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Rising Stars of Separation Science: Katelynn Perrault

This month we interview Katelynn Perrault, Associate Professor of Forensic Sciences and Chemistry at Chaminade University of Honolulu in Honolulu, Hawaii, about her work translating one-dimensional (1D) gas chromatography (GC) methods to effective comprehensive 2D GC (GC×GC) methods for forensic applications and the benefits that GC×GC offers the analyst.

—Interview by Alasdair Matheson

Q. When did you first encounter chromatography and what attracted you to the subject?
A: I first encountered chromatography during my Bachelor’s degree while studying forensic science. We were introduced to chromatographic techniques in our forensic chemistry classes at the same time we were starting to learn about them in analytical chemistry. I remember my chemistry courses iterating the fundamentals—how and why separation works based on a mobile phase, stationary phase, and analyte affinity. What I remember most is my forensic classes iterating when chromatography would be used—drug analysis, arson analysis, explosive investigations, and more. I remember being introduced to the concept of using chromatography to solve problems and answer questions. This combined view on what chromatography was and how it could be used made me really excited about it.

Q. What did your Ph.D. focus on?
A: My Ph.D. focused on isolating, separating, and characterizing the
Katelynn Perrault earned a Ph.D. from the University of Technology Sydney and a Bachelor of Science (Hons) in forensic sciences from the University of Ontario Institute of Technology. She specializes in the development of multidimensional separations for the comprehensive characterization of odours of forensic relevance. Kate is the Principal Investigator of the Laboratory of Forensic and Bioanalytical Chemistry, which is supported through several federal grants, foundation grants, and industry support. She researches decomposition odour for forensic search and recovery, and mentors numerous undergraduate researchers as part of her integrated teaching and research programme. Her current interests include odour production from post-mortem microbes, development of data processing workflows for multidimensional chromatography, promoting the adoption of multidimensional separations in the forensic sciences, and producing curriculum on multidimensional separations to be taught in undergraduate chemistry classes.

Q. What chromatographic techniques have you worked with?
A: The main chromatographic techniques that I am currently using for my work are GC–MS and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF-MS) methods to better characterize the soil matrix. We also worked with cadaver-detection dogs at a local police agency to complement the analytical information we were obtaining with real-world application information.

Q. You were recently involved in a project translating a one-dimensional (1D) GC method to a comprehensive 2D GC (GC×GC) method for forensic applications (1). How did this project arise and what type of forensic applications did you study?
A: This project actually arose from a desire to implement GC×GC in the laboratory in which I was establishing my research group as a new faculty member. In looking towards GC×GC options, which I was really intrigued by the idea of converting a one-dimensional GC system with qMS to a multidimensional instrument. The motivation behind this was that I am really invested in promoting the adoption of multidimensional techniques in forensic laboratories, and there is precedent for GC–qMS already existing in forensic laboratories. We wanted to demonstrate that GC×GC is an accessible technology to implement and can be added on to a previously existing setup with great success. In this work we demonstrated the parameters we monitored in converting our system to a GC×GC by adding on a modulator and splitting device, and how we use simultaneous detection (qMS/FID) to overcome some of the limitations of qMS as a detector for GC×GC. We focused largely on compounds present in decomposition odour, but this work could certainly be applied to other areas of forensic and medical analysis, among others.

Q. What were the main challenges you encountered when translating this 1D method to a GC×GC method?
A: We used components from several different suppliers and manufacturers and therefore a large part of the challenge in this type of approach is to make sure that every component is communicating with one another effectively. Our sample introduction methods (liquid injection, SPME Arrow, and thermal desorption unit) have to speak to the GC system, the GC system has to speak to the modulator, and everything has to communicate with the detectors that are functioning simultaneously. In addition, the data we
generate have to be exported, converted to a different file format, and then imported into a GC×GC software for processing and analysis. Making each of these steps flow smoothly is one of the bigger challenges of a retrofitted instrument, but it is possible and can be made straightforward if you have the appropriate guidance. We also recently published a paper explaining our workflows and published open access datasets so that our workflow can be followed and reproduced by other researchers who are looking to learn these steps (2).

Q. What were the advantages of the comprehensive GC×GC approach you developed?
A: The advantages of using the GC×GC approach were that you can obtain improved peak capacity and detectability within complex samples. This is the main benefit of multidimensional chromatographic analyses in general—you see more peaks within your sample. There is a large misconception within the chromatography community that coeluting analytes can always be resolved using deconvolution techniques. In our experience, most of our complex samples have many coelutions (that is, sometimes upwards of five compounds with broad dynamic range). This makes deconvolution algorithms problematic and causes the potential for both false positives and false negatives in a peak table. Improving the physical separation of analytes through multidimensional chromatography is a much more feasible and successful approach to comprehensively characterizing your sample.

Q. Can you elaborate on how your new approach was applied to forensic analysis?
A: Our new approach was published to demonstrate how flow modulation can be used as a technology to convert a one-dimensional GC system within a forensic laboratory into a GC×GC instrument (1). We applied it to the analysis of chemical standards to monitor the translation workflow and be sure that no quality is lost in this translation, especially related to identification and quantification. Most often people raise concerns about losing data in doing this translation and switching to a new approach. There are also concerns about losing the ability to accurately quantify, and so in the chemical standards we analyzed we demonstrated the retention of standard calibration and other parameters. We mostly focused on volatile organic compounds (VOCs) of
forensic interest, but these concepts help to better understand how GC×GC might be used in the future in drug analysis, toxicology, arson investigation, ink analysis, and other areas of forensic science currently using GC.

