LCGC™
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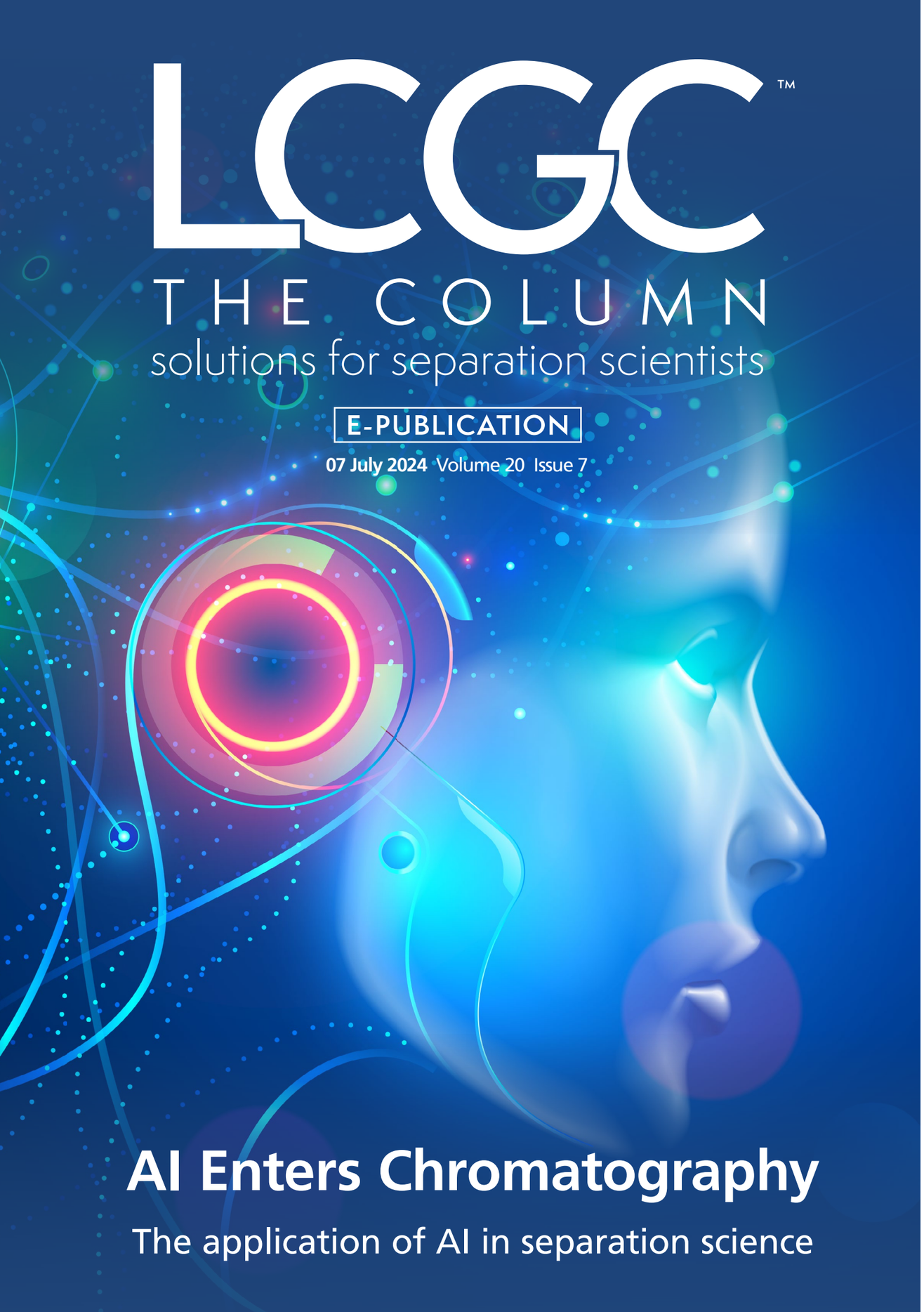
E-PUBLICATION
07 July 2024 Volume 20 Issue 7



AI Enters Chromatography

The application of AI in separation science

Cover Story

Features

Regulars

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Technology Trends in Separation Science: Data Handling

Patrick Lavery, Editor, *LCGC International*

LCGC International **spoke with Shawn Anderson, Associate Vice President of Digital Lab Innovations at Agilent Technologies; Marco Kleine, Head of the Informatics Department at Shimadzu Europa GmbH; Trish Meek, Senior Director, Connected Science, Waters Corporation and Todor Petrov, Senior Director, QA/QC, Waters Corporation; and Crystal Welch, Product Marketing Manager at Thermo Fisher Scientific about the latest trends in data handling.**

What is currently the biggest problem in data management for chromatographers?

