The Return of the HPLC Conference

After a pandemic-induced hiatus since the 2019 event in Milan, the HPLC conference is on schedule to return, 18–23 June 2022, in San Diego, California, USA, as an in-person conference. Frantisek (Frank) Svec, the chair of HPLC 2022, shares his excitement about the coming event.

High-Throughput Qualitative and Quantitative Analysis of Volatile Residues in Polymers

Vaughan S. Langford and Mark J. Perkins

Ensuring that volatile leachable impurities are at low levels in polymeric materials is very important for minimizing migration to food and pharmaceutical products.


Jonathan Edelman, Restek

The phenomenon known as the “green rush” outpaces all significant analytical market areas. Given all this excitement, it is worth stepping back to examine the overarching trends and nuances of the cannabis testing environment and offer some opinions about the key players and disruptive technologies gaining traction throughout this burgeoning marketplace.

My Career as a Separation Scientist

Michael Dong (唐纬中) looks back on his life as a China-born separation scientist coming to America as an international student, and his successful career in research and industry.

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A Final Word from Incognito—The Past, Present, and Future of Chromatography

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The history and future of chromatography
Polyethylene glycol (PEG) is a biocompatible compound with extensive use in a wide variety of medical, biopharmaceutical, and industrial applications. It is a popular compound for modifying peptides, proteins, and other biologics through covalent-bond attachment for therapeutic drug applications. PEGylation is the process of attaching PEG chains to a protein drug to shield it from proteolytic enzymes and improve pharmacokinetic properties. However, analytically characterizing PEGylated proteins can be a complex process, and many different methodologies are used.

Protein drugs are PEGylated for many reasons. “PEG has a number of unique properties: It’s a water-soluble polymer, bioinert, and non-immunogenic,” said Athena Papasodero, associate scientist, analytical development at the Pelican Expression Technology™ business unit of Ligand® Pharmaceuticals Inc. “When PEG is conjugated to a protein, it can provide many positive advantages that the native molecule lacks.”

Papasodero explained it increases half-life, reducing dosage frequency and extending the circulation lifetime in the human body. And because PEG is hydrophilic, when it attaches to proteins and other biomolecules, it decreases aggregation and increases solubility. PEG can lower toxicity, immunogenicity, and antigenicity and can also improve stability and decrease degradation. PEG conjugation masks the protein surface and increases the molecular size of the protein. It is transparent and nonfluorescent and therefore non-detectable by itself.

Current Methodologies

Currently, there are three major techniques for determining charge-variant analysis of proteins: ion exchange (IEX) chromatography, imaged capillary isoelectric focusing (iCIEF), and capillary zone electrophoresis (CZE). All three high-resolution techniques separate biomolecules based on differences in the net surface charge and enable for charge-variant characterization of both PEGylated and non-PEGylated proteins.

According to a 2020 study, “iCIEF has become the current industry standard for isoelectric-point (pI) determination and charged-variant quantification of proteins and antibodies.”
“Variants that can be separated and analyzed using IEX chromatography, iCIEF, and CZE include post-translational modifications such as glycosylation, as well as other modifications including deamidation and fragmentation of the protein,” Papasodero said. “For IEX chromatography, these types of separations are carried out using one of two gradient techniques: salt gradients or pH gradients.”

- Salt gradients have the advantage of historical use; therefore, they are more common and more familiar to scientists in the bioanalytical lab.
- pH gradients are a newer development and offer certain advantages, including the ability to be mass-spectrometry friendly when compared to salt gradients.

However, there are challenges with charge-variant analysis for PEGylated proteins. “The main challenge in characterizing charge variants of PEGylated proteins is the charge-shielding effect in which the steric-bulk of the PEG residue hinders the electrostatic interactions between the amino acid residues of the protein and the charges surface of the stationary phase of the IEX column,” Papasodero explained. “For lysine conjugated PEGylated proteins, the net cationic charge of the protein also decreases as the number of PEGylated lysine residues increase, which can alter selectivity and hinder resolution.”

As researchers from the department of biologics process development at Bristol-Myers Squibb discovered, iCIEF separation of PEGylated proteins creates broad peaks. “Charged variants of PEGylated proteins merge into one broad peak during iCIEF, most likely due to the masking of proteins by the surrounding PEG chain and the increased hydrodynamic volume cause by PEGylation.”

Papasodero explained it also produces distorted peak shapes and modification of pharmalyte contents, methylcellulose concentration, protein concentration, focusing time, and other additives do not yield reasonable peak shapes for iCIEF methods.

**The Experiment**

To address these challenges, the Pelican Expression Technology team assessed the ability of the YMC’s BioPro IEX SF column to analyze PEGylated protein charge variants reproducibly.

“We developed a characterization method for PEGylated biotherapeutic proteins that can provide increased information without compromising peak shape and resolution,” Papasodero said, which, as seen above, are two factors that at times are sacrificed when characterizing PEGylated proteins.

The stability indicating method developed by the Pelican Expression Technology team created exceptional separation of charge-variant constituents using BioPro IEX SF. The method can enable others who have similar separation requirements to develop methodology from a proven and effective protocol.
## Materials and Reagents

<table>
<thead>
<tr>
<th>Name</th>
<th>Brand</th>
<th>Catalog #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride</td>
<td>JT Baker</td>
<td>3628</td>
<td>(≥99.0%), mw 58.44</td>
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<tr>
<td>MES Hydrate</td>
<td>Sigma Aldrich</td>
<td>PHG0003</td>
<td>195.24 (anhydrous basis)</td>
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<tr>
<td>Ammonium Hydroxide</td>
<td>Sigma Aldrich</td>
<td>338818</td>
<td>28% NH3 in H2O, ≥99.99% trace metals basis</td>
</tr>
</tbody>
</table>

## Experimental Conditions

**Sample Preparation:** A sample of PEGylated protein was diluted to 10 mg/mL using Milli-Q water.

**Degraded Sample Preparation:** A sample of PEGylated protein was diluted to 10 mg/mL in formulation buffer. The sample was held at 40° C for 30 days.

**Column Specifications:** BioPro IEX SF; particle size 5 µm; column size length X.I.D. (mm) 100 x 4.6; flow rate 0.2 - 0.8 mL/min; pH range: 2 - 12; temp. range 4 - 60° C; column material PEEK.

## Chromatographic Conditions

<table>
<thead>
<tr>
<th>LC System</th>
<th>Thermo Scientific Vanquish UPLC</th>
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</thead>
<tbody>
<tr>
<td>Detector</td>
<td>Vanquish VF-P20-A</td>
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<tr>
<td>Autosampler</td>
<td>Vanquish VF-A10-A</td>
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<tr>
<td>Column Compartment</td>
<td>Vanquish VH-C10-A</td>
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<td>Pump</td>
<td>Vanquish VF-P20-A</td>
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<td>Viper Fitting</td>
<td>MP35N 0.18 x 350 mm</td>
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<td>Column</td>
<td>BioPro IEX SF 100 x 4.6 mm I.D. 5 µm P/N:SF00505-1046WP</td>
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<td>Mobile phase A*</td>
<td>20 mM MES, pH 5.9</td>
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<tr>
<td>Mobile Phase B*</td>
<td>25 mM MES, 0.5 M NaCl, pH 5.9</td>
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<td>Column temp.</td>
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<td>UV Wavelength</td>
<td>280 nm (reference OFF/16nm-bandwidths)</td>
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* Use ammonium hydroxide to adjust pH

## Gradient Table

<table>
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<tr>
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<th>Flow [mL/min]</th>
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<th>%B</th>
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<td>100.0</td>
<td>0.0</td>
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<td>7.00</td>
<td>0.750</td>
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<td>0.0</td>
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<td>14.00</td>
<td>0.750</td>
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<td>30.0</td>
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<tr>
<td>15.00</td>
<td>0.750</td>
<td>50.0</td>
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<td>16.00</td>
<td>0.750</td>
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<td>0.750</td>
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<td>0.0</td>
</tr>
<tr>
<td>20.00</td>
<td>0.750</td>
<td>100.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
The Results

The effect of pH on charge variant analysis of a PEGylated protein is shown in Figure 1. The panels show UV traces at 280 nm for (A) full-sized chromatograms of PEGylated protein during the mobile phase pH screen and (B) expanded chromatograms of PEGylated protein during the mobile phase pH screen.