Q. You have also used a GC×GC method in a forensic analysis to investigate decomposition odour in tropical climates (3). What is novel about this approach?
A: Since the start of graduate school I have always been interested in the idea of validating the work I was doing in different areas of the world. We have conducted studies in Canada, Australia, Belgium, and now for the first time in a tropical region—Hawaii. Before now, decomposition odour production was never studied in any tropical region. Therefore it is fitting and helpful that we can investigate the profile in this new region and verify the subset of the profile, which is reproducible from one location to another. One thing we are particularly interested in is seeing what the “core” VOC profile is from decomposing remains that can be detected anywhere in the world and with any instrumental setup—that is, what is actually common about this profile no matter what other factors are at play. We are certainly getting much closer to answering that key question, which lends a lot of validity to this active research area.

Q. GC×GC is often regarded as complicated. Is this the case and have there been any recent technology advances that have simplified ease of use?
A: In my opinion, GC×GC is no more complicated than many other currently used analytical techniques out there. That being said, I think many researchers using GC×GC tend to overcomplicate it when explaining it to new audiences—and I include myself in this statement too! Over the past few years I have been working on teaching GC×GC at the undergraduate level within my classes and over time I have developed a lot of new strategies on effectively communicating GC×GC concepts and incorporating project-based learning to solidify topics. New technology advances are happening so frequently for GC×GC, and I find that in teaching about this technique, sometimes we have to take a step back and just focus on the fundamentals of how each component works rather than all the different options that are currently commercially available. I think we are moving towards the availability of instruments that are more user-friendly and hopefully that will help with adoption. I do think the most important component is in making communication, training, and resources helpful for the next generation of GC×GC users.

Q. Are there any recent research projects using GC×GC that you find particularly innovative and exciting?
A: Most of the articles that draw my attention and excitement these days are ones that have different approaches for dealing with large batch data, usually with chemometric analysis. I recently read a very interesting paper by Favela et al. (4), which applied chemometrics on a large number of extracts from mask materials to see which chemicals people are exposed to during inhalation while wearing a mask. This study employed GC×GC to analyze the mask samples and two proprietary tools to extract data and classification information from mask samples. I found the application interesting and the approach to the data processing was also intriguing. I am always interested in seeing if there are new ideas from other applications that I can apply to forensic samples to help us get more out of our experiments. One thing that I am currently very interested in is incorporating statistical analyses on GC×GC data that have been collected from time series data because a lot of our studies involve analyzing the same samples repeatedly over a period of time. I think we can improve the way we incorporate time as a factor in our analyses and I am looking forward to exploring this area further.

References
Nominations Open for 2022 HTC Innovation Award

*LCGC Europe* is now accepting nominations for the 2022 HTC Innovation Award through to July 2021. The 2022 HTC Innovation Award winner will be selected by the HTC-17 Scientific Committee and the HTC-17 Industry Board, based on the following criteria:

- The winner has made a pioneering contribution to the field of separation sciences by introducing new methodologies, new instrumentation, or new techniques in the field, with a strong focus on applicability.
- Applications are open to scientists who have under 15 years of experience after completing their Ph.D.

Previous winners have included: Ryan Kelly of Brigham Young University, Utah, USA, who was nominated and awarded the honour based on his outstanding contributions to the field of microcolumn separations involving hyphenation, and Carolin Huhn of Eberhard Karls Universität Tübingen, Germany, who received the inaugural award for her work on a modular instrumental approach where the modular building blocks can be flexibly combined in relation to particular analytical tasks to implement a broad range of different elements of chemical analysis.

The 2022 HTC Innovation Award recipient will be presented with a plaque honouring their accomplishment at the HTC-17 conference, which will be held in Ghent, Belgium, at Conference Center Het Pand, the culture and congress center of Ghent University, from the 26–28 January 2022.


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**Agilent Presents Thought Leader Award to Professor Chris Elliott**

Agilent Technologies Inc. (Santa Clara, California, USA) has announced Chris Elliott as a recipient of an Agilent Thought Leader Award. A Professor of Food Safety in the School of Biological Sciences and Founder of the Institute for Global Food Security (IGFS) at Queen’s University Belfast in Northern Ireland, the award recognizes Elliott’s contributions to the field of food authenticity and the development of novel approaches to the detection of food fraud.

Food fraud is on the rise, impacting a vast array of food products and causing significant concerns for the food industry, regulators, and consumers. The Agilent Thought Leader Award provides the winner with an Agilent 1290 Infinity II LC system coupled with a 6546 LC-Q/TOF, a 7850 ICP-MS instrument, and sample preparation and analysis consumables, supporting Professor Elliott and his team in developing innovative analytical approaches that will help solve complex food authenticity challenges, focusing on making complex test methods more routine and easier to use.

“I'm deeply honoured to be the recipient of such a large and prestigious award,” said Professor Elliott. “The partnership between our institute and Agilent to provide cutting-edge scientific tools to help combat the growing menace of food fraud globally will go from strength to strength. Together we will help support the development of the global food supply system based on the core principles of integrity.”

“Providing this award to Professor Elliott is an honour as Agilent is committed to helping ensure that the world’s global food supply is safe,” said Sudharshana Seshadri, VP of Agilent’s Mass Spectrometry Division and executive sponsor of the award. “The development of new test methods that are fast and easier to use will enable much broader adoption and implementation of food authenticity testing in more labs.”

The Agilent Thought Leader Award programme promotes fundamental scientific advances by contributing financial support, products, and expertise to the research of influential thought leaders in the life sciences, diagnostics, and chemical analysis space. To learn more, visit the Agilent Thought Leader Award website.
Researchers have developed a QuEChERS method with liquid chromatography–quadrupole time of flight-mass spectrometry (LC–QTOF-MS) to determine 50 wastewater-borne pollutants utilizing earthworms, which were exposed to the treated wastewater (1).

