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How Switching from Helium to Hydrogen Carrier Gas in Thermal Desorbers Enhances Gas Chromatography–Mass Spectrometry Systems

Laura Miles and Elinor Hughes, Markes International, Bridgend, UK

Analysis of polyaromatic hydrocarbons (PAHs) in air demonstrates that a thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS) system can operate with hydrogen carrier gas as well as it does with helium.

Many semivolatile organic compounds (SVOCs) are considered hazardous to human health, including some phthalates (1) and benzo[a]pyrene (a five-ring PAH), a toxic air pollutant of interest to regulators both sides of the Atlantic (2,3).

However, traditional reference methods for measuring SVOCs in ambient air (4) are cumbersome and prone to high uncertainty (5,6). They typically involve using large pumps to draw large volumes (up to hundreds of millilitres) of air through filters backed by polyurethane foam, XAD-2, or other similar sorbents, then Soxhlet extraction and steam distillation followed by gas chromatography–mass spectrometry (GC–MS) analysis.
As thermal desorption (TD)–GC–MS technology has evolved over the years, its compatibility with increasingly higher-boiling compounds has improved (7). Now, TD-based methods provide a reproducible, readily automated, and convenient alternative approach for monitoring SVOCs in ambient air in non-dusty environments (8–16). Sample volumes in the order of 500 L are...
typically collected over a 24-h period using conventional small (personal monitoring) pumps at a rate of 350 mL/min. Subsequent TD–GC–MS analysis harnesses the SVOC recovery of modern TD technology and is suitable for GC-compatible SVOCs with volatilities above n-C_{44}(b.p. > 500 °C). Example compound classes include polychlorinated biphenyls (PCBs), phthalates (up to and including didecylphthalate), polycyclic aromatic hydrocarbons (PAHs), high-boiling hydrocarbons, and some polybrominated flame retardants (8–16).

With the latest enhancement to TD technology being certification for operation with hydrogen carrier gas, an investigation was carried out to see if there were any benefits or drawbacks of using hydrogen when measuring SVOCs. PAHs were selected as test compounds as they are particularly “sticky” and challenging. A PAH study using conventional helium carrier gas provided the basis for comparison (17).

**Why Consider Switching to Hydrogen Carrier Gas?**

Helium is a finite resource that is increasingly expensive and difficult to source as a GC carrier gas. In addition, it must be extracted and stored before being shipped around the world, giving it a high carbon footprint. Hydrogen, on the other hand, is simple to generate using water and electricity and would seem to be an obvious environmentally friendly alternative, securing against helium shortages in the long term and offering immediate cost savings. It also promises shorter analytical cycle times and faster sample throughput. It is important to test the impact of hydrogen carrier gas on system performance with SVOCs. No TD features or functions are compromised by using hydrogen and all multi-gas thermal desorbers can also be used with helium or nitrogen carrier gas without changing system hardware. Also, they can be connected to any hydrogen-ready GC and MS instruments.

**Experimental**

Using the chromatographic conditions reported in reference 17 as a starting point, clean sorbent tubes (Markes International “High-boiler” tubes: part number C2-CAXX-5138) were loaded with a 16-component PAH standard (10 ng/µL) using a calibration solution loading rig (Markes International; see reference 18 for method). The TD method was developed and optimized in a short iterative process (see reference 19 for method). The GC conditions selected for use with helium were translated to conditions for hydrogen carrier gas using software that is available free-of-charge online (20). The objective of this phase of the study was to achieve analyte recovery levels and chromatographic resolution with hydrogen carrier gas equivalent to those achieved with helium previously (17). The results are shown in Figure 1.

**TD–GC–MS Conditions Using Helium:**

- TD: instrument: TD100-xr (Markes International), focusing trap: ‘high-boiler’ tubes, tube desorption: 320 °C (15 min) with 50 mL/min tube and trap flow (no inlet split), trap desorption: –10 °C to 350 °C (10 min) at maximum heating rate, outlet split flow: 50 mL/min, flow path: 250 °C; GC: column: 30 m × 0.25 mm, 0.25-µm, DB-5ms (Agilent), column flow:
3 mL/min, oven ramp: 50 °C (1 min), then 15 °C/min to 100 °C, then 20 °C/min to 240 °C, then 10 °C/min to 260 °C (6 min), then 50 °C/min to 310 °C (5 min), inlet: 320 °C; MS: aux heater: 320 °C, ion source: 250 °C, SIM/scan analysis: m/z range 35–300.

**TD–GC–MS Conditions Using Hydrogen:** TD: instrument: TD100-xr Multi-Gas (Markes International), focusing trap: ‘high-boiler’ tubes, tube desorption: 320 °C (10 min) with 50 mL/min tube and trap flow (no inlet split), trap desorption: −10 °C to 350 °C (5 min) at maximum heating rate, outlet split flow: 50 mL/min, flow path: 250 °C; GC: column: 30 m × 0.25 mm, 0.25 µm, DB-5ms (Agilent), column flow: 3 mL/min, oven ramp: 50 °C (0.65 min), then 22.4 °C/min to 100 °C, then 30 °C/min to 240 °C, then 15 °C/min to 260 °C (6 min), then 74.5 °C/min to 310 °C (3.35 min), inlet: 320 °C; MS: aux heater: 320 °C, ion source: 250 °C, SIM/scan analysis: m/z range 35–300.

**Results and Discussion**

**Analysis Time:** The data obtained showed the expected significant gain in chromatographic efficiency without compromising resolution or sensitivity. Furthermore, it was found that complete recovery of the highest-boiling six-ring PAHs through the desorber could be achieved with 40% shorter desorption times using hydrogen compared with helium carrier gas—15 min with hydrogen versus 25 min using helium (Figure 2).

As the automated thermal desorber offered overlap mode—that is, it allowed the desorption of a subsequent sample to begin while GC–MS analysis of the previous sample continued—GC cycle times became the most important factor dictating system productivity. Figure 3 illustrates the improvement in sample throughput that can be achieved by using hydrogen carrier gas for this SVOC application. Broadly speaking, deployment of hydrogen carrier gas instead of helium allowed the analysis of at least one extra SVOC sample per hour.

**Assessment of Method Performance:** System performance was evaluated for peak shape at low levels, linearity, limits of detection and quantification (LOD and LOQ), and reproducibility. The use of quantitative sample re-collection and repeat analysis was also evaluated as a means of validating analyte recovery.

The results showed exceptional peak shapes, even at low picogram (pg) levels for each compound of interest. Figure 4
This demonstrates comparable results to those previously found with helium (17), and therefore no loss in sensitivity. Assuming air was sampled at 333 mL/min, allowing the collection of 480 L over 24 h (17), all LOQs were below 0.08 ng/m³ and linearity (R²) was greater than 0.995 for all compounds across the full volatility range for a concentration range of 0.01–10 ng standards (Figure 5). The results also showed exceptional sensitivity. Assuming air was sampled at 333 mL/min, allowing the collection of 480 L over 24 h (17), all LOQs were below 0.08 ng/m³ and all LODs were below 0.025 ng/m³ (Table 1). This demonstrates comparable results to those previously found with helium (17), and therefore no loss in sensitivity.
Excellent reproducibility was obtained, as demonstrated in Figure 6 where 10 replicate analyses are overlaid and show a high degree of consistency for both retention time and intensity. To validate analyte recovery, a standard (10 ng/µL) was re-collected and the analysis repeated several times. This process tests the entire TD–GC–MS workflow and is recommended in international standard methods such as ISO 16000-6 (21). If compound losses do occur, for example due to compound decomposition, hydrogenation, adsorption, or condensation, it would result in the affected peak area being smaller than expected in the repeat analysis, that is, smaller than that predicted from the split ratio or relative to other peaks in the mix. The data is shown in Figure 7 and demonstrates excellent recovery (17), that is, > 99% recovery across the PAH volatility range when using hydrogen as a carrier gas.