Table 1 reports the sum of the acidic variants, the peak area percentage for the main peak (MP), and the sum of the basic variants. Resolution (R) between the acidic residues and main peak and the ratio of peak height to valley height (End p/v) for the main peak are also reported.

<table>
<thead>
<tr>
<th>pH</th>
<th>Retention Time</th>
<th>Peak area acidic variants (%)</th>
<th>Peak area main peak (%)</th>
<th>Peak area basic variants (%)</th>
<th>USP Resolution (Acidic-MP)</th>
<th>Peak-to-Valley Ratio</th>
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<tbody>
<tr>
<td>5.5</td>
<td>10.910</td>
<td>6.8</td>
<td>81.1</td>
<td>12.1</td>
<td>1.1</td>
<td>2.7</td>
</tr>
<tr>
<td>5.9</td>
<td>10.387</td>
<td>7.2</td>
<td>85.7</td>
<td>7.1</td>
<td>1.4</td>
<td>3.7</td>
</tr>
<tr>
<td>6.0</td>
<td>10.253</td>
<td>7.0</td>
<td>86.1</td>
<td>6.9</td>
<td>1.6</td>
<td>5.1</td>
</tr>
<tr>
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<td>10.160</td>
<td>7.1</td>
<td>86.8</td>
<td>6.1</td>
<td>1.8</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Figure 2 displays the effect of pH on IEX of a PEGylated protein. Chart A shows the pH effects on the main peak area percentage. Chart B shows a heat map visualization of the method attributes: area percent of the acidic and basic charge variants, retention time, USP resolution between the major acidic peak and the main peak, and the peak-to-valley ratio between the major acidic peak and the main peak as a function of mobile phase pH.
In Figure 3, the IEX of a PEGylated protein characterized with the BioPro IEX SF column is shown. The panels show UV traces at 280 nm for (A) full-sized and expanded trace of mobile phase A blank, (B) full-sized and expanded trace PEGylated protein, and (C) full-sized and expanded trace degraded PEGylated protein. The optimized cation-exchange (CEX) high-performance liquid chromatography (HPLC) chromatogram for the PEGylated protein is shown in Figure 3B. One major acidic peak with a slight shoulder is observed in the pre-peak region of the chromatogram. Two small peaks are observed in the post main peak region. Six small basic peaks are eluting between 11-14 minutes, and one large basic peak eluting at 13.3 minutes. Over 100 injections were performed in a single run with no loss of resolution or peak shape of the PEGylated protein and charge variants. Figure 3C also shows the chromatogram following heat degradation of the PEGylated protein. The effects of heat degradation on the sample are clear: The dominant major acidic peak, the two smaller post main peaks, and the major basic peak increase following heat degradation.
Figure 4 shows peak-to-valley ratios used to judge peak separation for CEX-HPLC chromatograms of a PEGylated protein. The panels show UV traces at 280 nm for (A) an example showing a peak that is integrated as a main peak without baseline contact. It is separated by two neighboring peaks with the peak delimiters being on a valley, (B) expanded trace PEGylated protein, and (C) expanded trace degraded PEGylated protein.

Carryover from injection to injection can be a significant problem in PEGylated-protein analysis. As shown in Figure 5, the BioPro IEX SF column minimizes carryover: In the 118th injection using this column, there is minimal carryover. The percentage of carryover was calculated using this equation:

\[
\text{PEGylated protein area in blank injection for carryover} \times 100 / \text{PEGylated protein area in sample injection}
\]
Conclusion

YMC’s BioPro IEX SF column provides a tool for the analysis and confirmation of PEGylated protein and PEGylated protein charge variants, increasing mobile phase pH results in earlier retention times. From visual inspection of the data, similar chromatographic profiles are visible; however, a buffer pH 5.9 results in the elution of two small acidic variant species not present when differing pH mobile phases were screened. The column also exhibited good retention time, area, and relative-area reproducibility.

“Conjugation of the PEG group to a protein increases the shielding effect, causing the protein to elute early before the salt or pH gradient begins,” Papasodero said. “Our results suggest the combination of BioPro IEX SF column and optimized salt and pH buffer compositions can enhance charge-variant characterization of PEGylated molecules. The difference in profiles between non-PEGylated and PEGylated variants of the protein can offer rapid insight into the degree of PEGylation during PEGylation optimization and development.”

Overall, this method is superior to current charge-variant analyses of PEGylated proteins because it can be easily replicated and yield repeatable results; it also has little carryover and the potential to be used as a platform method for charge-variant characterization of PEGylated proteins, lowering both cost and the development time required.

The BioPro IEX SF column provides an illuminating analytical approach for charge-variant drug characterization.

REFERENCES:

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Incognito’s last article takes a look at what has changed over a career in chromatography, but it predominantly focuses on what the future might hold in terms of theory, technology, and working practices.

In the past 14 years of contributing to this column, I’ve written about just about every aspect of chromatographic life. From a trip to a sushi bar that inspired a conversation about the analysis of inks, to the acetonitrile and helium crises, which almost broke the editor’s email inbox. I’ve considered questions such as “Is chromatography boring?” and “Is there a future for chromatography?”, written about the threat of the academic supremacy of the East, and discussed fraud in the laboratory. The whole gamut has been considered and I hope you have found at least some of these musings interesting or even stimulating. As I pen this last editorial, I’m caught between writing about the fantastic advances I have witnessed during my career in chromatography and what the world of the future holds for the chromatographer. Perhaps a mix of both topics is a fitting way to bow out.

The Past and the Present
The chromatographic world I entered some 35 years ago was pioneering and emergent. Conferences were exciting and literature regularly heralded highly significant new advances. We packed our own high performance liquid chromatography (HPLC) columns and to this day I can remember the recipes we used to make the silica...
slurries and the packing pressures and times for many of the columns we used back then. I also remember a column blank (an unpacked column) blowing off the slurry tube during a pre-wash at around 11 metric tonnes of pressure and recall it bouncing off the laboratory floor prior to being launched through the tiles in the laboratory ceiling. When column efficiency dropped as a result of bed collapse, we simply took off the end fitting, topped up the bed with a high concentration slurry, waited for the solvent to evaporate, and closed it up again—usually with a new frit fitted. Good again for another 100 or more injections.

We “built” HPLC systems from modular components, usually from different manufacturers, and joined them all together with various remote start and signal cables. The computing integrator allowed us to record chromatograms on rolls of paper and these technological wonders could integrate chromatographic peaks on the fly, which was a real advance on cutting out peaks from standard-grade chromatography chart paper and weighing them on a balance to get a quantitative result. In the earlier models, there was no option to “reintegrate” and unless one got the integration parameters just right, the analysis had to be repeated. Autosamplers brought significant freedom and the ability to not have to stand by the instrument each time an injection was required. Mind you, the complexity of some of these early systems was literally mind-boggling and I was very much on first-name terms with the instrument company engineers, who seemed to always be on-site to fix and maintain these marvels of engineering.

Five micrometre particle size packing materials brought high efficiency and the possibility of reducing column lengths from 25 cm to 15 cm and the commensurate benefits in reducing chromatographic run times. Detectors were fairly rudimentary, and many UV instruments needed to be balanced or auto-zeroed between injections, and certainly over long analysis campaigns to stop the baseline wandering so much that the computing integrator chart pen ended up permanently bouncing off the end stop—and off the chart paper too! We also had very exotic refractive index, electrochemical, and fluorescence detectors too. Highly innovative, very useful for certain applications, and all of them required the skill of an artisan to get a stable baseline within a day of powering them up! LC–mass spectrometry (MS) had not been invented and the prospect of electronic storage of data was but a twinkle in the eye of the latest generation of whizz kids. Very few laboratories had computers.
and those that did had one or two at most for administrative or managerial use only.

The world of gas chromatography (GC) was similarly neophyte. Columns were all packed glass or stainless tubes, again mostly packed by the end user and restored after efficiency dropped by topping up with packing material. It was commonplace to have to replace the silylated glass wool plugs at the inlet end for every campaign of analysis to avoid gross peak tailing due to analyte interactions with the wool, which quickly lost its deactivation. Manual injection was the norm, and your injection technique was honed over many hundreds of injections, until qualitative peak shape was satisfactory and quantitative reproducibility assured. Flame ionization detection was also the norm, but we used nitrogen-phosphorus detection or electron-capture detectors much more frequently in those days to get the required sensitivity for various applications. I clearly remember the day we took delivery of our first GC–MS system; the excitement was palpable, and I remember the fear I felt when the vacuum system was explained as the oil-filled roughing pump started to pump down for the first time. It took me months to be truly comfortable with venting and pumping down the instrument, shaking with trepidation as I imagined the consequences of an oil backstream event, or a catastrophic vacuum loss leading to mangled blades of the very sensitive turbomolecular pump. The data system (yes, a whole computer dedicated to one instrument) ran on a turbo-pascal operating system and took around six months to be able to operate. Each spectrum was printed and manually searched against the many printed volumes of the McLafferty reference library, by either molecular ion or base peak searching. There were no autosamplers and there was certainly no automation of sample preparation.