Insects and invertebrates have proven to be valuable sources of information in many fields of science, from forensic investigations to medical studies. In the case of monitoring soil pollution, soil-dwelling earthworms (*Lumbricus terrestris*) can act as sentinels and be exposed to a variety of organic micropollutants, such as wastewater reused for agricultural irrigation.

A number of guidelines have been published on the reuse of water for agricultural purposes, which are necessary to not only protect the environment but also human health. Contaminants such as heavy metals and pesticides are the traditional targets of analytical methods, but emerging substances of concern, such as pharmaceuticals and other sewage-borne pollutants, also need to be monitored. In this scenario, earthworms have been previously shown to be a suitable species for analysis because of their soil-dwelling activity and their ability to bioaccumulate persistent compounds, as well as their ease of collection (2,3,4).

Despite this suitability, few studies have utilized earthworms, primarily because of the lack of analytical methods that offer the required sensitivity and selectivity. To rectify this, researchers developed a targeted ultra-fast high-resolution multiple reaction monitoring (MRM\(^*\)) method using LC–QTOF-MS and an extraction method for 52 compounds based on a QuEChERS methodology.

The method was then applied to the analysis of 36 earthworm samples that originated from laboratory experiments and a field that had been irrigated with treated wastewater. The method detected eight compounds from the field samples, with the highest concentration levels detected belonging to gemfibrozil and caffeine. The authors believe this is the first reported transfer of wastewater-borne contaminants to earthworms following irrigation under natural farming practices.—L.B.

**References**

• **The LCGC Blog: Killer Gas Chromatography Variables—and Other Insidious Ways to Destroy your Chromatography!**—I’d like to concentrate on variables that can really impact our chromatography, but may be on hidden, supplementary, or advanced pages of our software, or may appear on the main software acquisitions menus, but are poorly understood or rarely altered. These variables are often not specifically referenced in laboratory methods documents or, if they do appear, are poorly understood. [Read Here>>]

• **Advances in Asymmetrical Flow Field-Flow Fractionation (AF4)**—Lars Nilsson from the Department of Food Technology, Engineering and Nutrition, Faculty of Engineering, in Lund, Sweden, discusses the evolution of asymmetrical flow field-flow fractionation (AF4) in bioanalysis for the analysis of therapeutic proteins. [Read Here>>]

• **Defining Material Used in Biopharmaceutical Analysis**—There is a need to use more descriptive and precise terms to describe some of the properties of the products used for biopharmaceutical analysis. It is probably more correct to say “low adsorption” and “corrosion-resistant” systems. [Read Here>>]

• **Tools for Functional Assessment of Biotherapeutics**—Anurag S. Rathore, Rozaleen Dash, Ritu Jain, and Jared Auclair present the main analytical techniques for performing functional characterization of biotherapeutic products. Such assessments are particularly critical for biosimilars, where analytical testing must ensure functional comparability with the innovator product. [Read Here>>]

• **An Ant-Man Perspective for Chromatography: My Stochastic World**—The stochastic theory of chromatography allows one to connect mathematics to separation science in an intelligible form. We take a “walk” through the column at the level of an Ant-Man, where we can see that chromatography is mathematics and mathematics is chromatography! [Read Here>>]

• **Waters Corporation Celebrates the Life and Legacy of its Founder, Jim Waters**—Waters Corporation founder James L. Waters, a legendary inventor and pioneer in liquid chromatography, has passed away. [Read Here>>]

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**Coriolis Pharma Expands Facilities**
Coriolis Pharma (Martinsried-Munich, Germany) has announced the expansion of its advanced therapy medicinal products (ATMP) development facilities under biosafety level S2 (BSL2 / S2). Reconstruction of an existing building near the Coriolis headquarters started in March this year and the laboratories are planned to be operational in Q4 2021. The new facilities will increase Coriolis’ total floor space to 7800 m². The new labs will host the formulation development of ATMPs, cell culture activities, particle characterization and identification, analytical ultracentrifugation, and a lyophilization development centre for ATMPs. For more information, please visit: www.coriolis-pharma.com

**Veraxa Biotech and Quadira Biosciences Announce Partnership**
Veraxa Biotech GmbH (Heidelberg, Germany) has announced a partnership agreement with Quadira Biosciences AG (Zug, Switzerland) to jointly develop a suite of novel antibody-drug conjugates (ADCs) for the treatment of oncological conditions. The collaboration will leverage both the advantages of Veraxa’s drug conjugation technology in combination with Quadira’s 3D cellular assay and assessment systems. For more information, please visit: https://veraxa.de/
Meeting Regulatory Needs in the Characterization of Lipid Nanoparticles for RNA Delivery via FFF-MALS

Fanny Caputo1 and Christian Sieg2, 1Department of Biotechnology and Nanomedicine, SINTEF Industry, Trondheim, Norway, 2Wyatt Technology Europe GmbH, Dernbach, Germany

Field-flow fractionation coupled to multi-angle light scattering (FFF-MALS) is a powerful analytical approach for the advanced characterization of nanomaterials (sizes < 1 µm). Contrary to size-exclusion chromatography (SEC), no stationary phase is required during the fractionation process, and the optimal separation range can be fine-tuned by varying flow parameters. This flexibility enables reliable measurement of complex pharmaceutical products, such as nanomedicines, monoclonal antibodies (mAbs), and vaccines. For quality control purposes, the FFF-MALS protocol must satisfy regulatory needs and accepted standards. This article describes a validated FFF-MALS method aligned with technical specification ISO/TS 21362 for the analysis of lipid-based nanoparticles (LNPs) encapsulating siRNA and mRNA.