**Table 1:** LOQs and LODs for each target analyte calculated as a concentration in 480 L

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LOQ for 480 L Air Sample (ng/m³)</th>
<th>LOD for 480 L Air Sample (ng/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.015</td>
<td>0.004</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>0.029</td>
<td>0.008</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.013</td>
<td>0.004</td>
</tr>
<tr>
<td>Fluorene</td>
<td>0.035</td>
<td>0.010</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.042</td>
<td>0.013</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.050</td>
<td>0.015</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>0.025</td>
<td>0.006</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.021</td>
<td>0.006</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>0.033</td>
<td>0.010</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.029</td>
<td>0.008</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>0.031</td>
<td>0.010</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>0.040</td>
<td>0.013</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>0.077</td>
<td>0.023</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>0.042</td>
<td>0.013</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>0.023</td>
<td>0.006</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>0.027</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Pioneering new fields in ultra-trace analysis, the new GCMS-TQ 8050 NX triple quadrupole couples the powers of a world-leading GC and a newly designed detector. Both provide outstanding sensitivity at femtogram and even sub-femtogram levels.

Superior performance of NX technologies e.g. new flow controller and time management for maintenance

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The GCMS-TQ 8050 NX complements the Shimadzu NX family, coupling the Nexis GC-2030 with the quadrupole series TQ-8050, TQ-8040 or QP-2020. Shimadzu’s NX series provides high-end GCMS solutions for every analytical challenge.
In conclusion, it is important to consider that many other important advantages can be realized by switching to hydrogen. These include significant cost savings—hydrogen cylinders are between 6 and 10 times less expensive than helium cylinders and can be replaced by hydrogen generators for no significant downsides in terms of loss in sensitivity, reactivity, or method reproducibility. Moreover, important analytical quality control checks, such as quantitative sample re-collection for repeat analysis and validation of analyte recovery, have been shown to work just as effectively on the TD systems configured with hydrogen carrier gas as with helium.

Conclusion
This study has shown that the excellent performance of multi-gas-enabled thermal desorption for PAHs can be comfortably replicated using hydrogen carrier gas. Further, the results from this study have shown that using hydrogen significantly speeds up the overall analysis, offering both improved desorption efficiency and faster chromatographic separations with no significant downsides in terms of loss in sensitivity, reactivity, or method reproducibility. Moreover, important analytical quality control checks, such as quantitative sample re-collection for repeat analysis and validation of analyte recovery, have been shown to work just as effectively on the TD systems configured with hydrogen carrier gas as with helium.

In conclusion, it is important to consider that many other important advantages can be realized by switching to hydrogen. These include significant cost savings—hydrogen cylinders are between 6 and 10 times less expensive than helium cylinders and can be replaced by hydrogen generators for no significant downsides in terms of loss in sensitivity, reactivity, or method reproducibility. Moreover, important analytical quality control checks, such as quantitative sample re-collection for repeat analysis and validation of analyte recovery, have been shown to work just as effectively on the TD systems configured with hydrogen carrier gas as with helium.
efficiency of hydrogen can either be used to speed things up (as described in this study) or it can be used to achieve the same recovery within the same time but at lower temperatures. This is often preferred in trace-level studies, for example, where the most important factor is minimizing background rather than productivity or cycle times.

References
11. Markes Application Note 053, The performance of thermal desorption for the analysis of high-boiling SVOCs.
13. Markes Application Note 137, Quantitative determination of semi-volatile polychlorinated biphenyls (PCBs) using TD–GC–MS.
18. Markes Application Note 007, Preparing and introducing standards using thermal desorption.
19. Markes Application Note 021, Developing and optimising tube-based thermal desorption methods.
20. EZGC tool, Restek: https://ez.restek.com/ezgc-mtfc

Laura Miles is a senior application specialist in the thermal desorption business unit at Markes International. Laura is responsible for developing new methods and testing Markes’ suite of thermal desorbers for new and emerging applications. Laura joined Markes as a customer support specialist in 2014 before moving to work in application development. As part of her current role, Laura works closely with key opinion leaders in collaborations across a variety of market areas, and she has a particular specialism in environmental analysis, breathomics, and defence and forensics.

Elinor Hughes obtained her B.Sc. in chemistry and Ph.D. in organic chemistry at Bangor University, UK. After working for a chemical manufacturing company for three years, she moved to the Royal Society of Chemistry where she worked in journals publishing for six years and on Chemistry World magazine for four years. This was followed by five years as a freelance copyeditor and science writer. Her current role is technical copywriter at Markes International.

E-mail: enquiries@markes.com
Website: www.markes.com
Thermo Fisher Finalizes PPD Acquisition
Thermo Fisher Scientific (Waltham, Massachusetts, USA) has announced the acquisition of global clinical research and laboratory service provider, PPD (Wilmington, North Carolina, USA). The acquisition was first announced in April, with Thermo Fisher agreeing to purchase PPD for $47.50 per share, a total of $17.4 billion, plus the assumption of around $3.5 billion of net debt.

Speaking back in April, David Simmons, Chairman and Chief Executive Officer, PPD, said, “Thermo Fisher is a world-class company with a very similar culture and values and will provide a great foundation for our colleagues to continue to deliver for our customers and to develop their own skills and careers.”

PPD has more than 26,000 employees operating across 50 countries, and it produced revenue of $4.7 billion in 2020. Following the transaction, PPD has now become part of Thermo Fisher’s Laboratory Products and Services Segment.

“We are very excited to officially welcome our PPD colleagues to Thermo Fisher Scientific,” said Thermo Fisher Chairman, President, and CEO Marc N. Casper. “Expanding our value proposition for our biotech and pharmaceutical customers with the addition of PPD’s leading clinical research services advances our work in bringing life-changing therapies to market, benefiting patients around the world.”

For more information, please visit: www.thermofisher.com

Gerstel Group Changes Ownership
Holger R. Gerstel, Co-Owner and General Manager of Gerstel (Mülheim an der Ruhr, Germany), has acquired all remaining shares of the company from his brother, Eberhard G. Gerstel, completing a change of ownership for the German company. The transaction sees the retirement of Eberhard Gerstel from all roles and functions within the company.

“The foundation has now been laid to transfer Gerstel to the third generation of the family. I would like to expressly thank my brother Eberhard for making this possible, and for our excellent relationship over more than two decades of highly successful cooperation,” said Holger Gerstel.

Founded in 1967 by the late Eberhard Gerstel (1927–2004), Gerstel has grown under the stewardship of his sons Eberhard and Holger, who assumed control in 1998, and who, alongside Ralf Bremer, have developed Gerstel into a respected provider of laboratory analysis solutions, particularly in automated sample preparation.

“We will continue to invest significant amounts in development and production, in our research and application laboratories, as well as in our customer support and training facilities, to broaden our offering while continuing to provide our customers [with] best-in-class support,” continued Holger.

For more information, please visit: www.gerstel.com
Development of New Centroiding Algorithms for High-Resolution Mass Spectrometry

Researchers have developed two new algorithms capable of converting centroided data—generated during high-resolution mass spectrometry (MS) analysis—to mass peak profile data and vice versa (1).

Liquid chromatography and gas chromatography coupled with high-resolution mass spectrometry (LC/GC–HRMS) are ubiquitous when comprehensive chemical characterization of complex samples is necessary, generating extremely information-rich datasets on everything from environmental matters to biological problems. However, while the amount of data generated is often a benefit, the actual processing of that data becomes a challenge. This is particularly the case when dealing with unknown chemicals in highly complex sample matrices. One commonly employed strategy for the reduction of data size and information density is centroiding in which the distribution of the mass profile peak is represented with one point that is commonly associated with the mass peak apex. This approach is either performed by the instrument during data acquisition or as a step in a data processing workflow. Data can often be reduced 10-fold using this process, however, the price is the loss of information related to mass peak distribution—this provides valuable insights into the mass accuracy and precision. There is also a wide range of issues associated with the software packages used for centroiding, both vendor specific and open source. These issues can lead to uncertainty in results, and in particular analysis of complex matrices can be a struggle. The overall result is often a lack of reproducibility and issues with identification of unknown chemicals of interest.