It felt a little like the Wild West at times; we had to quickly learn how to keep a system running, perform maintenance to allow the next analytical campaign to proceed—frankly, how to be chromatographers. We have come such a long way in those 35 years, but I sometimes pine for those days and the intrepid voyages of discovery that occurred on a daily basis. However, as instruments and data systems have become more capable, robust, and reliable (one might say “industrialized”), there has been a paradigm shift in the chromatographic discipline and those who practice it. No longer do you have to think about the instrument and keeping it running, and so there is more time to consider the separation itself. Troubleshooting, development, and optimization of methods is much more commonly discussed in today’s laboratory because we can consider the separation rather than the equipment. That lack of insight into “what’s in the black box” can sometimes lead to a poorer understanding of the technique itself. Many of today’s chromatographers are users of chromatography rather than true chromatographers, but I’m not decrying that position. The demands of throughput, productivity, and capability have risen in line with the maturity of the science and the technology, and so the shift from a technique employed only by experts to one that is accessible to everyone in the analytical laboratory is perfectly natural. However, as I’ve often written in these pages, this can only be a defensible position if the level of training given to users is sufficient to allow them to recognize good data from bad.

Looking to the Future

Anyone working in an analytical laboratory today will recognize how far we have come since the “early days” of chromatography that I described earlier. But what of the future chromatography laboratory and the working lives of those within it? One may be flippant and muse on the possibility that manufacturers may consider
moving away from beige, dull blue, or cream and make instrument covers from more visually appealing colours. We might ask if HPLC system stacks will grow ever higher until even the tallest of folks in the laboratory will need a ladder to change solvents. But that would be facetious. I do see a future chromatography world where sensitivity of detectors pushes the problem of detectability to the sample preparation phase. We simply can’t inject large enough sample volumes to detect the vanishingly low analyte concentrations unless there is significant focus on sample preconcentration, and for this I forecast significant advances in the automation of sample preparation. Why automation? The sample preparation required will become ever more complex and to attain the reproducibility required in a reasonable timeframe automation is really the only way to advance in this area.

Micro- or nanoscale chromatography has been much heralded throughout my career. I had my first conversation on nano-LC in the very early noughties and was excited about the possibilities. But nanoscale or microscale systems have never really been fully made in system hardware, and—particularly in HPLC—extracolumn dead volumes need to be reduced to take full advantage of the improvements to efficiency and analysis time that the ever-decreasing particle sizes of column packings will offer. Perhaps we won’t use tubular packed bed formats, with all of their thermal disadvantages, at all, and the “chip” and microfabricated array formats will win out in the end.

For the last 20 years I’ve been told that, for liquid-phase separations at least, the chromatographer’s future is bleak because the mass spectrometric detector can select the required analytes from the sample “soup” without the need for a separation. This hasn’t yet come to pass, and the ability of an MS source and mass analyzer to effectively discern, for example, enantiomers or isomers remains a significant challenge. Perhaps advances in ion source technology that can overcome suppression or enhancement effects combined with the use of high mass accuracy mass analyzers and techniques such as ion mobility spectrometry as a pre-filter will eventually make all of us using HPLC effectively redundant.

Gas chromatography is a fairly mature technique, with good robustness and a plethora of highly sensitive mass analyzing detectors. One might think there is little room for improvement, but I would disagree. I see the future of GC focused on more advanced sample inlets that can overcome the fundamental “Achilles heel” of gas-phase separation techniques, which is sample volatilization and introduction into the analytical column. It is true that today’s GC inlet choices have expanded way beyond the standard split-splitless device, but in most laboratories this is still the default choice and internal standards are still used in many applications to account for the inherent disadvantage of losses or discrimination during sample injection. Cool on-column injection always held a lot of promise in my laboratory, as did thermal desorption, but I can’t help but feel there are areas of sample introduction technology that we have not yet fully explored, and whilst the current instrumentation is good, some inherent fallibilities remain.

Multidimensional separations are here to stay and indeed hold great promise for the future. However, to be truly game-changing, I think the reliability of the transfer mechanism (modulation) between dimensions needs to be more robust and reliable. The limitations on the physical dimensions of the second, or higher, dimension columns need to be minimized, perhaps with very highly efficient chromatographic media or devices that allow very rapid separations in the second dimension. Standardization of methodologies for various industries and application types also needs to be further considered. Software, datafile size, the ease of quantitative analysis, and the presentation and manipulation of data all need to be improved for multidimensional separations to reach global adoption.

Of course, our green credentials will also be a future focus. Think of the amount of plastic that we use in the laboratory without recycling. Think of the number of plastic disposable pipettes or pipette tips that we get through. The number of glass Winchester bottles. The volumes of solvent. The volumes of non-renewable gases. I’m aware that the “green chromatography” movement has already been established, but I do believe the future chromatographer will have environmental impact very much at the forefront of their minds. I’ll give them a starter for 10: Why don’t we recycle pipettes, pipette tips, and solvent Winchester bottles? The future will no longer offer us the option of green comparison. The fact that our environmental impact is significantly less than industry X or another of our business operations will not wash. We need to act soon and unilaterally to have a viably sustainable future.
Software used to acquire and process data has been revolutionized during my career, but I feel the future will be even more highly dependent upon developments in the digital aspects of our work. The ability to deconvolve and process huge amounts of data in reasonable timeframes will be key. We already have highly complex data systems whose features are underutilized because the average chromatography user either does not realize their potential or is not given the time and support to employ them in a compliant fashion. I don’t know many data systems that cannot take you from sample injection to final result given time and expertise, yet few of us really take advantage of these systems. Perhaps this brings us back to a discussion on training and expertise within the laboratory, but time and word count won’t allow yet another discourse on these particular topics.

I believe “industrialization” will also lead us to a future direction based on the Pareto principle. Consider how many different HPLC and GC phases are available and the number of manufacturers of these phases. Where do they fit? I recently had a conversation with a learned associate in which we mused on the possibility of the majority of separations—given an assumed level of system efficiency—that could be completed with only a handful of chromatographic phases from reliable vendors. Yes, there will always be “difficult” separations that will prove the exception to this rule, but perhaps 80% of all separations will be undertaken by the chromatography users, whilst the remaining 20% are undertaken by the chromatographers. Heck, your laboratory may even be set up this way currently. Maybe the future multidimensional system, when fully developed, may negate the need for this approach entirely. Let’s face it, a 5 × 5 array of columns in either liquid- or gas-phase techniques in the first and second dimensions, and which could be quickly screened in all of their combinations using automated method development platforms, could probably solve most of the selectivity issues that I’ve ever encountered. Method development software exists today that can help to evaluate the initial results of these screens and help with the heavy lifting of phase selection and initial solvent choice when using HPLC.

I could go on with future musings, but I’m afraid that I’ve already begged your indulgence for long enough.

So here we are then, the end of an era, and of a column in which I’ve had the privilege of sharing my thoughts and sometimes controversial viewpoints with you all, primarily to spark a reaction, which it has often succeeded in doing. I suspect it will not be much of a spoiler to admit that the opinions I have given in this column were not always my firmly held beliefs, rather a stance that I have taken to encourage debate and I hope that I have managed to achieve this.

We certainly have a long way to go, and to answer one of my own questions, chromatography certainly isn’t dead, but the future is in our own hands. We must continue fundamental research in separation science, both academically and industrially. We must respect and encourage our vendor partners to continue to push the capabilities of instrumentation, software, and consumables. We should continue to foster and develop the knowledge and skills of our younger colleagues and to promote our industry to the wider scientific and industrial world.