Lipid-based nanoparticles (LNPs) for nucleic acid delivery, especially for short interfering RNA (siRNA) and messenger RNA (mRNA), have recently attracted extraordinary attention, and are expected to revolutionize the medical
At the end of 2020, a milestone was reached with two vaccines against the ongoing COVID-19 pandemic, based on mRNA strands encapsulated in LNPs, approved by regulatory authorities in the USA and Europe: the BioNTech/Pfizer’s tozinameran and Moderna’s mRNA-1273. Apart from COVID-19 vaccines, further nucleic acid-based therapies are in development for a broad range of applications spanning immune-modulating agents, protein replacement therapies, regenerative medicine, and gene-editing complexes, amongst others. Nanocarriers such as LNPs can protect active pharmaceutical ingredients (APIs), enhance bioavailability, and thus improve safety and efficacy of novel therapies (1).

The use of lipid nanocarriers to deliver nucleic acid increases the complexity of the formulation and consequently introduces the need for sophisticated analytics to ascertain a stable and safe drug product. Based on guidelines for drug products containing nanomaterials, including liposome characterization, the following parameters can be regarded as plausible critical quality attributes (CQAs): particle concentration, particle average size and polydispersity, nucleic acid loading levels, and chemical stability and physical stability (aggregation propensity). Clearly, the advancement of robust analytical methods for CQA determination that are compliant with regulatory requirements is essential to streamline development and quality control.

Field-flow fractionation (FFF) physically separates species of different sizes or, more precisely, hydrodynamic volumes (Figure 1). First, the unseparated sample is injected into the channel. In conventional channels, the sample attains equilibrium height above the membrane in a focusing step; this action tends to concentrate the sample in close proximity to the membrane. The dispersion inlet channel (also known as frit inlet channel) uses the “hydrodynamic relaxation” principle for which no focusing step is required, diminishing unwanted effects of high local concentrations. A parabolic channel flow then transports the sample towards the channel outlet. Since small particles have a higher diffusion coefficient, they will on average be higher above the bottom membrane and hence exposed to a higher flow rate. Thus, small particles elute first, followed by larger species, inversely to the order in size-exclusion chromatography (SEC).

The particle retention time, $t_r$, is directly proportional to the hydrodynamic radius, $R_h$, and the tunable cross flow/channel flow ratio. Subsequent multidetector...
analysis including UV, multi-angle light scattering (MALS), online dynamic light scattering (DLS), and differential refractive index (dRI) detection elucidates several key sample parameters in the same measurement. This was recently demonstrated for pharmaceutical products including liposomal drug formulations (2,3). Based on the 2021 publication by Mildner et al. (4), we outline here the establishment of a robust FFF-MALS method for the characterization of lipid nanoparticles encapsulating RNA (RNA-LNP). Key parameters that can be measured with FFF-MALS are average particle size, polydispersity, morphology, physical stability, and particle concentration.

To fulfill regulatory requirements, the repeatability, reproducibility, and robustness of the analytical methods used to characterize a drug product need to be addressed according to the harmonized guideline ICHQ2R1 (5). Even if ICHQ2R1 addresses mostly classical chromatographic methods and does not explicitly mention FFF-MALS, its principles can be applied to ensure the quality and reliability of the analytical methods used for drug product characterization.

**Figure 2:** Comparison of conventional and dispersion inlet channel separation and recovery of a siRNA formulation. (a) The UV fractograms of conventional long channel and dispersion channel are shown with rms radius as an overlay. The main species has a size of 26–27 nm. (b) Here, the results averaged over three measurements are reported including standard deviation of the mean. Spread: RMS max–RMS min across the full width half maximum (FWHM) of the peak.

- **Conventional long channel:**
  - R%: 91 ± 1
  - rms radius: 27 ± 0.5
  - Spread: 11 ± 2

- **Dispersion inlet channel:**
  - R%: 99 ± 1
  - rms radius: 26 ± 1
  - Spread: 6.5 ± 0.5

**Figure 3:** Comparison of mRNA-LNP samples with different mRNA content and empty particles. The LS fractograms at 90° MALS angle are shown overlaid with the ratio of rms radius and hydrodynamic radius, \( \frac{R_g}{R_h} \). The ratio tracks the degree of encapsulated RNA.
not specify the requirements for sizing measurements such as FFF-MALS, ISO/TS 21362 (6) could be used as a suitable reference for the validation of FFF-MALS methods. The following criteria need to be met:

1. Recovery of the analyte ≥ 70% (performance separations, such as particle concentration, would require recovery ≥ 90%);

### Figure 4: Particle size distribution by number and total particle concentration values obtained by FFF-MALS and NTA on a formulation of LNPs encapsulating siRNA.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Total particle/mL (SD)</th>
<th>Mode Radius nm (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTA</td>
<td>3.9E+12 (3E+11)</td>
<td>36 (2)</td>
</tr>
<tr>
<td>FFF-MALS</td>
<td>6.77E+12 (1.5E+10)</td>
<td>33 (2)</td>
</tr>
</tbody>
</table>
This article describes FFF method development, including channel-type selection, and demonstrates the power and versatility of FFF-MALS for the characterization of siRNA-LNP and mRNA-LNP according to regulatory needs.

**Experimental**

The experimental procedure is described in depth in reference 3. In brief, the samples were analyzed using an Eclipse FFF instrument (Wyatt Technology) operated with an isocratic pump, degasser, and autosampler from the 1260 Infinity II series (Agilent Technologies). Fractionation was performed in the Eclipse conventional long channel and dispersion-inlet channel. Sample recovery was calculated by integrating the main UV peak area at 260 nm for each sample, with and without the applied cross flow and focusing step.

Detection was accomplished by a DAWN MALS instrument with embedded DLS module (Wyatt Technology). Data were collected and analyzed in Astra software (Wyatt Technology) to determine particle size and concentration. Further data processing calculated the particle size distribution in particles/mL/nm for 2 nm bin sizes (4).