MARCO KLEINE: One of the biggest problems in data management for chromatographers is the huge volume of data generated during analyses. Chromatography techniques, such as liquid chromatography (LC), high-performance liquid chromatography (HPLC), and gas chromatography (GC), produce large amounts of data that need to be organised, stored, analysed and in some cases transported through a network. This can be a time-consuming and error-prone task. Additionally, the lack of standardized data formats and the compatibility issues between different chromatography software systems (CDSs) can make data management even more complicated.

SHAWN ANDERSON: Thoughtful inclusion of the chromatography results in a larger data set, to allow for insights into purity and yield improvements. After all, separation and detection combined are only one step (often the last) in what is usually a process to produce a molecule. There are many other steps as well, and correlating the purity and yield results with other factors in this process can drive true innovation. As a prerequisite for this, many chromatographers are yearning

for more widespread adoption of vendor-neutral standards for data. The Findability, Accessibility, Interoperability, and Reusability (FAIR) data principles are useful for guiding this journey (1).

CRYSTAL WELCH: The largest problem continues to be reducing the time and effort spent to manage data. It is still common that files are spread across multiple storage locations, with the effort to compile information together being manual and time-consuming. People want their chromatography systems to work like their phone software, with a more stable platform and easy-to-use applications, with all data secured into a central location so they can use, view, and download it onto their next tablet or cell phone without having to transfer it from device to device.

TODOR PETROV: If we look at the chromatography data as something that has a lifecycle, there are different challenges in the different phases the data goes through. For example, once a multitude of chromatograms are acquired and quantitative results are calculated, the first challenge an analyst faces is with screening the data to determine which data sets are in line with the expectations and which are outliers. In today's technology, machine learning can be utilized for anomaly detection to make the data review process more efficient by focusing on the exceptions.

Once the data passes the first review gate, the next challenge may often be with data sharing for collaboration purposes. Companies have large networks of partners that generate chromatography data that the sponsors need to review as well. The growth of contract services demands efficient solutions for data sharing with minimum delays. In today's technology, cloud-based solutions offer the best mechanisms to achieve that.

Once the chromatography data has been reviewed and has served its primary purpose, it needs to be made available for extracting analytical insights across other processes the sample in question has been subjected to. The data format standardization is the main challenge in this phase.

The data gets archived eventually and while the amounts of it accumulated over time can be challenging to manage, a major challenge is the expectation that data sets can be resurrected at any time in the software application that has produced them originally. This implies data format compatibility that goes back decades or having to maintain dated application instances.

TRISH MEEK: Throughout each of the steps in the lifecycle that Todor described, laboratories need to be able to share laboratory data and methods with their internal and external colleagues, show

auditors that they are following regulatory guidance and Good Laboratory Practice (GLP), and use their data to make decisions about whether water is safe to drink or if a product can be released.

While organizations often rely on systems like electroniclab notebooks (ELNs) and laboratory information management systems (LIMS) to aggregate and share to handle final results, like peak concentrations and amounts, across the enterprise it does not include all of the chromatography data, so it is often evaluated without the context of how that data was acquired. As we work with laboratories, their biggest challenge is getting the complete picture of their data.

What is the future of data handling solutions for chromatographers?

ANDERSON: We believe that we are seeing the limits of the current LIMS-oriented model, and we are likely to see an advancement in insight generation that is distinct and separate from the LIMS wheelhouse of sample management and test execution. There are numerous innovations around this that are becoming popular. One is data format standardization in a vendor-neutral way, likely based on ASM, the allotrope simplified model. This provides a common input language for organizations to develop and maintain their own data lakes. Another is cloud/prem hybrid storage, which balances redundancy and backup security with low-latency, real-time access. This hybrid model can also allow for more powerful (and cheaper) data processing operations in the cloud while keeping control and stepwise analyses on premises and close to the instrument and end user.

KLEINE: The future of data handling solutions for chromatographers is likely to involve advances in automation, cloud-based storage, data analytics and standardisation.

In terms of automation the increasing volume of data generated during a measurement means automation will play a key role in data handling. AI-driven algorithms can automate data processing and analysis, reducing the amount of work and minimizing (human) errors.

Cloud-based technologies will enable chromatographers to store and access their data remotely from everywhere. Cloud-based solutions also enable data sharing and collaboration with other researchers. Advanced data analytics techniques, such as machine learning and artificial intelligence, will help to extract more detailed information from chromatographic data.

Additionally, standardisation will become important. Efforts have already been undertaken to establish standardized data formats and protocols for chromatographic

data to ensure integration and compatibility between different instruments and software platforms.

WELCH: Solutions in this space are looking to take the hard work out of data analysis and management—whether that is by enabling software to process data holistically and offer things like consensus reporting for multi-omics, reduce manual processes with automation, or leverage new AI tools with a goal of getting closer to the truth.