I leave you with the thoughts of one of my own personal heroes, the British comedian and author Spike Milligan: “A sure cure for seasickness is to sit under a tree”. Take to the boats my friends and don’t fear the sea, but push your boundaries to ensure that the next generation of chromatographers have better boats and better cures for sea sickness. I wish you all a fair wind and calm waters.
Lonza and Agilent Announce Cell Therapy Collaboration

Contract development and manufacturing organization Lonza (Basel, Switzerland) and Agilent Technologies (Santa Clara, California, USA) have agreed a collaboration deal to transform the manufacturing process for personalized cell therapies.

Cell therapies are a rapidly growing and developing industry with a number of products in clinical development. Inherently complex with highly specific patient needs, cell therapies represent an uncompromising challenge to researchers because ensuring product consistency requires real-time information on critical quality attributes (CQAs) and analytics throughout the production process. Minor changes during manufacturing could potentially cause major changes in the quality and efficacy of the final product.

The deal will see Agilent integrate its analytics technologies with Lonza’s Cocoon cell manufacturing platform. The companies hope that by integrating Agilent analytical technologies both at-line and in-line within the Cocoon platform automated manufacturing workflows manufacturers will be able to ensure that in-process controls and analytics can be employed on-demand to deliver a more consistent drug product.

“There is a huge opportunity through this partnership to determine what the ideal critical quality attributes are for manufacturing the most effective cell therapy, and drive the manufacturing, optimally in real-time, to meet those criteria,” said Nicholas Ostrout, Senior Director, Business Strategy and Implementation, Personalized Medicine, at Lonza.

For more information, please visit: www.lonza.com or www.agilent.com

Illicit Drug Analysis Using GC–IMS and Oral Fluids

Researchers from the University of Valencia have developed a gas chromatography-drift tube ion mobility spectrometry (GC–IMS) method for the analysis of illicit and psychoactive substances in oral fluids (1).

The determination of illicit drugs is needed across many industries, from toxicology reports in hospitals to traffic control or doping prevention in professional sports. Traditionally these determinations have been carried out through the analysis of urine and blood; however, each have issues associated with them. In the case of blood, the sampling procedure is quite invasive, whereas with urine there have been numerous incidents of falsification. Therefore, interest in alternative biological fluids has increased, with oral fluids emerging as one such target. The main advantages of oral fluids are the non-invasive sampling and the potential analysis of unmetabolized drugs.

Another important consideration is the technique used to analyze the oral fluids. Hyphenated mass spectrometry techniques, and especially liquid chromatography (LC)–MS/MS, have gained in popularity because of their selectivity, specificity, and sensitivity, but they require expensive instrumentation not accessible to all laboratories. Solid-phase extraction (SPE) or liquid-phase microextraction (LPME) followed by LC–MS and GC–MS are also possibilities, requiring slightly more in terms of sample preparation than the proposed method, which utilized a simple centrifugation step prior to injection.

Other limitations of GC–IMS were acknowledged by the researchers in terms of the method’s ability to identify unknown signals. There are no global available databases for the reduced ion mobility constants ($K_0$), and therefore, for unequivocal identification, the MS spectra should be verified. This could be a major drawback to the method’s acceptance by possible end users. However, researchers also point to a recent database of $K_0$ values that has been published with approximately 200 psychoactive compounds, including illicit and new psychoactive substances (2), which could be used to obtain preliminary information regarding unknown substances.

As such, researchers wanted to explore the possibility of utilizing GC–IMS to determine illicit and psychoactive substances in oral fluids. Amphetamine, methylene, α-PVP, ketamine, lidocaine, MPHP, cocaine, THJ-2201, and 5F-ADB were employed as model compounds in the study. The developed method was tested through the analysis of two oral fluid certified reference materials, with the obtained relative percentage errors lower than 8.4%. This indicated that the proposed GC–IMS procedure could be a reliable, selective, and sensitive technique for the determination of psychoactive substances in oral fluids.

References

1. A. Denia et al., Talanta 238, 122966 (2022).
Markes International Sponsors “Forget Me Knot” Charity Expedition

Markes International (Bridgend, UK) has sponsored a cross-Atlantic rowing expedition in support of Dementia UK Admiral Nurses. The “Forget Me Knot” crew will row almost 4000 miles of the Atlantic Ocean from Portugal to French Guiana, aiming to break two world records in the process—the first trio and the fastest boat to row from continental Europe to South America. However, their core mission is to raise over £100,000 in funding for Dementia UK Admiral Nurses.

Admiral Nurses work alongside people with dementia and their families, giving them one-to-one support, expert guidance, and practical solutions. Over 700,000 families in the UK care for people with dementia. The relentless demands of care commonly lead to depression and mental health problems, but few carers seek support. When things get challenging or difficult, Admiral Nurses provide expertise and invaluable experience for people with dementia and their families.

“One of our teams has experienced first-hand the physical and emotional ordeals of coping with dementia within their families,” commented Markes’ Managing Director, Tim Hawkins, adding: “During these Covid times when mental health has been so challenged, we felt that Forget Me Knot’s cause was one we wholeheartedly wanted to support.”

In June 2021, crew member and expedition organizer Johnnie Ball approached the company to see if it would be interested in supporting the cause.

“We wish Johnnie and his crew the very best of luck on their epic challenge. To say that they will have to work hard is somewhat of an understatement; they will certainly have to face extreme physical and mental challenges of their own in the coming days. In raising awareness of dementia, an often under-represented issue in society, perhaps they can help all those carers who also steadfastly put in so much hard work, day in and day out.”

If you would like to donate to the expedition please visit their GoFundMe page: [https://www.gofundme.com/f/forget-me-knot-atlantic-row](https://www.gofundme.com/f/forget-me-knot-atlantic-row)

For more information on Dementia UK Admiral Nurses, please visit: [www.dementiauk.org/get-support/dementia-helpline-alzheimers-helpline](http://www.dementiauk.org/get-support/dementia-helpline-alzheimers-helpline)

Thermo Fisher Announces PeproTech Acquisition

Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA) has completed the acquisition of PeproTech, Inc. (New Jersey, USA), a developer and manufacturer of recombinant proteins, for a total of approximately $1.85 billion.

PeproTech is a privately held provider of bioscience reagents known as recombinant proteins, including cytokines and growth factors. Recombinant proteins are used in the development and manufacturing of cell and gene therapies as well as in broader cell culture applications, especially for use in cellular research models.

“PeproTech will be an excellent strategic fit within our biosciences business and will allow us to even better serve our pharma and biotech customers by adding new capabilities to our existing offering,” said Marc N. Casper, Chairman, President and Chief Executive Officer of Thermo Fisher Scientific.

PeproTech will become part of the biosciences business within Thermo Fisher and will be integrated into the Life Sciences Solutions Segment.

For more information, please visit: [https://corporate.thermofisher.com](https://corporate.thermofisher.com)
Peaks of the Month

• The LCGC Blog: Laboratory Accreditation is Not a Cloak of Infallibility—If your decision making relies on analytical chemistry, then you want to be confident that the measurements are an accurate representation of the matrix that is being analyzed, and that they are of “publication” quality. But how can you know for sure if the analytical laboratory that you’ve selected is producing reliable data? Read Here>>

• Analysis of Proteins, Biologics, and Nanoparticles in Biological Fluids Using Asymmetrical Flow Field-Flow Fractionation—Aspects of applying AF4 to plasma, serum, milk, and cerebrospinal fluid in the field of analysis and characterization of proteins, biologics, and nanoparticles in biological fluids are reviewed. 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>>

• Two-Dimensional Liquid Chromatography (2D-LC): Analysis of Size-Based Heterogeneities in Monoclonal Antibody-Based Biotherapeutic Products—Anurag S. Rathore, Jared Auclair, Sanghati Bhattacharya, and Deepika Sarin review different approaches and coupling strategies for analyzing monoclonal antibody aggregates with 2D-LC. Read Here>>

• How Switching from Helium to Hydrogen Carrier Gas in Thermal Desorbers Enhances Gas Chromatography–Mass Spectrometry Systems—Analysis of polyaromatic hydrocarbons (PAHs) in air demonstrates that a TD–GC–MS system can operate with hydrogen carrier gas as well as it does with helium. Read Here>>

• 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. Read Here>>

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News In Brief

Preomics GmbH, a Martinsried, Germany-based developer of automation and sample preparation tools, has raised €13.5 million in a Series B financing round provided by Bruker Corporation. The funding will be used to accelerate the research, development, and commercialization of their proteomics tools and consumables. Preomics was founded in 2016 by Garwin Pichler and Nils A. Kulak as a spin-off company from the laboratory of proteomics researcher Professor Matthias Mann at the Max Planck Institute of Biochemistry in Martinsried. For more information, please visit: www.preomics.com