**High Recovery as Key Measurement Quality Indicator**

Sample recovery (R%) is the key parameter to consider for initial method optimization. It is determined by main UV peak integration with and without cross flow and focusing step. During method development, the conventional setup with a standard FFF long channel was compared to a method using the dispersion inlet channel, where the sample is injected directly into the carrier stream. Here, no focusing is required, reducing the risk of sample destabilization and increasing recovery for delicate samples such as LNPs. Figure 2 shows the results obtained by measuring the same siRNA-LNP formulation by using the two channels. In both channels the sample is eluted and fractionated according to the particle size. Although in the tested configuration both channels fulfill the quality criteria in terms of R%, the dispersion inlet method was used for advanced characterization of LNPs due to the higher recovery and its benefits for sensitive samples.

**Determination of Size, Morphology, and Particle Concentration by Using an Optimized Dispersion Inlet Method**

Using the optimized fractionation method, with MALS and DLS detectors online, several LNP key attributes can be determined, including: (i) average size and...
polydispersity, (ii) particle morphology, and (iii) particle concentration. As proof of concept, three formulations with variable mRNA payload were measured to determine both the sample size, polydispersity, and particle morphology: empty particles, particles with low (N/P = 8) RNA content, and particles with high (N/P = 3) RNA content. The particle size and polydispersity were higher for lower mRNA content, and even more pronounced for empty particles, indicating that a minimum amount of mRNA is needed to obtain a monodisperse formulation. In fact, it is known that in the absence of a sufficient amount of RNA, the LNP nanostructure where the mRNA is complexed by the ionizable lipids and the other particle components cannot be formed.

Differences in particle size and polydispersity are therefore associated with differences in particle morphology, another important parameter that can be determined by FFF-MALS. MALS with simultaneous online DLS to determine hydrodynamic radius ($R_h$) elucidates particle morphology (shape, core density) associated with the RNA loading. For a hollow sphere, $\rho$ (the ratio of rms radius [determined by MALS] to $R_h$) is unity. For a sphere with a dense core, however, the scattering centres are distributed closer to the centre of mass and $\rho \leq 0.77$ is expected. Figure 3 shows $\rho$ vs. retention time of mRNA-LNP samples with different RNA content, and of the empty LNP control sample. Interestingly, the empty particles possess $\rho = 1$, typical of a hollow or irregular sphere, while increasing mRNA contents reduces the ratio. The value of the monodisperse mRNA particles match the ratio associated with a dense spherical particle. Note, the empty LNP and the mRNA particles loaded with a lower payload show a larger proportion of aggregates, and are less stable, showing the correlation between particle morphology, monodisperse size distribution, and the physical stability of the LNP formulations.

As demonstrated by Mildner et al. (4), the data obtained by MALS, in combination with knowledge of particle shape, structure, and refractive indices of the particles and solvent, determine particle concentration and thus derive the number-based particle size distribution. As a proof of concept, the results obtained by FFF-MALS were compared with particle concentration measured by nanoparticle tracking analysis (NTA) and showed remarkably comparable results (Figure 4). Notably,
FNN-MALS detects and quantifies LNP particles below 30 nm in radius that are too small for NTA.

**LNP Stability Assessment**

A critical parameter for RNA-LNPs is their stability. As FNN-MALS can determine size, morphology, and concentration with high resolution, it is one of the methods of choice for evaluating the physical stability of the particles, and to determine their tendency to aggregate. Here, a freshly prepared LNP sample loaded with siRNA was compared to a sample prepared three months earlier and stored at 4 °C. The freshly prepared sample showed one main particle population (Peak 1: $R_t = 17$ min) and a small number of larger particles eluted as a second peak (Peak 2: $R_t = 31$ min). After 3 months of storage, the size associated with the main population was slightly decreased, while the intensity of the peak associated with larger particles and/or aggregated was significantly higher (Figure 5). The change in the particle size distribution and substantial increase in sample polydispersity is called Ostwald ripening and is induced by the migration of lipids and particle components from smaller particles to larger ones. Higher sample polydispersity immediately following synthesis corresponds to faster Ostwald ripening and thus larger particle instability. This highlights the power of FNN-MALS to study changes in size, polydispersity, and concentration of LNP formulations over time.

**Conclusion**

Thorough method development and validation are crucial to move FNN-MALS into standard QC procedures in the nanomedicine field. Here, we demonstrated method development following the ISO/TS 21362 technical specification and showed results for RNA-LNP. We presented a robust FNN-MALS approach that can be used by the pharmaceutical industry to characterize nucleic acid-based nanotherapeutics during drug development and for quality control purposes. Importantly, FNN-MALS measures multiple CQAs of LNP nanoparticles encapsulating mRNA or siRNA, such as particle size distribution, particle morphology, and particle concentration, as well as physical stability and aggregation propensity.

**References**


Fanny Caputo is a research scientist at SINTEF (Norway). Her main interests lie in the physical-chemical assessment of nanomaterials, nanoplastic, medical devices, and pharmaceutical products containing nanomaterials, including lipid-based nanoparticles for nucleic acid delivery.

Christian Sieg works as an application scientist for Wyatt Technology and is responsible for instructing customers to utilize Wyatt’s instrumentation. His work includes visiting customers onsite, establishing applications, and training.

Website: www.wyatt.com/LNP
Enhanced Evaluation of the Authenticity of Perfumes Using GC×GC–TOF-MS

Laura McGregor, Aaron Parker, and Elinor Hughes, SepSolve Analytical, Peterborough, UK

The global perfumes market was valued at over $30 billion in 2019 and is expected to continue growing. It is perhaps inevitable that this has been accompanied by a rise in counterfeit products, sold at significantly lower prices compared to the luxury brands they imitate. Comprehensive data acquired using two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOF-MS) combined with tandem ionization—simultaneous hard and soft electron ionization—data can be used to uncover subtle differences between brand and imitation perfumes.