One solution to these problems would be to introduce access to information related to the peaks in both time and mass domains, which has been shown to improve reproducibility and reliability in other techniques. Currently, most existing centroiding algorithms do not produce such information, and there is no algorithm that can estimate the peak mass widths from centroided data. As such, researchers have sought to develop and validate new algorithms capable of being seamlessly converted to profile data and vice versa.

The successfully developed algorithms, named the Cent2Prof package, was developed in Julia language and tested using seven previously analyzed datasets from three different vendors in both positive and negative modes. For evaluation purposes, the new algorithms were tested against an existing algorithm called MZmine.

Researchers found rates of false detection were reduced by ≤5% with the new algorithm package, with the MZmine algorithm having a 30% rate of false positives and 3% rate of false negatives. The error in profile predication was found to be ≤56%, independent of the mass, ionization mode, and intensity. This was six times more accurate than the resolution-based estimate values, according to the authors.

All of the algorithms are open source and open access, with the current model only being based on quadrupole time-of-flight (QTOF) data, which limits the application of the algorithms for orbital trap data. Researchers believe an additional model is needed for orbital trap data and will work on this in the future alongside optimization to improve the currently required time to run the package, which is around 16 min for a chromatogram consisting of approximately 2000 scans.

Reference
Novasep Invests €6 Million into Chasse-sur-Rhône Site

Novasep (Lyon, France) has announced a €6 million investment of its Chasse-sur-Rhône site, in Lyon, France. This investment will increase and modernize its manufacturing capabilities to support new generation active pharmaceutical ingredients (APIs), in areas such as oncology, central nervous system (CNS), and infectious diseases.

Continuous, or flow chemistry, is a growing area of importance in API manufacturing, offering advantages such as improved productivity, reduced energy and solvent consumption, better reaction control, increased process safety, and reduced exposure for personnel over traditional batch chemistry.

Novasep has invested heavily into flow chemistry since the establishment of a research platform at the Chasse-sur-Rhône site in 2013. This latest investment sees the installation of a cGMP flow reactor, and strengthens the site's ability to develop methods for efficient process development under continuous flow conditions.

“The addition of a cGMP flow capability at manufacturing scale, a technology which Novasep has been working on for several years in R&D, is an important step forward to strengthen our competitiveness in the active pharmaceutical ingredients market,” said Catherine Vindevoghel, Site Director of Chasse-sur-Rhône.

“We are proud of this investment, which will increase employment opportunities in France whilst improving responsiveness and reinforcing manufacturing capacities for essential therapeutic drugs for our customers, and ultimately for patients,” continued Vindevoghel.

In parallel, Novasep continues to reinforce its capabilities on the site for low to medium volume APIs, in response to increased customer demand (through the debottlenecking of drying and purification capacities, and the addition of a new clean room). These investments will enable Novasep to accommodate customers' growing needs for outsourced API manufacturing services, increasing flexibility, reinforcing state-of-the-art technologies, and strengthening its competitiveness.

The investment project was also selected and supported by the French government as part of the “France Relance” recovery plan. The programme rewards innovative and promising industrial investments in France, with the aim of reinforcing production capabilities of APIs for essential therapies, increasing economic growth and boosting employment.

For more information, please visit: www.novasep.com
News In Brief

Biognosys and Thermo Extend Agreement
Biognosys (Schlieren, Switzerland), a developer and provider of proteomics technology and solutions, and Thermo Fisher Scientific (Waltham, Massachusetts, USA), have announced an extension to a co-marketing agreement to provide life science researchers with comprehensive mass spectrometry solutions to gain deep proteomics insights at a large scale. Following the extended co-marketing agreement, laboratories with in-house proteomics facilities will continue to have access to mass spectrometry-based proteomics workflows combining Thermo Scientific mass spectrometry instruments with Biognosys’ proteomics data analysis software and kits. For more information, please visit: www.biognosys.com

Sotio and LCB Announce Agreement
Sotio Biotech (Prague, Czech Republic) has announced an exclusive, target-specific license and option agreement with LegoChem Biosciences (LCB) (Daejeon, Republic of Korea) for five new antibody–drug conjugate (ADC) programs. Rights to LCB’s ADC platform were acquired for a total of up to $1027.5 million payable based on certain developments and regulatory achievements. The deal enables Sotio to combine its proprietary antibodies with LCB’s ADC technology platform to deliver novel therapeutics for the treatment of solid tumours. For more information, please visit: www.legochembio.com or https://sotio.com

Peaks of the Month

• The LCGC Blog: ACS SCSC: What We Do and Who We Are—Amber Hupp provides information about the Subdivision on Chromatography and Separations Chemistry (SCSC) of the Analytical Division of the American Chemical Society (ACS), sharing their main goals and introducing their newly appointed executive board members. Read Here>>

• Tips & Tricks: Influence of Sample Dispersity on the GPC/SEC Separation of Multi-Component Systems—This installment of Tips & Tricks focuses on whether a complete separation can be achieved using GPC/SEC and, if so, under what conditions. Read Here>>

• LCGC Employment and Salary Survey 2021: A Year of Recovery—Pandemic, Back to the Office, Economic Changes, Hybrid Meetings, and More—Our annual LCGC salary survey takes a look at employment conditions, salary, benefits, and other workplace concerns affecting scientists and technicians working in the chromatography and separation sciences field, and assesses where things stand after more than 18 months since the start of the coronavirus pandemic. Read Here>>

• Rising Stars of Separation Science: Jelle De Vos—This month we interview Jelle De Vos, Senior Postdoctoral Researcher at the Department of Chemical Engineering, Vrije Universiteit Brussel, Belgium, about his recent work to develop a chromatographic method to characterize nucleocapsid proteins from SARS-CoV-2, and the development of spatial three-dimensional liquid chromatography (3D-LC) as a separation concept. Read Here>>

• The Role of Two-Dimensional Gas Chromatography in Arson Investigations—The Column spoke to Nadin Boegelsack and Gwen O’Sullivan from Mount Royal University in Calgary, Canada, about their work creating a workflow for use in the two-dimensional gas chromatography (GC×GC) analysis of ignitable liquid residues in arson investigations. Read Here>>

• Trouble Brewing: Categorizing Beers Using LC–QTOF-MS—Researchers used an untargeted LC–QTOF-MS method to analyze 32 beers across five different styles. Read Here>>

Like us  Follow Us
In Memoriam: Michael J. Hennessey, Sr.

Mike Hennessy Jr, President and CEO, MJH Life Sciences™

Michael J. Hennessy, Sr., was the beloved chairman and CEO of MJH Life Sciences, parent company of The Column.

Hennessy spent his career turning his passion for building businesses and creating jobs into a run of successful ventures and brands. Following his graduation from Rider University in 1982, he started his career in medical publishing as a sales trainee. In 1986, Hennessy became chief operating officer of Medical World Business Press, which was part of the launch of medical newspapers and other media products. The company prospered and was eventually sold to a Boston-based venture capital firm.

Hennessy launched Multimedia Healthcare, LLC, in 1993 and built a portfolio of award-winning clinical journals. In 2001, Freedom Communications, Inc., acquired Multimedia Healthcare, about the time that Hennessy was pioneering a new approach to print and digital publishing with Intellisphere, LLC (now part of MJH Life Sciences). Guided by the principles of innovation and entrepreneurial spirit, and reflecting its founder’s dedication to improving quality of life through health care research and education, Intellisphere publishes a variety of integrated print and digital products focusing on a range of topics in research and clinical medicine.

To build a comprehensive multimedia and education platform, Hennessy added additional companies and capabilities to the MJH Life Sciences portfolio. In 2004, he acquired Healthcare Research Analytics, which has been the leader in health care market research for over 30 years. In 2005, Hennessy acquired ArcMesa Educators, LLC, leaders in online certification for physicians, pharmacists, nurses, and other health care professionals.