ProBioGen (Berlin, Germany) has announced that Innovent Biologics, Inc. (China) has signed a multi-product license for ProBioGen’s GlymaxX technology. Under this license agreement, Innovent receives non-exclusive rights to use the afucosylation technology for multiple candidates in their pipeline. “We are very pleased to have Innovent as a new partner for a GlymaxX license. Since its foundation, Innovent has established itself in a very short time as an innovative biopharmaceutical company with its own fully-fledged antibody development platforms and a robust pipeline of antibodies. I am very much looking forward to this collaboration to benefit Innovent’s products by the ease of use and high efficacy of our GlymaxX technology,” said Gabriele Schneider, Chief Business Officer, ProBioGen. For more information, please visit: www.probiogen.de
High-Throughput Qualitative and Quantitative Analysis of Volatile Residues in Polymers

 Vaughan S. Langford1 and Mark J. Perkins2, 1Syft Technologies Limited, Christchurch, New Zealand, 2Anatune Limited, Cambridge, United Kingdom

Ensuring that volatile leachable impurities are at low levels in polymeric materials is very important to minimize migration to food and pharmaceutical products. Sustainability initiatives have led to more recycled polymers being used by industry and therefore more frequent testing is required. Direct-injection mass spectrometry (DIMS) techniques such as selected ion flow tube mass spectrometry (SIFT-MS) provide high throughputs at a low cost for each sample. This article reviews polymer analysis applications of automated headspace SIFT-MS. Rapid quality assurance/quality control (QA/QC) screening of approximately 250 samples a day was conducted using targeted or untargeted analysis, while quantitative volatile impurity analysis was achieved efficiently using multiple headspace extraction (MHE)-SIFT-MS.

Polymers are primarily synthesized by polymerization of low-molecular-weight organic compounds, and therefore monomer residues and other volatile byproducts can be trapped in the polymeric material. These volatiles then migrate through the polymer...
and transfer into adjacent materials (such as pharmaceuticals and consumer products, if the polymer is used as a packaging material). The reverse process can also occur, in that compounds—especially more volatile ones—can migrate from the contained product into the polymeric packaging. This, of course, is not an issue if the polymeric packaging is disposed of but may be an issue if it is being recycled. If the residual volatile does not wash out or otherwise migrate out during reforming into granules for the next use, then it may migrate into the next product. Hence, analysis of potentially toxic or taint-causing volatile impurities is important for both virgin and recycled polymers. However, while impurities in virgin polymers are well characterized, recycled materials can vary markedly and therefore testing frequencies need to be increased.

**Figure 1:** Rapid automated static headspace-SIFT-MS analysis of styrene, formaldehyde, and acetaldehyde in three polymers.
Conventional volatiles analysis is conducted using gas chromatography (GC)-based techniques. These have the advantage of maximizing specificity by resolving volatile components temporally, but consequently result in a relatively low sample throughput. Further, analysis of very polar, low-molecular-weight volatiles is often a significant challenge for GC, requiring derivatization and/or use of a specialized column. Direct-injection mass spectrometry (DIMS) techniques, which—through elimination of the chromatographic column—analyze samples continuously as they are introduced into the instrument, may provide the means by which higher sample throughputs can be achieved. Selected ion flow tube mass spectrometry (SIFT-MS) is a DIMS technique that has been demonstrated to be readily automated and adapted to routine analysis (1). In this work, headspace analysis was conducted using a SIFT-MS instrument coupled with a multipurpose autosampler (MPS Robotic Pro, Gerstel). Details of analysis conditions are given in the cited literature. In general, headspace analysis was conducted using 20-mL headspace vials, with sample extracted using a 2.5-mL headspace syringe, followed by steady injection (usually at a rate of 50 μL/s) into a flow of nitrogen makeup gas (around 10-fold dilution in the sample inlet).

**High-Throughput Targeted Analysis**

Targeted analysis is the preferred approach for volatiles that are likely to be present and are known to be toxic or have an odour impact. Because virgin polymers exhibit lower variation in their volatile impurities, they are usually more effectively screened using targeted SIFT-MS analysis than recycled polymers are. It should be noted that method development needs to ensure that the matrix is compatible with SIFT-MS so that false positive results are not reported for lower-grade polymer products. These may produce elevated measurements for low-threshold target compounds if a target compound is interfered with by other volatile impurities.

Figure 1 demonstrates the flexibility of the SIFT-MS technique for targeted residual monomer analysis using three ground polymer samples incubated at 80 °C for 15 min (2.5 mL of headspace injected at 100 μL/s). Polystyrene (PS), polyoxymethylene (POM), and polyethylene terephthalate (PET) emit styrene, formaldehyde, and acetaldehyde, respectively. In contrast to chromatographic approaches, via highly controlled soft chemical ionization SIFT-MS can directly analyze routine VOCs, such as styrene, in the same run as low-molecular-weight aldehydes (that is, no sample preparation is required for formaldehyde or acetaldehyde). Figure 1 also shows that high sample throughputs are achievable with SIFT-MS. Incubated samples can typically be analyzed with a throughput of 12 samples an hour (or over 250 samples a day), meaning that SIFT-MS can provide rapid quality assurance/quality control (QA/QC) of polymers.
Quantitative Analysis of Volatile Impurities in the Polymer

Static headspace (SH)-SIFT-MS analysis of polymeric samples yields quantitative measurements of impurities in the headspace. However, relating this value to the mass of volatile in the polymer is very challenging because the nature of polymers means that preparation of standard, homogenous concentrations of impurities is not possible. Quantitative determination of speciated residual volatiles relies on the stripping of all volatiles using, for example, dynamic headspace analysis (DHA). Because exhaustive extraction using DHA is a very long procedure, Kolb and Ettre (3) developed the multiple headspace extraction (MHE) technique to achieve this from extrapolation of a limited number of headspace measurements that follow headspace purge and regeneration cycles.

Figure 2 shows MHE-SIFT-MS results obtained for styrene emitted from ground polystyrene pellets incubated at 140 °C for 60 min (4). Six replicates were analyzed, with each data point on the graph being the mean across the replicates for that injection. Calculation of the area under the curve enabled the residual styrene concentration to be determined for each replicate. The mean concentration of styrene was 270 μg/g. The low relative standard deviation (RSD) of 1.4% demonstrates the excellent repeatability of SIFT-MS analysis.

MHE is a very protracted procedure when coupled with GC due to multiple analyses on the same sample. Direct headspace analysis in MHE-SIFT-MS means that significant efficiencies can be gained due to the much shorter analysis time: One sample can be analyzed while the headspace is generated in up to 11 other samples (4). The number of samples that can be analyzed in parallel is dependent on the time taken for the headspace analyze-and-purge cycle relative to the optimized headspace generation time (the time to reach equilibrium). For polystyrene, an eightfold throughput enhancement was achieved with SIFT-MS compared to the equivalent GC method.
Untargeted Analysis

Targeted SH-SIFT-MS and MHE-SIFT-MS methods work well for known volatile residues (especially in virgin polymer), but recycled plastics have varied usage histories and therefore have a substantially higher risk of releasing other, nontargeted compounds. In this scenario, with its potential for significant inter-sample variation, an untargeted SH-SIFT-MS approach is

Figure 4: Classification of 10 recycled HDPE products (S1 to S10) using untargeted SIFT-MS analysis with the NO$^+$ reagent ion, coupled with SIMCA multivariate statistical analysis. Class projections, interclass distances, and discriminating powers of the 10 most important variables (mass-to-charge ratio) are shown.

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Mass-to-charge ratio | DP
---|----|
98 | 5151
136 | 2169
129 | 1306
101 | 1282
113 | 1132
114 | 977
112 | 940
99 | 916
152 | 672
126 | 671

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preferable. Nonconforming samples can be detected quickly by utilizing rapid full-scan SIFT-MS analysis with multivariate statistical data processing.