Counterfeit perfumes, and their packaging, are often so similar to the authentic perfumes that consumers may be unable to distinguish between genuine and imitation goods. Reliable quality and authenticity tests are important to ensure consumers do not receive an inferior product, to protect brand reputation, and to reduce loss of revenue.

However, the diverse range of chemical classes in perfumes requires advanced separations to resolve coelutions to provide identification of the analytes present. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOF-MS) can tackle this challenge, as it is capable of separating compounds that would normally coelute with...
one-dimensional GC. This is because with GC×GC, two columns of different selectivity separate the analytes based on two different chemical properties (for example, volatility and polarity).

Another challenge is that perfumes contain structurally similar compounds (such as terpenes) that can be difficult (or impossible) to speciate when using conventional 70 eV electron ionization (EI), even with the increased separation capacity of GC×GC. Tandem ionization, which provides both hard and soft EI data in a single analysis, can overcome this problem. The complementary soft EI spectra improves chemical selectivity to enhance untargeted “discovery” workflows, as required in quality and authenticity evaluation, where you may not know what compounds can be used to differentiate between genuine and imitation products.

Here, we show how the comprehensive data acquired using GC×GC–TOF-MS with tandem ionization can be processed in a single, automated workflow to uncover the subtle differences between brand and imitation fragrances for quality and authenticity evaluation.

**Experimental**

Three brand perfumes and three imitation perfumes were analyzed in triplicate by GC×GC–TOF-MS. The perfumes had three distinct scents: blackberry, pomegranate, and lime. GC×GC: Insight flow modulator (SepSolve Analytical); Modulation period (P_M): 3.6 s. MS: Instrument: BenchTOF2 - TI (SepSolve Analytical); Mass range: m/z 35–600; Acquisition rate: 100 Hz.

Figure 1: GC×GC–TOF-MS colour plots for the three brand and imitation fragrances.
in tandem ionization mode at 70 and 14 eV. Software: Full instrument control and data processing by ChromSpace with ChromCompare+ (SepSolve Analytical) for untargeted chemometrics.

**Results and Discussion**

In this study, three luxury brand perfumes and their imitation versions were analyzed. As seen in Figure 1, the brand and imitation fragrances share similar compositions; however, there are differences that can be uncovered easily by GC×GC–TOF-MS.

Here, tandem ionization was also utilized to improve the selectivity for compounds that share similar spectra at 70 eV (such as terpenes). This enabled both hard and soft EI data to be acquired simultaneously in a single workflow for complementary chemical information with no added analysis time. Figure 2 shows the “tandem” data file, which contains both MS data blocks. Using the tandem format, both sets of spectra were searched simultaneously against hard and soft ionization libraries.

Tandem ionization is especially useful in the case of isomer identification, such as the diverse range of terpenes and terpenoids found in fragrances. For example, Figure 3 shows spectral comparisons for two isomers of guaiene, with enhanced differences in ion ratios obtained when using soft EI.

In this study, the tandem ionization data was also used to improve discovery of true differences between the brand and imitation perfumes, using novel automated workflows in the software.

This approach divides the chromatogram into small tiles and sums the signal for every individual $m/z$ channel in each tile for comparison between samples. Time-consuming pre-processing steps, such as integration and identification, are eliminated and all the raw data is used, minimizing the risk of missing important details. By utilizing both sets of MS data in a single workflow, discovery of true differences...
is improved through reduction of false positives.

The principal components analysis (PCA) score plot in Figure 4 shows the comparison of all six perfumes (run in triplicate). The brand and imitation versions of the pomegranate fragrance appear to be most similar in composition as they cluster closely in the PCA plot, while those of lime and blackberry appear to be more distinct.

Using a feature discovery tool in the software, the top 10 differentiating features between the brand and imitation versions were found for each fragrance and were identified using the TOF-MS (Figure 5).

Interestingly, the imitation pomegranate fragrance was found to contain the two isomers of lyral (also known as 3- and 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde, or HICC). Lyral is now banned under Regulation (EU) 2017/1410 (an amendment to Regulation 1223/2009/EC), due to the high risk of contact allergy associated with this compound (1). The amendment calls for all non-compliant cosmetics to be withdrawn from the market by 23 August 2021.

On the other hand, many of the differentiators found only in the brand perfumes are described as “long-lasting” base notes—such as kephalis and muscencene—and as such, these are potential markers of quality when comparing brand and imitation products.

It is important to note that the use of tandem data to confirm positive hits during feature discovery improved the discovery of subtle, trace differences by suppressing false positives. When the top 50 differences for the brand and imitation fragrances were compared using the tandem data and then using 70 eV data alone, two true
true differences have been found, by reducing the number of false positives for review.

**Conclusions**

This study has shown that GC×GC–TOF-MS with tandem ionization and chemometrics software provides comprehensive fragrance profiling to aid quality and authenticity evaluation. The study demonstrated the enhanced separation of GC×GC, combined with the spectral fidelity of TOF-MS, enabling confident identification of analytes in fragrances. Tandem ionization added an extra level of confidence through complementary hard and soft EI data. Finally, the software enabled fast and simple identification of key quality markers using automated, untargeted data analysis.

**References**


Laura McGregor received an M.Chem. in chemistry from the University of St Andrews, UK, followed by an M.Sc. in forensic science at the University of Strathclyde, UK. Her Ph.D. in environmental forensics focused on the chemical fingerprinting of environmental contamination using advanced techniques such as GC×GC–TOF-MS. In her current role at SepSolve Analytical, she specializes in the application of GC×GC and TOF-MS to challenging applications.

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The International Conference on Non-Target Screening 2021 (ICNTS21) will take place 4–7 October 2021 and be a hybrid event, meaning it will be both online and on-site in Erding, Germany. Here’s a preview of what attendees can look forward to.

