
- Mass Spectroscopy
- Size Exclusion Chromatography
- Intrinsic Viscometry

www.postnova.com

Asymmetrical Flow FFF ▪ Electrical Flow FFF ▪ Centrifugal FFF ▪ Thermal FFF
Centrifugal Partition Chromatography

Your cost-effective purification alternative

- Environmentally friendly, silica-free technique
- One-step purification process
- High recovery and purity

www.gilson.com/cpc