PETROV: Many organizations are moving or have moved their IT infrastructure to the cloud, including data handling solutions like CDS. There are multiple reasons for the increasing interest in software as a service (SaaS) solutions for chromatography data. The primary reasons are to simplify the management of the applications and to make the data accessible to the organization. SaaS solutions provide benefits such as secure worldwide access, up-to-date application and infrastructure security, scalable IT infrastructure, economies of scale, competitive operational costs, and lower initial costs compared to non-subscription deployments on premise.

MEEK: In addition to the infrastructure changes, techniques such as machine learning will become critical to data acquisition, processing, and analytics. There are many opportunities to improve on traditional data processing algorithms and support review by exception by deploying artificial intelligence.

What one recent development in "Big Data" is most important for chromatographers from a practical perspective?

ANDERSON: It is difficult to not answer "Generative AI" for this question. An obvious use case might be to train a model on chromatographic methods for categories of molecules and then ask the AI to generate ideal yet broadly applicable separation methods. Another area that is intriguing (but not as fashionable as AI) is using Big Data for real-time decision-making. One example is using chromatographic data from bioreactor sampling to trigger changes in media composition or temperature settings. Another example is setting limits for hardware metrics such as pump cycles to automatically trigger preventative maintenance scheduling.

KLEINE: For a long time, chromatographers have relied on manual data analysis methods, which can be time-consuming and lead to errors. With the latest development in (big) data analytics, chromatographers now have access to powerful tools, like databases, that can support and automate data analysis. These data analytics tools utilise machine learning algorithms, pattern recognition techniques, and statistical analysis methods to analyse large volumes of chromatographic data quickly and accurately. They can help in identifying peaks, quantifying compounds, detecting outliers, and optimising experimental conditions.

WELCH: Big Data can mean different things to different people, but one practical example would be utilizing trending over time to inform on when to perform maintenance, replace instrumentation, or just manage practical utilization of instrumentation better. Tools like schedulers, control charting, or predictive modelling can help plan for events and keep the whole lab moving forward.

PETROV: The term "Big Data" is typically used to describe large, unstructured data—think random text, images, and videos—where searching for an item of interest is not trivial and pattern recognition and training models are utilized instead. The chromatography data is structured for the most part, except for the chromatograms themselves, and therein lies the opportunity for using machine learning algorithms originally developed for Big Data. Detecting anomalies using such algorithms can substantially increase the efficiency of traditional methods for comparing chromatograms.

If we extend the scope beyond chromatography and consider the data lakes storing data from multiple phases a substance goes through during its development or manufacturing process, unstructured data is how that can be described. From that standpoint, anomaly detection algorithms can be beneficial, as well as another type of machine learning algorithms, known as classifiers. The classifiers identify clusters of similar data, and once clusters are associated with outcomes, the algorithms can predict an outcome for a set of data exhibiting similarities to a known cluster.

What obstacles do you think stand in the way of chromatographers adopting new data solutions?

ANDERSON: Primarily the pain and time investment to change. Data will need to be transformed and migrated into these newer paradigms and this will often be a lower priority than the many day-to-day laboratory business demands. A large contributor to this daunting effort is (re)validation, which is required in regulated environments. In non-regulated environments it is also becoming more commonplace because these organizations also recognize the value of truly FAIR data.

KLEINE: There are five main obstacles today:

Fear of change: Users may be accustomed to their existing data management and analysis methods and may be hesitant

to adopt new solutions. They may be comfortable with manual processes or may have concerns about the reliability and accuracy of automated data solutions.

Costs: Implementing new data solutions often requires investment in hardware, software, and training. Chromatographers may be concerned about the upfront costs and ongoing expenses associated with adopting new technologies, especially if they are working with limited budgets.

Compatibility: Decision-makers may face challenges in integrating new data solutions with their existing instruments, software, and laboratory infrastructure. Compatibility issues can make the transition to new solutions difficult and time-consuming.

Data security: Chromatographers work with sensitive data and may have concerns about data security when adopting new data solutions systems. They must be sure that their data will be protected, especially when using cloud-based solutions.

Training: Adopting new data solutions requires additional trainings for chromatographers and laboratory staff. It will take time to acquire the necessary skills and knowledge to effectively use and leverage the new tools and technologies.

WELCH: There is always a lag seen between new technology and adoption due to it not fitting exactly into the prior solution footprint. For example, moving software to cloud-hosted took a change in everything from architecture to validation approaches. But the only way to move forward is to challenge whether we keep procedures for familiarity or functionality.