In recent years there have been several very successful workshops on non-target screening (NTS), starting in 2016 with the Non-Target meeting in Ascona (Switzerland), continuing with “SWEMSA 2016” in Garching (Germany), the many Society of Environmental Toxicology and Chemistry (SETAC) meetings, American Chemical Society (ACS), Royal Society of Chemistry (RSC), and Gesellschaft Deutscher Chemiker (GDCh) events, “SWEMSA 2019” in Erding (Germany), and most recently a digital meeting at Analytica 2020, as well as the “GC meets NTS” workshop in 2021.

Now, for the first time, non-target screening and its applications are the focus of their own international conference. This conference will bring together leading international scientists from various consortia and disciplines. It is the ideal location for free laboratory, industrial, and academic researchers to exchange information with colleagues from all around the world and across disciplines. NTS users and vendors from the field of instrumental analysis and software developments will present their latest results and ideas in keynote lectures, lecture sessions,
and poster sessions throughout the meeting.

The International Conference on Non-Target Screening 2021 (ICNTS21) will be a hybrid conference, meaning it will be both online and on-site in Erding, Germany, and will take place 4–7 October 2021. The conference will promote discussion on NTS topics, such as:

- NTS with quality standards, data standardization, and harmonization
- NTS in doping and forensics
- NTS in environmental analysis
- NTS in food(omics)
- NTS in metabolomics
- NTS in commercial solutions
- Computational mass spectrometry (MS), with various instrumental setups and strategies, such as liquid chromatography–mass spectrometry (LC)–MS/MS, gas chromatography (GC)–MS/MS, supercritical fluid chromatography (SFC)–MS/MS, multidimensional chromatography, and other techniques in NTS such as nuclear magnetic resonance (NMR) and infrared (IR).

You still can apply for a late poster presentation. The deadline to submit an abstract is 31 August 2021.

ICNTS21 intends to inform, combine, and harmonize the NTS strategies and workflows from each single discipline to extend the NTS horizon and to give us all the chance to “take a look over the edge”. Participants of various disciplines such as chemistry, environment, food, forensic, informatics, metabolomics, water, and instrumental analysis will discuss the latest developments in NTS with a focus on quality standards in analysis and data handling. The programme will feature a solution-focused discussion strategy including overview talks and panel discussions in each slot. Each panel discussion—a ICNTS21 specialty—is guided and integrates the participants strongly.

The overall aim of this meeting is to condense, standardize, and harmonize various common aspects of NTS, to extend the use and understanding of software and workflow strategies, and to learn about the potential of NTS applied in various disciplines. The organizers are strongly encouraging the active participation of younger scientists in the conference with posters, and are offering a discount to those that qualify.

In (post)pandemic times, a hybrid format is essential (online talks, posters, and discussions alongside on-site talks, posters, and discussions). The interaction of both types of participants is an exciting challenge. A rich social programme awaits participants in Erding with sightseeing and three evening events planned. The evening events will also bring you closer to the Bavarian style of life and offer some culinary delights.

Erding has a wide range of accommodation to suit all budgets, and with its international airport it is easily accessible by plane, as well as by car or train for those closer by.

The organizers are looking forward to welcoming you to Erding later this year.

For more information and registration, please visit the website at: https://afin-ts.de/icnts-21
E-mail: education@afin-ts.de
The LCGC Blog: Water-Immiscible Solvents as Sample Diluents in Reversed-Phase HPLC—You Must Be Joking!

Tony Taylor, Arch Sciences Group, Manchester, UK, and CHROMacademy

Many of us have faced the situation where we have analytes that vary widely in their polarity or LogP(D) values, and encounter issues with analyte solubility when choosing a suitable sample diluent for our high pressure liquid chromatography (HPLC) analysis. The more polar analyte will favour aqueous solvents, and the less polar will be more highly soluble in organic solvent—so which do we choose?

We often plump for an intermediate solvent such as 50:50 water–acetonitrile (or methanol) to make the diluent as favourable as possible to ensure all analytes are fully dissolved prior to analysis. However, this is often where we run into problems, with peak shape deformation of early eluting peaks—particularly when gradient high performance liquid chromatography (HPLC) is employed. There is also the possibility that neither acetonitrile nor methanol are able to solubilize the hydrophobic analytes of interest. Further, we may have an organic eluent from a sample preparation technique that we wish to inject directly into our HPLC system without risking drying down and
re-constituting in an aqueous-based diluent (solvent exchange). What are our options under these circumstances?

Well, contrary to popular belief, it is possible to use water-immiscible solvents or solvent combinations as injection solvents when the solubility of analytes is limited or if we wish to introduce an organic extract directly into the HPLC system. However, there are a few basic rules that we need to follow for successful analysis.

So, let’s start by examining the so-called “general elution” problem, where we need to dissolve our analytes in a diluent that contains a higher proportion of organic solvent than the mobile phase at the start of the gradient.

Figure 1 shows two model analytes during “ideal” gradient analysis circumstances. Here the diluent has the same or lower elution strength than the starting composition of the eluent in a gradient analysis. For example, if the eluent composition at the start of the gradient is 90:10 water–acetonitrile, then the diluent will contain 10% or less acetonitrile by volume. As the sample plug is injected into the HPLC system and enters the column, and the sample diluent and eluent mix, then the elution strength of the solvent environment for each analyte will be approximately equal. The analyte will travel through the column and there will be no solvent-based driving force for peak broadening or splitting; this is the ideal situation for gradient HPLC analysis. For clarity, all of the other intrinsic band-broadening processes associated with HPLC will still be in play, however, there will be no further solvent-based band broadening.

As I’ve explained above, there are several circumstances in which this ideal situation cannot be achieved, and often we need to increase the “elution strength” of the sample diluent above the composition of the starting gradient. This situation risks both peak broadening and deformation, especially for earlier eluting analytes.