In February 2008, Hennessy acquired the rights to the journals Pharmacy Times and The American Journal of Managed Care, both recognized in their respective markets as authoritative, trusted media platforms that provide essential information to a large audience of health care professionals.

In April 2011, MJH Life Sciences acquired Physicians’ Education Resource, LLC (PER), an accredited continuing medical education company that is an industry leader in producing high-quality, first-rate oncology and haematology meetings and conferences. The PER acquisition included a variety of multichannel enduring educational activities, as well the rights to legacy medical meetings, such as the annual Miami Breast Cancer Conference.

In 2019, MJH Life Sciences made its largest acquisition to date when it acquired the Healthcare and Industry Sciences divisions of UBM Medica, nearly doubling the size of the organization and adding European legacy titles such as LCGC Europe, Pharmaceutical Technology Europe, and Ophthalmology Times Europe to this already impressive portfolio. This acquisition made the organization the largest independently owned medical communications company in North America. In addition to acquisitions, Hennessy organically developed ancillary in-house agency divisions with Proximyl Health, Truth Serum NTWK, and MJH Global Medical Affairs.

Later in 2019, Hennessy elevated his own role to Chairman while naming his son, Mike Hennessy Jr, to assume the leadership role of the organization and carry on the family legacy. Under Mike Jr’s leadership, the company enhanced its global potential by entering into a long-term partnership with BDT Capital Partners, LLC in November 2021.

Due to his broad business and educational experience and understanding of the challenges facing New Jersey, Hennessy’s counsel and insight had been sought by several organizations, including his alma mater Rider University, where he served on the Board of Trustees and was elected to the executive committee. In addition to being active in state and national politics, Hennessy also had a long record of service at the local level, where he was a strong advocate for veterans and environmental issues.

Hennessy was preceded in death by his wife, Patrice Hennessey, who bravely battled cancer for almost 10 years until her death in January 2020. Hennessy donated $4 million to Rider University to expand the Science and Technology Center at their alma mater. The Mike & Patti Hennessy Science and Technology Center is set to be completed in 2022.
The 13th Multidimensional Chromatography Workshop: Why Does Multidimensional Chromatography Matter?

Katelynn A. Perrault¹, Pierre-Hugues Stefanuto², and Dwight Stoll³, ¹Chaminade University of Honolulu, Honolulu, Hawaii, ²University of Liège, Liège, Belgium, ³Gustavus Adolphus College, St. Peter, Minnesota, USA

The 13th Multidimensional Chromatography Workshop is a free event involving keynote presentations, contributed presentations, and discussion groups on all multidimensional techniques, and is happening virtually on 31 January–2 February 2022. The workshop welcomes both experts and new users to the field for training, networking, and sharing the latest trends. Keynote lecturers will present on major trends in both multidimensional gas chromatography (GC) and multidimensional liquid chromatography (LC), from the point of view of both academic and industry research.

Participants will be able to choose from several focus group discussions intended to stimulate discussion on key areas of the field. The full programme has
been released and indicates concurrent sessions on popular topics including modulation technologies and data analysis. The programme will also include concurrent poster sessions on different application areas. Presenters will be eligible for three poster awards:

- **Multidimensional GC Poster Award**—Sponsored by the American Chemical Society’s Subdivision on Separations Chemistry and Chromatography—$250 USD Student Award
- **Multidimensional LC Poster Award**—Sponsored by the American Chemical Society’s Subdivision on Separations Chemistry and Chromatography—$250 USD Student Award
- **Industrial Research and Development Poster Award**—Sponsored by CerTech—€250

**Keynote Lectures**
This year, the workshop will highlight the work of four keynote speakers. The speakers come to us with experience in either multidimensional gas chromatography (GC) or multidimensional liquid chromatography (LC), and they represent both academic research and industry sectors. In addition, we have asked all the keynote speakers to address the following questions in their presentations to get their perspective on the evolution of multidimensional chromatography:

- What sparked your interest in multidimensional chromatography?
- What was the moment you were convinced that multidimensional chromatography was a valuable solution to adopt?
- What is the biggest challenge facing the field of multidimensional chromatography?
- What is the most interesting application of multidimensional chromatography you have seen in the last year?
- Why does multidimensional chromatography matter?

**Robert E. Synovec**
P. E. Synovec is Professor of Chemistry at the University of Washington (UW) in Seattle (Washington, USA). He obtained his Ph.D. in 1986 from Iowa State University, and then joined the UW faculty that year. He served as Associate Chair of the Chemistry Graduate Education Programme from 2007–2020. Synovec has graduated 45 PhDs, 4 thesis masters, and 8 non-thesis masters students, with 10 postdocs and 60 undergraduate researchers. His group pioneers the development of novel analytical instrumentation and methodology based upon chemical separation science coupled with chemometric data analysis. The group investigates the basic principles of separation science, detection, and data analysis at both a fundamental and problem-solving level. He has over 270 publications and over 620 research presentations, which includes over 250 invited lectures and invited presentations. In May 2013, Synovec was awarded the GC×GC Scientific Achievement Award at the 10th GC×GC International Symposium. This award has been instituted to recognize the pioneering contributions of key scientists in promoting GC×GC instrumentation, method development, and/or applications. In May 2016, Synovec received the Marcel Golay Award at the 40th ISCC meeting in Riva del Garda, Italy, which is presented annually to a scientist in recognition of a lifetime of achievement in capillary chromatography.

**Petr Česla**
Associate Professor, University of Pardubice

**Optimization of Microcolumn Two-Dimensional LC—Towards Highly Sensitive Separation Methods**
Petr Česla is Associate Professor of Analytical Chemistry at the Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice. His research work focuses on the development of separation techniques in the liquid phase, mainly LC and capillary electrophoresis (CE), with special attention on two-dimensional separations, optimization procedures, data processing, and coupling of LC and CE to mass spectrometry (MS). He has been engaged in many research projects and acts as Principal Investigator for several projects for the Czech Science Foundation. He is author of more than 45 papers in scientific journals with 850 citations, and co-author of one book and two book chapters. He was supervisor of 20 diploma and 14 bachelor theses and currently supervises three Ph.D. students.
Melissa Dunkle  
Research Scientist, Dow Benelux BV  
Implementing GC×GC for Industrial Applications: The Good, The Bad, and The Ugly  
Melissa received her Ph.D. degree in 2007 under the direction of Luis A. Colón from the Department of Chemistry at the University at Buffalo, New York, USA. She then completed a two year postdoc at Ghent University in Belgium under the direction of Pat Sandra. In 2009, Melissa accepted a role as GC Specialist at the Research Institute for Chromatography (RIC) in Kortrijk; she then joined Dow Benelux BV in 2015 in Analytical Science, Core R&D. Her expertise includes liquid chromatography–high-resolution mass spectrometry (LC–HRMS, MS/MS), gas chromatography coupled to various detector technologies (GC–flame ionization detection [FID], GC–MS, GC–[HR] time-of-flight [TOF]–MS, GC–vacuum ultraviolet [VUV]), supercritical fluid chromatography (SFC), and multidimensional chromatography (LC×LC and GC×GC). In her current role, Melissa leads various research projects to advance analytical capabilities and improve the evaluation of natural gas and circular feedstocks.

Alexandre Goyon  
Senior Scientist, Genentech  
2D-LC Analysis of Oligonucleotides: From Their Conventional Analysis at the Intact Level to an Integrated Bottom-Up Approach  
Alexandre Goyon is Senior Scientist in the Genentech Research and Early Development (gRED) organization. He received a Ph.D. degree in pharmaceutical analytical chemistry in 2019 from the University of Geneva, Switzerland. He has published 32 peer-reviewed papers and he is the first author of 19 of them. His team supports early- and late-stage research.

Focus Groups  
This year, the workshop will host small focus groups to discuss the landscape of three narrower topics appearing to gain attention in the field of multidimensional chromatography. Attendees will choose one of the sessions to attend and contribute to the discussion. This will also be an opportunity for individuals to interact more easily in smaller groups to learn more about certain topics or to contribute their expert opinions. Focus groups will be moderated by experts in multidimensional separations to lead the conversation.