A recent study investigated variation in virgin and recycled high-density polyethylene (HDPE) pellets using this approach (5). Four virgin and 10 recycled samples (from six manufacturers) were obtained. Replicate samples (10 of 1 g each) were incubated at 80 °C for 20 min, then full-scan spectra were acquired with all three SIFT-MS reagent ions within one 40-s analysis. Figure 3 shows SIFT-MS full scan spectra obtained using the NO⁺ reagent ion for selected virgin and recycled HDPE samples (these are the mean of 10 replicates for each sample). Selected spectra span the range of variation observed in the sample set. Mass spectra of virgin HDPE samples generally have fewer spectral peaks and emit lower levels of volatiles than recycled HDPE samples (unless hydrocarbon residues are present). Recycled products exhibit a range of features derived from residues are present). Recycled products were differentiated. These data are visualized on the class projection plot, while the discriminating power indicates the most significant variables (m/z here) that differentiate the products. Hence untargeted SIFT-MS analysis coupled with multivariate statistical analysis can be applied to rapid differentiation of recycled HDPE—for example, to assure pellet volatile emissions conform to the standard required for their intended use. It should be noted, however, that definitive identification is not a strength of DIMS techniques such as SIFT-MS (outlier samples should be reanalyzed offline by GC–MS), whereas rapid and sensitive differentiation of products is.

Conclusion

Increased use of recycled plastic in consumer packaging means that wider testing of volatile residues is required. Automated SH-SIFT-MS has the potential to accommodate new analytical demands while also tackling existing analyses for volatile impurities in polymer because throughputs of greater than 250 samples per day are achieved. Moreover, SIFT-MS instruments can run targeted and untargeted analyses in a single sequence. Quantitative analyses of volatile residues in polymers are readily conducted using MHE-SIFT-MS, with an eightfold throughput enhancement for six-injection MHE.

SIFT-MS also provides a unique breadth of analysis because of direct, soft chemical ionization of sample headspace, as exemplified by simple analysis of formaldehyde. SIFT-MS is complementary to chromatographic methods, providing high-throughput qualification and quantitation, and broad VOC analysis, rather than detailed compound identification.

References

Vaughan Langford is a principal scientist at Syft Technologies in New Zealand. He joined Syft in late 2002 after completing his Ph.D. in physical chemistry at the University of Canterbury, and postdoctoral fellowships at the Universities of Geneva, Western Australia, and Canterbury. He has over 30 peer-reviewed publications on a wide range of SIFT-MS applications, plus he has contributed numerous conference papers.

Mark Perkins is a senior applications chemist and SIFT-MS expert at Anatune Limited, based in Cambridge, United Kingdom. Mark graduated from the University of Southampton, UK, with a Ph.D. in electrochemistry. He was with the Malaysian Rubber Board’s UK research centre for 12 years, first as a senior analyst, then as head of the analytical section. He joined Anatune in early 2015 in a role that supports and expands the analytical capability of SIFT-MS—with a particular focus on autosampler integration and the development of automated test methods.
The Return of the HPLC Conference

After a pandemic-induced hiatus since the 2019 event in Milan, Italy, the HPLC conference is on schedule to return, 18–23 June 2022, in San Diego, California, USA, as an in-person conference. Frantisek (Frank) Svec, the chair of HPLC 2022, shares news about the conference and his excitement about the coming event.

Q. What are you most excited about for the HPLC 2022 conference?
A: We must remember that HPLC conferences have a long tradition. The upcoming HPLC 2022 event is the 50th conference in this series. The first conference was held in 1973 in Interlaken, Switzerland; ever since, the event has alternated between Europe (in odd years) and the United States (in even years). The first Asian location was Kyoto, Japan, in 2001 and the regular biennial conferences in Asia started in 2008. The Asian events are held as the second HPLC meeting in the odd years. However, because of the Covid pandemic, the last symposium was held in Milan in 2019. Thus, between the Milan event and the upcoming 2022 conference, three years will have passed. Can you imagine what has happened during these three years? Clearly, a lot—yet nothing has been reported or discussed at any in-person conference. Also, how many new scientists entered the field of chromatography who have never been able to attend such a large symposium and meet with their peers? So, I feel really excited that we are finally (and hopefully for good) coming back to normal, to once again hold three in-person HPLC conferences every two years. The community of both old friends and newcomers will congregate again. We will see each other, shake hands, hug, talk—you name it. Isn’t that exciting?

Q. What is new or different in this year’s programme?
A: The HPLC 2022 programme will be certainly full of new thrilling developments. Obviously, we plan to retain all the goodies of past conferences. This means

Q&A HPLC Conference Chair
we are going to have sessions concerning column technologies, sample preparation, detection with focus on mass spectrometry, chromatography theory, and a plethora of applications, to name just a few. We will see the poster competition in which young participants will demonstrate their skills in designing their presentations. The young speakers selected to compete for the traditional Csaba Horvath Award will present in the oral sessions. In addition, some other new features, mostly focused on younger attendees, are also coming. For example, the Separation Science Slam is a new competition for young scientists that highlights short scientific presentations and aims to not only inform but also to engage the audience. Another new competition open for everyone is the HPLC Tube contest, where short videos related to high performance liquid chromatography (HPLC) will be presented. People contributing to both these new activities will get oral time to present their creations. But I don’t have enough space here to name everything. You can find details on our website.

Q. One of your goals is to encourage more dialogue and collaboration between academic and industrial scientists. Can you talk more about that?

A: The biotechnology industry is developing rapidly these days. New highly effective biological drugs pop up like mushrooms in the forest. Monoclonal antibodies move the world. RNA has come to the forefront of our interest thanks to vaccines fighting the Covid pandemic. Thus, the biopharmaceutical industry urgently needs chromatography both in the development phase as well as during the production cycle and quality control. Therefore, it is not uncommon for company employees to participate in the symposium. Many of them will be part of the oral programme and will have the opportunity to show their priorities and communicate their results. And conversely, many of the presentations by academics will relate to basic research with an impact that extends beyond the walls of university laboratories directly into industry. For instance, the development of new methods might be very useful in quality control.

Q. What aspects of the conference will be of particular value for young scientists?

A: I think I have already mentioned several aspects that are designed to attract young scientists to actively participate in the symposium. In addition, the symposium programme will include 15-min presentations that will be mostly reserved for students and postdoctoral researchers. These speakers will be selected based on submitted abstracts. Also, all the competitions are attractive for the new generation and are very popular because they include an opportunity to give a talk.

Q. There is talk in the scientific community about taking a fresh approach to poster presentations. Are you doing anything new in that regard?

A: Sure. The presentation of posters is developing with the development of tools available for their design and preparation. Remember the pre-PowerPoint and pre-large colour printing era? What a pain it was. Posters were created from individual panels printed in-house by simple printers on letter-sized paper. Now, we create the posters in a single piece on screen and then print it in beautiful vivid colours to enhance their visual appeal. However, the options that current technology provides do not guarantee high-quality posters. Their content does.

Unfortunately, not all presenters know how to create an award-winning poster. I have seen posters crowded with small features that are difficult to see clearly even up close; these posters require way too much time to read. Another bad habit is filling the poster with excessive text. A poster should not be a manuscript attached to a board. A poster is a visual art. Thus, we plan to prepare detailed guidance for the preparation of posters and post it on the symposium website. These instructions may also include a sample template to help beginners. We are also going to advise authors to show only the most important features of their research and place a QR code on the poster to guide those interested to a page where the authors will post related information—such as experimental details, large tables, links to accepted and published papers on the topic, and even videos—that could not fit on the poster.

Q. Does the HPLC conference have a value that is distinct from that of other conferences?

A: The HPLC symposia are the largest of their kind. Hundreds of attendees. An extensive exhibition. Where else can you find all this in a single place? Other conferences covering liquid-phase separations are certainly important as well, but they typically bring together only a smaller fraction of people working in the field. We have a conference where all the gurus are present and often also presenting; one can connect the name with the face, and it is even possible to talk to those colleagues during coffee breaks, at the
The large conference gives you a unique opportunity to do so. I know what I am talking about: I started as a “greenhorn” in the early 1990s and the HPLC symposia were my first door into the chromatographic community.

Q. In today’s digital world—particularly given the many virtual events held since the start of the pandemic—do you think people need to be convinced of the value of attending an in-person scientific meeting?

A: The ancient Greek philosopher Aristotle wrote, in 350 BCE, “Man is by nature a social animal”. People need to gather and communicate. The internet is certainly a wonderful technology that often makes our lives easier (but not always). But the internet cannot replace personal encounters. Virtual events are just what they are: virtual events. Speaking to an audience you see in front of you is irreplaceable. The energy of the people there, the eye contact, the body language, the feedback. Did you ever try to tell a joke when presenting virtually? You don’t hear the attendees laugh, and they probably don’t laugh anyway. And now imagine a real, in-person audience. It’s electrifying. Thus, I do not think that people need to be convinced of the value of meeting in-person. The only limiting factor can be economic—whether they can afford it. But the desire to meet in person never ceases.