Figure 2 shows why this might be the case. The red solvent is the more highly eluting sample diluent, and the blue is the mobile phase, which has a lower elution strength. As the injected sample mixes at the head of the analytical column, the analytes are distributed across a zone that has markedly different elution strength characteristics, and so some analytes travel down the column faster and some will travel slower. The chromatogram in Figure 2 highlights the issues with peak broadening and splitting due to the “differential” sample elution speed of analytes in either the faster or slower eluting solvents. These peak shapes are far from ideal and will certainly give us problems with peak integration—and destroy the faith any reviewer of the data has in our abilities as chromatographers!
There are several precautions that we can take to reduce these effects, including:

- Using the lowest amount of organic solvent within the diluent that will produce the required solubility characteristics for the analytes of interest;
- Using the lowest injection volume possible to ensure the optimum mixing of eluent and diluent prior to the sample reaching the head of the analytical column (for example, within the tubing, eluent pre-heating chambers, and connections between the autosampler and the analytical column);
- Using a low-volume inline filter at the outlet of the injection valve to promote the mixing of the diluent and eluent, again achieving a more homogeneous solvent plug prior to arrival at the head of the analytical column.

However, what if we need to revert to an alternative organic solvent as the sample diluent for solubility reasons? Or what if we wish to avoid a solvent-exchange step from our sample preparation eluate? Well, it is possible to use much less conventional, even water-immiscible, solvents as diluents if we bear one primary rule in mind, and that is, the solvent we use must elute later than our analytes of interest, or, if this is not possible, then much earlier than them.

Figure 3 demonstrates this principle with our model analytes. Unfortunately, we can see that a solvent has been chosen that elutes between the two analytes and whilst the earlier eluting analyte has regained its peak shape, the analyte eluting later than the solvent front gives us a very poor peak shape. Again, we can rationalize this behaviour in terms of the distribution of analyte between the two solvent regions. Where the organic solvent—ethyl acetate in this case—elutes more slowly than the analyte, there is no driving force for the analyte band to broaden as the front of the analyte band is not moving faster than the back of the analyte band. Whilst the organic solvent still has a higher elution strength, as long as it does not carry the front of the analyte band with it, then no broadening will occur. Conversely, for the later-eluting analyte, we are back to the same position we had in Figure 2, where the front of the analyte band is carried significantly further down the column than the back of the band and peak deformation occurs.

In Figure 4, we have chosen methyl isobutyl ketone (MIBK) as the sample diluent, which elutes later than both of the model analytes. Here we achieve the ideal situation in which both analyte bands encounter weaker mobile-phase strength at the front of the band and a more strongly eluting mobile phase at the rear of the analyte band as the sample enters the column. As described earlier, neither analyte experiences
a driving force for severe peak broadening or splitting, and one could argue strongly that these peak shapes would be acceptable for integration and save our reputation as decent chromatographers!

We should be realistic here and comment that in any situation where our analytes experience differential elution strength environments as they enter the HPLC column, there is a risk of a small degree of band broadening, and this risk increases as the volume of the sample injection increases. As such, we should still seek to limit the volume of sample injected to minimize any peak broadening.

Many different organic solvents can be used in this manner—the important point being the relative elution time between the diluent solvent and your analytes, and this may need a little trial and error to optimize. I have had success with iso-propanol, ethyl acetate, MIBK, and toluene to name but a few diluent solvents. It is also possible to mix small amounts of acetonitrile with these solvents, in various proportions, depending upon the solubility characteristics required for your sample mixture.

So, the next time you are considering moving to hydrophilic interaction chromatography (HILIC) using a solvent-exchange step for your organic eluate from your sample preparation step or, even more radically, thinking of moving to normal-phase chromatography, ask yourself if using an organic solvent as your diluent might just solve your problem with a great deal less fuss!

Tony Taylor is the 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.

Website: www.chromatographyonline.com
## Training Courses

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- **Website:** [www.chromacademy.com/channels/gc-training-courses/troubleshooting/gc-troubleshooter](http://www.chromacademy.com/channels/gc-training-courses/troubleshooting/gc-troubleshooter)

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### Applied Interpretation of GC–MS
- **Mass Spectra**
  - **28–30 July 2021**
  - **Online—virtual classroom**
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### HPLC/LC–MS
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- **Fundamental LC–MS**
  - **Website:** [www.chromacademy.com/channels/lc-ms/principles/fundamentals-of-lc-ms-video-training-course](http://www.chromacademy.com/channels/lc-ms/principles/fundamentals-of-lc-ms-video-training-course)

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Please send your event and training course information to Kate Jones
KJones@mjh lifesciences.com
Event News

5–7 October 2021
14th Congress of the French-Speaking Association for Separative Sciences (SEP21)
Porte de Versailles, Paris, France
E-mail: info@sep2021.fr
Website: www.sep2021.fr

5–7 October 2021
Forum Labo
Paris Expo - Porte de Versailles, Paris, France
E-mail: infos@forumlabo.com
Website: www.forumlabo.com/paris/en-gb.html

2–5 November 2021
The 10th International Symposium on Recent Advances in Food Analysis (RAFA 2021)
Clarion Congress Hotel, Prague, Czech Republic
E-mail: RAFA2021@vscht.cz
Website: www.rafa2021.eu

26–28 January 2022
The 17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-17)
Het Pand Conference Center, Ghent, Belgium
E-mail: htc17@kuleuven.be
Website: https://htc-17.com/

11–14 October 2022
The 18th International Symposium on Preparative and Industrial Chromatography and Allied Techniques (SPICA 2022)
Lisbon, Portugal
E-mail: secretariat@LDOrganisation.lu
Website: www.spica2022.org/
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