Topic 1: Simultaneous Detection: Who is doing it? What are the combinations being used? What are the benefits? What are the drawbacks? What is the difference in how they are used most commonly for multidimensional GC vs. multidimensional LC?  
Topic 2: Optimization: How do you optimize your separation? Who is performing full optimization for every study? Are users moving towards a standard column set for various applications? Do newcomers need to know how to do robust optimization to get started?  
Topic 3: External Software and Freeware Options: Who is using freeware written using languages such as R or Python? What part of data processing can be done in these programs? What are the pros and cons of using freeware (validation, standardization)? What are the benefits and drawbacks of external software vs. embedded approaches being developed?

Final Comments  
In preparing for the conference, we released a survey to ask participants—why does multidimensional chromatography matter? We hope to understand this question in greater detail at the end of the conference, but so far, respondents felt that multidimensional chromatography was helpful to:  
• Uncover the hidden contents of a sample;  
• Diversify the tools they are able to use to solve chemical problems;  
• Find what is unknown in the most complex samples.

Registration is completely free of charge and can be completed at www.multidimensionalchromatography.com. We hope that you will join us for this exciting event, whether you are looking to find out what multidimensional chromatography is all about, looking to improve your skills in multidimensional separations, or hoping to improve your network in the field of multidimensional chromatography.

Katelynn A. Perrault is at Chaminade University of Honolulu, Hawaii.  
Pierre-Hugues Stefanuto is at University of Liège, Belgium.  
Dwight Stoll is at Gustavus Adolphus College, St. Peter, Minnesota, USA.

Website: www.multidimensionalchromatography.com
Tips & Tricks: A Few Things Molar Mass Distributions Can (and Can’t) Tell

Wolfgang Radke, PSS Polymer Standards Service GmbH, Mainz, Germany

Gel permeation chromatography/size-exclusion chromatography (GPC/SEC) is the standard technique to derive molar mass distributions of polymers and macromolecules. However, there are several ways to display molar mass distributions, depending on the information one needs to extract from it. This instalment of Tips & Tricks will explain the benefits of a molar mass distribution over molar mass averages and the differences between the different representations of molar mass distributions.

Macromolecular samples are disperse in molar mass. Thus, specifying a single molar mass to a macromolecular sample is insufficient to describe its characteristics. Certain required information can only be obtained from the molar mass distribution. For example, registration of chemicals according to the Regulation concerning the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) in the EU requires determination of the amount of material below specific molar masses (1). USP and EP monographs for dextrans require the weight average molar masses to be calculated for the high and low molar mass fractions of the sample.

Gel permeation chromatography/size-exclusion chromatography (GPC/SEC) is the most important chromatographic separation technique for macromolecules,
allowing the determination of molar mass distributions. From the molar mass distributions the molar mass averages can be calculated. Often uncertainties in the interpretation of distributions exist because of their different representations. The information provided by molar mass distributions—in addition to molar mass averages—will be described in the following text.

**Benefit of Molar Mass Distribution Compared to Molar Mass Averages**

Macromolecular samples are often characterized using averages, such as weight-averages ($M_w$) and number-averages ($M_n$) molar masses. The ratio of weight- to number-average molar mass, known as the *dispersity index* $D = M_w/M_n$, is typically used to describe the width of a molar mass distribution.

Molar mass averages are often applied to compare samples. Differing molar mass averages correspond to different samples. However, identical averages do not necessarily indicate the existence of identical or even similar samples.

Furthermore, $D$ only provides a very rough indication on the width but not on the shape of the molar mass distribution. As an example, it is not clear if there is a uniform distribution with a single maximum or if there are several maxima due to a multimodal distribution.

In fact, for a given combination of number- and weight-average molar mass as well as the resulting dispersity, there exists an infinite number of possible distributions.
molar mass distributions. This is shown in Figure 1, where three different molar mass distributions are displayed. All three distributions correspond to the same weight- and the same number- average molar masses of 50,000 g/mol and 15,000 g/mol, respectively, resulting in the same dispersity index of 3.3.

While the average molar masses are identical, the molar mass distributions are clearly not. This shows that the information content of a molar mass distribution is much higher than that of simple molar mass averages.

In summary, the molar mass distribution contains much more information than simple molar mass averages and allows:

- Distinction between mono- or multi-modal distributions;
- Identification of inflection points or shoulders in the molar mass distributions, which might provide information on the polymerization process, for example;
- Determination of the amount of material below or above certain molar mass limits. Such information is often required for registration purposes and cannot be derived from average molar masses, but only from the distribution;
- Determination of mass fractions or even average molar masses within certain molar mass ranges, allowing much more precise material specifications for critical applications.

Differential and Cumulative (Integral) Molar Mass Distribution

Obviously, the molar mass distribution tells us something about “the relative portions of molecules with a specified molar mass or with molar masses within a specified range” (2). Whether the relative portions relate to a specific molar mass or to a molar mass range depends on whether the molar masses are assumed to be discrete or continuous. In the former case, the molar masses can take only discrete specified values, while in the latter case they can take any intermediate value.

In GPC/SEC, continuous molar masses are assumed, though in reality this is not the case. However, the assumption of a continuum of molar masses is frequently applied in polymer science as it eases calculations.

The term molar mass distribution is not precisely defined at this point. The distribution can be based on the number fraction of molecules or on the mass portion of molecules with molar masses within a specified range.

In GPC/SEC, the distribution function typically refers to the mass portion of material. The molar mass range of a typical industrial polymer sample usually covers orders of magnitude. Therefore, in GPC/SEC a logarithmic molar mass axis is usually chosen. The above discussed “mass portion of molecules” within a specified range
yields a so-called *differential molar mass distribution* and results in the often-found, nearly bell-shaped curves. It should be stressed that the molar mass distribution derived by GPC/SEC usually does not represent the number of molecules within specified molar mass ranges (frequency distribution) but the mass (weight) fraction of these, though they can be transferred into each other.

Frequently, besides the differential molar mass distribution, the cumulative or integral distribution is displayed. This typical sigmoidal-shaped curve represents the integral across the differential molar mass distribution from zero to the selected molar mass. Thus, the sigmoidal curve represents the area under the differential molar mass distribution curve. Consequently, the integral molar mass distribution I(M) describes the mass fraction of material with a molar mass less than M, within the sample (Figure 2).

Cumulative or integral distributions are used frequently to determine information required to decide on whether new products fall into the category “polymer of low concern” (PLC) or whether a product needs to be registered according to REACH (1).

To allow for better data comparison, the molar mass distributions can be plotted using different scaling on the ordinate. As mentioned above, the molar mass distribution \( w(\log M) \) represents the mass portion of material of a given molar mass. The area of \( w(\log M) \) is normalized to unity. In Figure 3(a), the different peak heights are a consequence of the different peak widths. As the distributions are normalized to unity, the different peak widths are compensated by the peak height.

Some people prefer a normalization such that the peak maximum corresponds to 100% (norm. \( w(\log M) \)) (Figure 3[b]). As an example, this allows the easy determination at which molar mass the mass fractions have decayed to a certain percentage of the sample’s most prominent molar mass fraction.

In contrast, the relative molar mass distribution (rel. \( w(\log M) \)) (Figure 3[c]) provides a non-normalized distribution. The total area under the curve varies with the amount of injected sample. Such a representation can be useful, for example if molar mass distributions are to be compared at different conversions. In that case rel. \( w(\log M) \) allows visualization of the change in molar mass distribution and conversion within the same graph.

It should be noted that the peak shapes and molar mass averages are not affected by the selection of the ordinate. Furthermore, the peak maximum of the molar mass distribution does not represent the molar mass of the most abundant molecules in the sample. It represents the molar mass of the molecules contributing the highest weight fraction to the sample.