Q. What Covid safety measures will be in place for the meeting? Are you planning any online aspects of this meeting, for those who may not be able to attend in-person, or who are reluctant to do so?

A: Unfortunately, Covid is here, so we cannot neglect measures to prevent its spreading. Clearly, it is difficult to predict what rules will apply in mid-June. But again, all measures will be updated as needed to meet regulations required by the CDC at the time. We deeply believe that the epidemiological situation in June will be good. That is why we are not planning any online presentations.

Q. Putting together a large conference is a lot of work. How will you judge if it was a success?

A: I will consider it a big success if the HPLC 2022 symposium is held in-person. That by itself will be an achievement because we all know well that things can change rapidly. Then, I hope the symposium will breathe new life into the community of chromatographers—the vitality we almost lost during the last three years. I also trust that we will welcome many academic, industrial, and governmental leaders in separation science. A success will be the presence of many students and postdocs—the younger generation of chromatographers. And finally, success will also be judged by the attendees themselves. All the efforts we make should lead to a conference that the participants will remember for a long time. We have a unique opportunity to create a wonderful professional agenda framed by a strong social programme in a charming city abounding in all tastes of attractions. I am looking forward to seeing many friends and colleagues in San Diego in the summer.
My Career as a Separation Scientist

Michael Dong (唐纬中) looks back on his life as a China-born separation scientist coming to America as an international student, and his successful career in research and industry.

In his book *My Career as a Separation Scientist*, Michael Dong chronicles his early interest in science and writing, which has led to many books and articles in analytical chemistry and liquid chromatography (LC). This three-part series also describes his living experience in New York, New England, and California.

Part 1 describes his birth in Shanghai and childhood in Hong Kong, where he developed an interest in science, leading to a journey to America as an international student.

Part 2 chronicles his graduate studies in analytical chemistry and early career in research in chemical, instrument manufacturing, and pharmaceutical industries in the New York areas.

Part 3 describes his relocation to the Bay Area in California, working for Genentech in new cancer drug development, and returning to Connecticut as a consultant.

Michael W. Dong is a pharmaceutical consultant from Connecticut, USA. He was formerly a senior scientist at Genentech, a research fellow at Purdue Pharma, and a staff scientist at Applied Biosystems/Perkin-Elmer. He has 130+ journal publications and four books, including a bestselling book on chromatography. Michael was born in Shanghai and raised in Hong Kong. He is multilingual, an Eagle Scout, and a long-term Toastmaster. He holds a Ph.D. in analytical chemistry from the City University of New York.

To order a copy of the book: English version (e-book: $5.00 USD, paperback: $12.99 from Amazon USA), M. Dong, *My Career as a Separation Scientist: The life of a China-born analytical chemist and his career in research and industries in America.*

Virtual Event Preview: EuSP2022 and GSAC2022

The 2nd European Sample Preparation e-Conference (EuSP2022) and the 1st Green and Sustainable Analytical Chemistry e-Conference (GSAC2022) will be held virtually 14–16 March 2022. Here is a sneak preview of what to look forward to.

In March 2021, the 1st European Sample Preparation Conference, organized by DAC-EuChemS Sample Preparation Study Group and Network, was successfully concluded. The key theme of “green sample preparation” opened the gate for all challenging ideas, aspects, and problems in sample pretreatment. A plenary lecture on green and sustainable chemistry was presented by Paul T. Anastas (Yale University, USA). In numbers, the impact of this conference is reflected in three plenary lectures, 42 oral presentations, 65 poster presentations (in two parallel sessions), a satellite event dealing with the preparation of manuscripts, and 309 registrations from 35 different countries. In addition, five awards were given to young scientists. Following the success of the 1st European Sample Preparation e-Conference, the EuChemS-DAC Sample Preparation Study Group and Network announced the second edition of this event. This year a new series of conferences are initiated, focusing for the first time on green and sustainable analytical chemistry.

The 2nd European Sample Preparation e-Conference (EuSP2022) and the 1st Green and Sustainable Analytical Chemistry e-Conference (GSAC2022) will be fully virtual and occur simultaneously between 14–16 March 2022. The EuSP2022 and GSAC2022 e-conferences will aim to bring together top experts to discuss new advancements in the sample preparation and green analytical chemistry areas. In each conference,
contributed sessions will showcase innovative research, cutting-edge technology, as well as new products and techniques in a broad range of analytical and applied areas in sample preparation and green analytical chemistry. The two events are recognized by the European Chemical Society (EuChemS).

The scientific programmes will include plenary lectures, oral presentations, 2-min flash communications for posters, and discussion sessions to stimulate intense scientific exchange. The annual theme for 2022 is "Interdisciplinary Sample Preparation". This year the EuChemS-DAC Sample Preparation Study Group will collaborate across science and engineering boundaries to transfer knowledge and integrate the processes, skills, and concepts from other disciplines into sample preparation.

Plenary lectures in the two events will be given by Professor Damià Barceló, Professor Klaus Kümmerer, and Professor Zhugen Yang. For EuSP2022, relevant researchers of the sample preparation community are confirmed speakers and include: Manuel Miró, Stig Pedersen-Bjergaard, Javier Hernández-Borges, Elefteria Psillakis, Jared Anderson, Gangfeng Ouyang, Rafael Lucena Rodríguez, Victoria Samanidou, María Eugènia Queiroz Nassur, Emanuela Giofriddo, Verónica Pino, Giorgia Purcaro, Cecilia Cagliero, Lorena Vidal, and Barbara Bojko. GSAC is pleased to announce as confirmed speakers: Chiara Cordero, Luigi Mondello, Gert Desmet, Lourdes Ramos, Miguel de la Guardia, Marek Tobiszewski, Mariosimone Zoccali, Miguel Herrero, Davy Guillarme, Andreas Brauer, Chalotta Turner, Teresa Casimiro, and Nora Ventosa.

Registration in the EuSP2022 and GSAC2022 e-conferences is free for the network members. Online registration and abstract submission will take place via the following link. The deadline for abstract submissions for oral presentations is 5 February 2022, while the deadline for posters to be included in the book of abstracts is 12 February 2022. For more information, please visit the webpage of the joint online events.

The European Chemical Society-Division of Analytical Chemistry (EuChemS-DAC) Sample Preparation Study Group and Network was created in 2019. Although it started as a “task force”, prompted after its annual evaluation, it was upgraded to a “study group” owing to its remarkable activity. Study groups are devoted to topics of particular relevance for EuChemS-DAC, and sample preparation is one of the eight study groups in this field. The DAC-EuChemS Sample Preparation Study Group and Network is divided into four working groups (WG): 1. Science and Fundamentals; 2. Automation, Innovation, and Entrepreneurship; 3. Information Exchange and Networking; and 4. Green Analytical Chemistry, as a follow-up of the previous year annual theme.

This scientific study group and network is headed by Professor Elefteria Psillakis from the Technical University of Crete and comprises more than 400 members from 37 countries in Europe, America, Asia, Africa, and Oceania working in academia, research institutes, industry, and private laboratories.

The aim of the network is to promote the science of sample preparation, the facilitation of the exchange of information between research teams, the facilitation of collaborations and networking, the linking of research with innovation, the support of young scientists, and the organization of scientific activities, such as conferences, courses, webinars, and support of publications and special issues to disseminate new ideas in this field. Therefore, the network welcomes new regular members professionally engaged in or associated with sample preparation. More information on this action and how to become a member is available at: https://www.sampleprep.tuc.gr/en/home.

The organizers look forward to e-welcoming you!

Jonathan Edelman, Restek Corporation, Philadelphia, Pennsylvania, USA

The dynamic world of cannabis testing is examined in this instalment of the LCGC Blog.

The buzz at the 2021 Cannabis Science Conference in Baltimore was palpable: People of widely disparate backgrounds were excited to address the ever-growing challenges and opportunities afforded by this burgeoning marketplace. The phenomenon known as the “green rush” outpaces all significant analytical market areas. Given all this excitement, it is worth stepping back to examine the overarching trends and nuances of the cannabis testing environment and offer some opinions about the key players and disruptive technologies gaining traction throughout this burgeoning marketplace.