**Molar Mass Distribution and Detector Signal**

To derive the molar mass distribution of an unknown sample, the sample’s detector signal is recorded as a function of elution volume. Based on the calibration curve, the elution volume axis is converted into a logarithmic-scaled molar mass axis (logM). At the same time, a transformation is applied to convert the detector signal into a relative concentration as a function of logM, in this case the molar mass distribution (3).

There are various detectors that can be hooked up to a GPC/SEC instrument.
At first glimpse the term *concentration detector* seems to be clear, as the higher the eluting concentration, the higher the signal. However, a second look is needed. Concentrations can be defined either in mass concentrations (units of mass per unit volume) or as molar concentrations (units of moles per unit volume). Again, at first it does not seem to matter if one elutes a given number or mass of molecules, as the eluting mass is given by the number of molecules multiplied by their molar mass. However, when performing a GPC/SEC analysis, if identical numbers of molecules (identical molar concentrations) are eluted at different elution volumes, they correspond to different molar masses. Consequently, the mass concentrations differ.

This situation is depicted in Figure 4 where three different polymethyl methacrylate (PMMA) molecules have a different number of repeating units. Each molecule carries an UV-active phenyl group at the chain end. The phenyl group gives rise to a strong UV absorption at 254 nm, where the repeating units do not absorb. Thus, the UV-signal at 254 nm will be proportional to the number of eluting molecules, namely to the *molar concentration* of eluting species. In contrast, the signal of an RI-detector is proportional to the number of eluting repeating units, ignoring the effect of the end group as a first approximation. Thus, the RI-detector’s response is proportional to the eluting *mass concentration*. Consequently, the term *concentration detector* is not as clear as it seems to be.

The question of detector response has a significant influence on the resulting detector signals. Figure 5 shows the chromatograms of a mixture of two PMMAs started using an UV-active initiator. Clearly, the shapes of the detector traces differ from each other. At higher molar masses (low elution volumes), the intensity of the UV-signal relative to the RI is lower when compared to higher elution volumes (lower molar masses). For the same UV-intensity at low elution volume (high molar masses), the number of repeating units is higher than at higher elution volumes (lower molar masses). Therefore, the relative RI-signal is higher at low elution volumes (lower molar masses). Since the signal traces of RI and UV differ, the molar masses calculated from the respective signals differ as well. The molar mass, however, is a sample property that is not expected to depend on the type of detector applied. Thus, if UV- and RI-detector traces differ, a second thought should be given to identify which signal to use for molar mass calculation.

Figure 5: Normalized RI- (green) and UV-traces (red) for a mixture of two PMMA samples initiated with an UV-active initiator.
Commercial GPC/SEC software expects the signal of the detector to be proportional to the mass concentration. This means that the detector needs to recognize the number of repeating units rather than recognizing the number of molecules eluting at a particular elution volume. In other words, a single chain of a given chain length has to yield the same signal strength as two molecules of half of the chain length.

Thus, care must be taken if detector traces between UV- and RI-detectors differ. The often-applied approach of simply choosing the less noisy signal is not recommended. As UV-signals tend to be more specific than RI-signals, the universal RI-signal should be preferred.

**Summary**
- The information content of the molar mass distribution is much higher than that of the molar mass averages.
- There exists an infinite number of distributions with the same combination of number- and weight-average molar mass.
- A molar mass distribution provides the mass fraction of molecules within a specified molar mass range. It does not show the number portion of molecules—though it can be calculated from the molecular weight distribution (MWD).

• GPC/SEC programs assume signals proportional to the mass concentration of eluting species.
• If UV- and RI-detector traces differ from each other, the RI-signal should be evaluated to derive molar mass distributions.

**References**

**Wolfgang Radke** studied polymer chemistry in Mainz (Germany) and Amherst (Massachusetts, USA) and is head of the PSS application development department. He is also responsible for instrument evaluation and for customized trainings.

E-mail: WRadke@pss-polymer.com
Website: www.pss-polymer.com
Rising Stars of Separation Science:
Hedvika Raabová

This month we interview Hedvika Raabová, a graduate student at the Charles University, Faculty of Pharmacy in Hradec Kralove, Czech Republic, about her work using novel micro- and nanofibres during sample preparation, and the concept behind composite micro-/nanofibre extractions.

—Interview by Kate Jones

Q. When did you first encounter chromatography and what attracted you to the subject?
A: The first time I came across a chromatographic instrument was when I was working on my master thesis five years ago. At that time, it was mostly a “black box” where I inserted my samples in one end and the results came out the other. It was only during my graduate studies that I began to learn more about this separation technique. This understanding resulted from the topic of my Ph.D. thesis where liquid chromatography (LC) played an important role. Thus, I cannot tell for sure when I genuinely chose to work in this field of separation sciences, but I definitely do not regret it. I realized very quickly how flexible liquid chromatography can be depending on which approach is chosen. The repetitive, large-scale analyses can be done quickly and precisely. One just needs to place the samples in the instrument and push the start button. Or one can be more creative and try different mobile and stationary phases, add more columns, use various detectors, and thus move the work to the next level.

Q. Can you tell us more about your Ph.D. thesis?
A: My thesis dealt with the application
of nanofibrous polymers as innovative sorbents for solid-phase extraction (SPE) coupled online with LC. The various nanofibrous polymers were manually filled into an extraction cartridge and this extraction unit was introduced into the chromatographic system. Then, the extraction and chromatographic analysis was performed and the extraction efficiency of the sorbents was evaluated from the peak areas. My Ph.D. thesis summarized all the theoretical and practical aspects of this topic. It included a description of sorbent fabrication procedures, modifications of the sorbent, and the comparison of the effectiveness of the polymer sorbents for different analytes. I described the challenges of the preparation of the extraction unit and discussed the pros and cons of nanofibrous extraction sorbents.

Q. What chromatographic techniques have you worked with?
A: I have to say, I am pretty conservative about the techniques I use. In my research I work mostly with high performance liquid chromatography (HPLC) or ultrahigh-pressure liquid chromatography (UHPLC) systems and reversed-phase separation mechanism. Depending on the project, I use them in one-dimensional or two-dimensional mode.

Q. Your primary focus concerns the use of novel micro- and nanofibres in sample preparation prior to separation via chromatography—what specifically attracted you to this area of research?
A: My current scientific focus was again more a lucky coincidence than a conscious decision. Since I have always enjoyed working in the laboratory, I wanted to complete my master thesis in the Department of Analytical Chemistry. The original task was just a simple analyte determination via HPLC. But at the same time, the initial experiments with nanofibres started in the department and my supervisor asked me to be part of the team. No one knew at that time if and how the extraction using nanofibres would work. This sparkle of uncertainty attracted me much more than the previous “safe” project and I started using nanofibres for the solid-phase extraction. First, the extractions proceeded separately, and the extracts were analyzed by LC to evaluate the extraction efficiency of the polymer fibres. The results were very promising, and it began to be clear to me that I would like to explore this topic a bit deeper. Therefore, I continued as a Ph.D. student.

Q. You have recently published a paper on polycaprolactone composite micro- and nanofibre material as an alternative to restricted access media (RAM) for extraction and separation of non-steroidal anti-inflammatory drugs from human serum (1). Could you tell us about this research? What do these novel polymer fibres offer over others available?
A: After all the elementary research with nanofibrous sorbents I had undertaken, I was curious to find some practical application. I finally had this opportunity when we confirmed that the composite micro/nanofibres extracted the analytes from cow milk and human serum with minimal prior treatment of those matrices. The analytes I chose were the non-steroidal anti-inflammatory drugs because they are common medication. It was also easy to obtain real-life samples from the local hospital. They are also administered in tens or even hundreds of milligrams doses, so the UV detector we used was sensitive enough to aid in detection. In the end, we had a method for online extraction of ibuprofen, ketoprofen, naproxen, and diclofenac from human serum that was used for diclofenac determination in a real-life serum sample. There is still a long way to go, but I feel that the nanofibrous sorbents are a bit closer to common application in...
analytical laboratories after publication of this study. We achieved great results with the composite micro/nanofibres that were comparable to the silica sorbents. Unlike purely nanofibrous sorbents, the composites have better mechanical stability and are therefore suitable for online extractions in high-pressure systems.

Q. Can you talk about the concept behind composite micro/nanofibre extractions?
A: As I mentioned before, the greatest advantage of this composite material is its mechanical stability. When we started using nanofibres for online extraction, it was hard to achieve a repeatable result as the sorbents could not withstand the high back pressure. The fibres collapsed in the extraction unit, leading to increase of the void volumes, and the extraction did not work well. We solved the problem by combining the nano- and microfibres in the sorbent. The technology for production of these fibres was invented at the Technical University in Liberec (Czech Republic) with which we cooperate. The composite micro/nanofibrous material combines the mechanical stability of microfibres and the enhanced surface area of nanofibres. These benefits significantly improved the results of our experiments.

Q. What application areas could these novel polymer fibre materials be useful for?
A: This is a tricky question, and it would probably cost me another four years of research to demonstrate other application areas, even just for analytical chemistry and separation sciences alone. But as far as I know, these composite materials are applied in tissue engineering, and I believe that they can also be used as a filtration material in the industrial sector.

Q. Are there any challenges involved with using these novel materials for sample preparation?
A: Of course there are, and as our research progresses new ones are emerging all the time. However, I think the most crucial issues concerning the preparation of extraction cartridges have already been overcome, for example, cartridge leaking, fluctuation of mobile phase flow and back pressure, sorbent dissolution. Now, we continue working on improvements in extraction efficiency and selectivity. Overall, in my opinion, dealing with the challenges is an inseparable part of the scientific research. If no challenges were left, it would be the end of the research.

Q. Anything else you would like to add?
A: I would like to point out that the nanofibrous sorbents should not be compared entirely with the commercial ones. The extraction using nanofibres has its own specifics, with some being beneficial while others are not. One day the fibrous sorbents may become an interesting alternative to the commercial products, but I do not think they can replace them completely.

Q. What are the emerging trends in sample preparation?
A: Sample preparation follows the global trend to do things better and faster. The trends are for miniaturized formats for sample pretreatment devices, which naturally results in the need for new sorbents. They must be very selective and provide a high extraction capacity as sample volumes decrease and so do the sorbent amounts. Scientists have already found many ways to achieve the desired sorbent properties, but the search for new materials is still ongoing. In addition, more sustainable approaches are becoming more and more valued in sample preparation. A reusability of nanofibrous sorbents and a biodegradability option for some of them support this trend.

Q. What projects are you working on next?
A: Actually, I also want to follow the trends in sample preparation and achieve more selective extractions using nanofibres. One idea is to link the specific antibodies on the fibre surface and proceed with the analyte extraction based on antigen-antibody interaction. I have not yet started because I have been busy with my Ph.D. thesis until now. Anyway, I want to give my full attention to this project at the beginning of 2022 and see how it goes.

Reference
The LCGC Blog: Critical Evaluation of Chromatography Methods—Essential Detective Skills

Tony Taylor, Arch Sciences Group and CHROMacade

In this series of blog posts, I’m going to explore how the challenges of adopting methods from the literature, or from internal or external clients, can often be made easier, and more enjoyable, by taking time for some detective work prior to even entering the laboratory.

I get excited when there is a new chromatographic method to implement or to transfer into the laboratory. I don’t understand why everyone doesn’t feel the same way.

With a sound understanding of chromatography principles and taking some time to properly understand the variables within each method (all of the variables not just the “headlines”) as well as considering those variables that have not been stated in the method, we can save ourselves a lot of trouble once we don our lab coats and cross the laboratory threshold. Of course, it takes time to build the knowledge and experience necessary to spot these potential issues, but once we are aware of the possible pitfalls within a method, we can pay close attention to them during the implementation of the method, or even change those parameters, where client or regulatory guidance allows.
The sources of “problems” within chromatographic methods are too numerous to list here; however, the starting point in any method detective work is a thorough reading of the method to gain a sense of whether or not it stacks up against our previous experience and knowledge. In this series, I’ll be covering some typical examples from problematic methods to highlight specific issues and areas to focus on but also expanding upon these a little, to give some general guidance on what does or does not, in my experience, constitute a clue that may be worthy of further investigation.

I’m going to start with a high performance liquid chromatography (HPLC) method, and we will concentrate on the mobile phase and HPLC column combination. I will consider the issues with column geometry and stationary-phase chemistry again in later blogs, so this example will serve as an illustration of what we might look for when investigating mobile-phase conditions.

I recently came across this information in a method that was being transferred:

- **Sample diluent:** Dilute sulphuric acid (approximately pH 2.8)–methanol 50:50
- **Column:** End-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R (1.7 µm)
- **Buffer solution:** Dissolve 1.36 g of potassium dihydrogen phosphate R in 900 mL of water for chromatography R. Add 0.15 g of sodium heptanesulfonate R, adjust to pH 7.0 with triethylamine R, and dilute to 1000 mL with water for chromatography R.
- **Mobile phase A:** Methanol R, buffer solution (10:90 v/v)
- **Mobile phase B:** Buffer solution, methanol R (15:85 v/v)
- **Gradient:** (See Table 1)

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<thead>
<tr>
<th>Time (min)</th>
<th>Mobile Phase A (% v/v)</th>
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You will note that I’ve not included any details regarding the analyte at this stage, we’ll consider this later.

For me, with over 30 years of practicing chromatography, the constituents of the mobile phase are recognizable but certainly not what one could call “modern.”

However, for some less experienced chromatographers, the mobile-phase additives (Figure 1) and the fact that the pH is titrated to a set value using one of the reagents will be, frankly, alien. Potassium dihydrogen phosphate is a traditional buffer that is used to resist small changes in pH when the sample in its diluent (approximately pH 2.8) mixes with the eluent within the instrument tubing.
and primarily at the head of the analytical column. As we wish to maintain a pH of 7.0 to enable reproducible separation, and because the $pK_a$ of the phosphate buffer is 7.21, this obeys the general rule that the buffer will be most effective when the desired solution pH is within 1pH unit of its native $pK_a$. This should lead to an improvement in the robustness of retention time and peak shape. We will discuss this particular reagent and the eluent pH later.

Sodium heptane sulphonate is a so-called ion-pairing reagent and has two modes of action. In the bulk mobile phase, the anionic reagent will form an ionic equilibrium with basic analyte functional moieties to form a neutral complex, which will be more highly retained on the highly hydrophobic stationary phase surface. The second mode of action of the ion-pair reagent is to strongly partition into the stationary phase via the highly hydrophobic moiety of the pairing reagent, which will effectively contribute anionic groups (SO₃⁻) to the stationary-phase surface, with which any unpaired cationic analyte species may undergo electrostatic attraction, and therefore improve retention.

Apart from its use to obtain the correct mobile phase pH, triethylamine is used as a silanol group masking reagent. Figure 2 can give us further insight into the mechanism and requirement for this reagent.

Species 5 in Figure 2 represents an acidic silanol group that has not been effectively treated with an end-capping reagent (such as that shown in species 8), which will be anionic under the mobile-phase conditions of our method (species 5 will have a $pK_a$ of around 4). If left untreated, these silanol anions will form electrostatic attractions with cationic analyte molecules and unwanted secondary interactions and can lead to issues with peak broadening and tailing, as well as extended column equilibration times and possible issues with peak area reproducibility. Therefore, ionogenic masking reagents such as triethylamine were traditionally added to mobile phases to effectively “end-cap” surface silanol groups towards interaction with basic analytes and was typically not treated to avoid the presence of lone silanol groups. The later development of Type B silica effectively reduced silanol activity by removing target metal ions from the silica matrix and used surface treatments to reduce the number of lone silanols and promote the amount of inter-hydrated or vicinal silanol groups (species 2 in Figure 2). These developments were very effective at reducing the interactions between basic analytes and surface silanol species, effectively negating the requirement for masking reagents such as triethylamine. The bridged ethylene phases (species 1 in...
complex, may render the masking reagent ineffective, and one needs to wonder what robustness issues this may create. Let’s now consider the role of the ion-pairing reagent in this separation. Modern stationary phases, such as those used in this method, are capable of withstanding high pH mobile-phase environments, and as such we must also call into question the use of such a large modifier within the mobile phase at the beginning of the mobile-phase gradient to facilitate retention of more polar or ionized analytes. The use of ion-pairing reagents, such as sodium heptane sulphonate, has not been necessary for many years, since the introduction of higher quality silica, combined with more polar (so-called polar embedded) and more pH-resistant stationary phases, can be used to gain sufficient reversed-phase retention, even for more polar analytes.