The Shifting Cannabis Testing Market
At the start of 2019, the global cannabis testing market totalled $1054.6 million, with an anticipated compound annual growth rate (CAGR) of 15.4% (more than doubling that of the pharmaceutical market as a whole) (1). This growth is primarily due to three factors: the increase in the global population’s access to and participation in legal cannabis, growing regulatory scrutiny, and consumer shifts from raw cannabis “flower” to processed goods. This shift in the cannabis industry and the effect on its testing market to my mind rests on a seemingly obvious truth: One
day the demand for raw cannabis (flower) will be less than a hundredth of that for its many processed forms, whether they are processed for recreational or medical devices, pharmaceuticals, nutraceuticals, or otherwise wellness products.

We can observe the overall effect of this trend as the cultivators’ dominant market share of the cannabis testing market (62.4% in 2019, at US $658 million) begins to shift to cannabis drug manufacturers (CDM) (21.7% in 2019, at US $264 million). The CDM segment exhibits very aggressive growth (17.3% CAGR in 2019) (2). This growth is driven by an increase in demand for medical cannabis; as CDMs have developed cannabis R&D programmes, their need for tools to process and analyze the products has grown.

Recent Developments in Medical Research about Cannabis
The increased success of cannabis-based medical products is strongly correlated to the relaxation of the previously insurmountable bureaucratic hurdles, as well as humans’ ancient relationship with this pharmacologically active plant (3). For instance, it is entirely possible, if not likely, that humans crossing the land bridge from Asia into the Western hemisphere brought cannabis along with them. Today, scientists from various disciplines are exploiting the endocannabinoid system in humans. Academic and governmental institutions, pharmaceutical companies, and the separations community are well into the hunt to utilize the plant’s unique chemistry and its relationship to our biology. To date, roughly one million scientific articles have been published on the topic. Research continues to flourish, aiming for some of the most important target markets in human health.

Below are highlights of the conclusions of some meaningful publications showcasing the current state of cannabis-based medical research and its enormous potential:

**Pain Management:**
- Cannabidiol (CBD) could reduce inflammation and pain in people affected by arthritis (4).
- CBD is effective as a natural alternative to opioids in patients suffering from chronic pain (5).

**Multiple Sclerosis:**
- CBD can be used effectively for improving mobility in patients suffering from multiple sclerosis (6).
- The use of CBD or mucosal spray, a CBD-based medicine, in patients effectively reduces spasticity—one of
the most common symptoms of multiple sclerosis (7).
• CBD was concluded to be an effective alternative to standard treatment available for multiple sclerosis because it improves refractory spasticity in patients (8).

Cancer:
• Cannabinoids such as CBD could be used effectively for relieving symptoms associated with pancreatic cancer (9).
• CBD-induced apoptotic cell death in patients suffering from gastric cancer (10).
• CBD could be used effectively to increase the survival rate in patients suffering from non-small cell lung cancer (11).

Epilepsy:
• Researchers reported around a 41.9% reduction in the number of seizures when CBD was provided to patients (12).
• In a study wherein CBD was administered in patients suffering from frequent attacks, it was concluded that CBD was quite effective in reducing the severity and frequency of seizures (13). One harbinger of a success story is the case of GW Pharmaceutical’s epilepsy drug, Epidiolex (which received FDA approval in 2018). Due to its high effectiveness in treatment, clinicians and patients have welcomed this drug, the first-ever approved cannabis-infused anti-epileptic drug. The commercial success of Epidiolex is primarily due to its lack of psychoactive tetrahydrocannabinol (THC) compounds—THC is explicitly addressed in many stringent government regulations. With successes such as this one many prominent governments, such as the United States, Brazil, France, Germany, and South Korea, are leading the world to adjust their laws and aid in developing these essential medicines. It is reasonable to assume that this alone will move our world; yet many factors complicate the development of cannabis-based medicines.

Analytical Chemistry Solutions
A few notable factors slowing the progress of cannabis-based medicines are the lack of certified professionals, the lack of harmonized regulations, and the high cost of analytical instrumentation—a frustration we know all too well. Fortunately, chromatography vendors are increasingly focusing on serving the analytical challenges of this burgeoning field—Shimadzu, in my opinion, is the most committed to winning this marketplace. They offer a version of simplified software on their benchtop system, application support, and many consumables to help any laboratory get off the ground. Gaining ground is Agilent, which introduced pesticide, mycotoxin, and potency-testing kits in July 2020. PerkinElmer’s most significant advance is owed to Avinash Dalmita, a principal R&D scientist. Dalmita wrote a proprietary derivatization method by which cannabis laboratories can analyze the complete California pesticide list using only liquid chromatography–mass spectrometry (LC–MS), eliminating the need for gas chromatography–mass spectrometry (GC–MS) to complete this task. With more companies entering this field, we will undoubtedly see advances in chromatography that suit the needs of the cannabis testing market.

Two other companies worth highlighting are Lucidity Systems and Icon Scientific. They both feature instruments that cost roughly half the “familiar” price. Both claim that the reduced complexity and easy-to-use interface of their systems do not diminish testing expectations. Icon’s ANSWR liquid chromatography instrument is fully portable and small enough to put in your carry-on luggage. Lucidity’s Mini LC and GC instruments are half the footprint of typical benchtop units and can be shipped overnight for service.

Others are developing even more affordable systems. A class of systems is emerging mainly targeting the fast-growing potency testing segment (US $229 million in 2019, with a 21.7% market share and a 17.3% (CAGR), and they are on offer for less than five thousand USD. These instruments are sold by GemmaCert, Orange Photonics, Purpl Scientific, and Sage Analytics. The efficacy of many of these instruments may come into question in the increasingly scrutinized cannabis marketplace. However, they are deemed sufficient for many typical applications and likely will always be useful for field testing performed by cultivators and law enforcement groups alike.

Navigating a marketplace as dynamic and often controversial as cannabis testing can be fraught with challenges. I hope this article has helped you understand and perhaps appreciate its value to our global community. On a personal note, I believe that the more visible and controversial areas of recreational cannabis unfairly encumber appreciation of the plant’s potential as a whole. This gap in understanding can only obscure the incredible potential of this ancient plant to alleviate human suffering, a gap that analytical chemists can help narrow. Delays in progress reduce the
pharmaceutical community’s ability to bring meaningful and lasting changes to improve and prolong human health—a most worthy mission.

This blog is a collaboration between LCGC and the American Chemical Society Analytical Division Subdivision on chromatography and separations chemistry.

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Jonathan Edelman began his career as an analytical chemist in the pharmaceutical industry. Inspired by his long-standing love of science and natural ease in its commercial aspect, he works towards strategic business solutions in the chromatography field. Currently in business development at Restek, he volunteers his time to various nonprofit organizations within the chromatography community.

Edelman serves as Treasurer of the ACS SCSC; Chair of the organizing committee for the ISCC/GC×GC 2021 conference; President emeritus of the Chromatography Forum of Delaware Valley and Washington Chromatography Forum; and Founder and President of the Separation Community Mixer.

Website: www.chromatographyonline.com
## Training Courses

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Online webcast from CHROMacademy  
Website: www.chromacademy.com/channels/infrared/principles/introduction-to-infrared-spectroscopy

**Absolute Basics of Powder X-ray Diffraction (PXRD)**  
22 February 2022  
Online—virtual classroom

Please send your event and training course information to Kate Jones kjones@mjlifesciences.com
Event News

18–20 May 2022
The 17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-17)
Ghent University - Aula, Ghent, Belgium
E-mail: htc17@kuleuven.be
Website: https://htc-17.com/

29 May–2 June 2022
The 19th International GC×GC Symposium
Virtual event
E-mail: gcxgcub@ualberta.ca
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21–24 June 2022
Analytica 2022
Messe München, Munich, Germany
E-mail: info@analytica.de
Website: www.analytica.de/en/

18–22 September 2022
The 33rd International Symposium on Chromatography (ISC 2022)
Budapest, Hungary
Website: https://isc2022.hu
Mission Statement
The Column (ISSN 2050-280X) is the analytical chemist’s companion within the dynamic world of chromatography. Interactive and accessible, it provides a broad understanding of technical applications and products while engaging, stimulating, and challenging the global community with thought-provoking commentary that connects its members to each other and the industries they serve. 

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