The column contains 1.7 mm particles that are designed for use with UHPLC systems, given the higher inherent back pressure created when using such small diameter particles. Intrinsically, these systems have very small internal volumes and very narrow internal diameter tubing and connectors, reducing the effects of extracolumn (bed) band broadening on the separation. The use of a solid, involatile buffer with UHPLC systems is to be avoided, as any precipitation of the buffer through the use of highly organic eluents or via evaporation of the mobile phase on standing will lead to significant problems with system blockages and overpressure situations. Further, the column end frits used with UHPLC columns containing 1.7 mm particles typically have a porosity of around 0.2 mm, which renders them more susceptible to blockages with solid particles. It is possible to filter the eluent solution to remove any particulate materials initially present in the eluent system; however, one must again be very mindful that the triethylamine additive is relatively volatile, and this may be partially lost if filtration is achieved using vacuum apparatus. The target eluent pH of 7.0 is also relatively problematic. Given that the $pK_a$ of at least one of the ionogenic analyte functional groups in each of the analytes was close to 7.0, we might expect a relatively large change in the ionic character of the analyte, and therefore its retention, unless the pH of the mobile phase is very accurately controlled each time it is made. To ensure the method is robust, it would have been better to quote a volume or weight of triethylamine required to achieve the desired pH, which could be controlled much more reproducibly.

It should be further noted that the detection is at a fixed wavelength of 254 nm, and this can be problematic when the ionic strength of the mobile phase changes to such a large extent during the analysis, as is the case here, where the aqueous component ranges from 90% to 15% during the course of the analysis. Without the judicious setup of the detector, for example, by employing an appropriate reference wavelength to account for differences in the refractive index of the eluent, such changes in ionic strength might well give rise to retention time shifts, peak shape changes, and unstable and rising baselines during the gradient unless sufficient time is given to allow re-equilibration of the column.

Further problems with this reagent also exist. Because it inherently alters the stationary-phase surface, one has to be mindful when equilibrating the HPLC column because extended flushing of the phase is often necessary to obtain reproducible retention times for the first injections of a sequence. One also must question the possible interaction between the anionic ion-pairing reagent and the triethylamine cation. The possibility of this interaction, forming a neutral triethylamine cation. The possibility of this interaction, forming a neutral triethylamine cation. The possibility of this interaction, forming a neutral triethylamine cation. The possibility of this interaction, forming a neutral triethylamine cation. The possibility of this interaction, forming a neutral triethylamine cation.

Given that the pH of the mobile phase will change as the triethylamine concentration lowers. In my own experience, it is also possible for the triethylamine to “settle” within the eluent, again leading to drifting or changing analyte retention. Further problems with this reagent also exist. Because it inherently alters the stationary-phase surface, one has to be mindful when equilibrating the HPLC column because extended flushing of the phase is often necessary to obtain reproducible retention times for the first injections of a sequence. One also must question the possible interaction between the anionic ion-pairing reagent and the triethylamine cation. The possibility of this interaction, forming a neutral triethylamine cation.

Further, triethylamine is relatively volatile and will be lost from the mobile phase to the atmosphere on standing, causing drift in analyte retention over time as the inherent pH of the mobile phase will change as the triethylamine concentration lowers. In my own experience, it is also possible for the triethylamine to “settle” within the eluent, again leading to drifting or changing analyte retention. Further problems with this reagent also exist. Because it inherently alters the stationary-phase surface, one has to be mindful when equilibrating the HPLC column because extended flushing of the phase is often necessary to obtain reproducible retention times for the first injections of a sequence. One also must question the possible interaction between the anionic ion-pairing reagent and the triethylamine cation. The possibility of this interaction, forming a neutral triethylamine cation.

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between sample injections. It may be far more judicious to use a ternary system in which the ionic strength is maintained using a third eluent system that contains the buffer and additives at a higher concentration (such as all reagents at 10× concentration and added at a constant 10% of mobile-phase composition during the analysis).

From this brief review it would very much appear that the current method has been “stitched together” using a very traditional mobile phase with a modern stationary phase, which should not require any of the now “exotic” mobile-phase additives to achieve a suitable separation. Without the requisite experience in critical evaluation of chromatography methods, this combination may be baffling and the resulting problems very difficult to interpret or overcome.

Let’s summarize some of the detective work that we have undertaken to start building our investigative toolkit.

1) The use of phosphate or other solid buffers suggests that the method is “older,” and these buffers usually preclude the use of mass spectrometry (MS) detection and the potential issues with baseline stability, especially when using UV detection. These phases will also require significantly extended column equilibration times. High ionic strength mobile phases that use solid buffers can present significant issues with system blockage, especially where eluent conditions reach >60% acetonitrile or >95% methanol during the gradient.

2) The use of ion-pairing reagents such as triethylamine should ring alarm bells in terms of the robustness of the method and the potential issues with peak tailing, due to their association with older, less inert, stationary-phase chemistries and substrates.

3) By studying the physicochemical parameters of the analyte, it may be possible to suppress analyte ionization using pH control and modern stationary phases that will withstand eluent pH values between (approximately) 2 and 11.

4) The use of “exotic” additives within mobile phases such as triethylamine should ring alarm bells in terms of the robustness of the method and the potential issues with peak tailing, due to their association with older, less inert, stationary-phase chemistries and substrates.

5) One should always consider the analyte structure and the polarity (LogP, LogD) and the pK_a of any ionogenic functional groups with respect to the mobile phase being used. It is a real skill to be able to visualize the degree of ionization of each analyte and anticipate any issues with retention and resolution under the mobile-phase conditions specified.

6) Eluent pH values that closely approach the pK_a of any ionogenic groups will present potential robustness issues with retention and resolution of the separation. Further, methods that specify the eluent components volumetrically rather than gravimetrically should, where possible, be converted to gravimetric specification to enable more accurate mobile-phase preparation.

7) The use of ion-pairing reagents can present real challenges with column equilibration. High ionic strength mobile phases can indicate that robustness can only be achieved by significantly altering the nature of the stationary phase, and there may be issues with a mismatch between the analyte diluent and the mobile phase. These phases will also require significantly extended column equilibration times. High ionic strength mobile phases that use solid buffers can present significant issues with system blockage, especially where eluent conditions reach >60% acetonitrile or >95% methanol during the gradient.

8) Eluent gradients that present a significant change in ionic strength can present issues with baseline stability, especially when using UV detection. These problems can be minimized through careful optimization of the reference wavelength; however, the general principle should be to minimize ionic strength of the eluent and the change in ionic strength throughout the gradient.

**Tony Taylor** is Chief Scientific Officer of Arch Sciences Group and the Technical Director of CHROMacademy. His background is in pharmaceutical R&D and polymer chemistry, but he has spent the past 20 years in training and consulting, working with Crawford Scientific Group clients to ensure they attain the very best analytical science possible. He has trained and consulted with thousands of analytical chemists globally and is passionate about professional development in separation science, developing CHROMacademy as a means to provide high-quality online education to analytical chemists. His current research interests include HPLC column selectivity codification, advanced automated sample preparation, and LC–MS and GC–MS for materials characterization, especially in the field of extractables and leachables analysis.

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