PETROV: I see two major obstacles standing in the way of adopting new chromatography solutions. One is the accessibility of such solutions in terms of deployment difficulties associated with software upgrades and validation. Solutions delivered as SaaS will help lower that barrier. Another obstacle is the willingness to accept that automated decision-making can displace the human factor in industries with critical outcomes as life sciences. If you think about it, humans are trusted with certain decisions because they have been trained appropriately and have proven that they can make such decisions as discerning good from bad chromatograms. Algorithms can be trained too, and they can prove in subsequent tests that they can make such decisions. The real difference is that once properly trained, algorithms can do that day in and day out with a lot higher efficiency and a lower failure rate than humans.

MEEK: There is an additional challenge, that adopting new technologies can be difficult in a regulated environment. Regulators have shown, however, that they are supportive of using technology to

eliminate manual processes such as manual integration to ensure consistent and reliable results. AI does pose a particular challenge given the natural drift that can occur in models, which is why, at least for the time being, a human in loop approach that leans on the expertise of chromatographers provides the best balance.

What was the biggest accomplishment or news in 2023-2024 for Data Handling?

ANDERSON: Some might mention the growth in popularity of ASM or the availability of generative AI tools; however, we don't think this area has seen the biggest accomplishment yet. Perhaps the coming months of 2024 will surprise us all.

KLEINE: It is becoming easier and easier to store and handle large amounts of data. Improved computing power and network connections make this possible. Measurement results no longer need to be stored locally, making the storage space for data scalable. The large amounts of data are therefore also available over a longer period. With the help of large amounts of data, an AI can support the user in chromatography in the evaluation and interpretation of measurements.

WELCH: The biggest thing in the last year must be AI. Who hasn't read something about ChatGPT? But the foundation for AI is not really in the algorithms or the user interface, but in how AI uses large banks of data. So, data architecture, classification, cataloguing, and the design of data tagging and master lists are really where the fundamental shifts are coming. Without stable structures, AI cannot utilize the available information in a productive way.

MEEK: While not "data handling" specifically, I think everyone would agree that, since its launch in November 2022, ChatGPT has dominated technology news. While generative AI may have been the focus of the media, any AI-based technology is only as good as the quality and volume of data that informs it. I think the biggest accomplishment over the past two years is the work companies are doing to build data lakes that enable them to use data science to look across research and development and from the lab to the production floor.

PETROV: Organizations in the pharmaceutical space have been able to use AI to develop novel therapeutics in drug discovery and development. Using AI to generate extremely complex molecules and then test their binding capabilities in the virtual space is a ground-breaking advancement to speed up drug discovery like never before. Over time, we expect to see this technology deployed across the product lifecycle through manufacturing.

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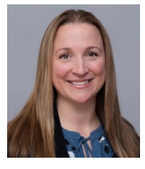
Shawn Anderson, Associate VP, Digital Lab Innovations, Agilent Technologies.

Marco Kleine, Head of the Informatics Department at Shimadzu Europa GmbH.



Trish Meek, Senior Director, Connected Science Waters Corporation.

Todor Petrov, Senior Director, QA/QC Waters Corporation.

Crystal Welch, Product Marketing Manager Thermo Fisher Scientific.

From the CEO

Welcome to the July 2024 issue of *The Column*. Artificial Intelligence is obviously "The" big topic of modern times, and in this month's cover story Chiara Cordero and Marco from the University of Turin, Italy discuss how artificial intelligence is entering — and will enter — the lives of chromatographers in practice.

Data generaton and analysis is obviously also a pressing concern for chromatograhers. How do you deal with the vast quantities of data being generated? A panel consisting of expert from Waters, Shimadzu, Thermo and Agilent discuss the latest technologies that help laboratory scientists achieve the results they are looking for.

Bruker officially openened the company's new manufacturing site and research facility in Bremen, Germany in May 2024. *LCGC* was at the launch to interview Jeff Zonderman, Senior Vice President of Bruker Applied MS about the rationale behind building the new site site and the company's plans for the future and answers The Eternal Questions: Are we moving to a chromatography-free world?

Biotherapeutics are an important driver in the modern life science sector and chromatography plays a key role throughout the drug development process. Szabolcs Fekete, Mateusz Imiołek, and Matthew Lauber from the Waters Cirporation explore the advantages of sequential or bracketed methods to obtain better results for biotherapeutic applications. Ann Marie Rojahn, Mathias Hehn and Daniel Eßer from YMC Europe describe a comprehensive approach to optimize ion-pair reversed-phase liquid chromatography for the analysis of protected and unprotected single-stranded DNA oligonucleotides

In our expanded news section we cover the latest company and research news and highlight that *LCGC's* 2024 HTC Innovation Award was awarded to Bob Pirok from the University of Amsterdam, The Netherlands in person at HTC-18 in Leiven, Belgium where he presented a lecture on his innovative advances in separation science. Bob was also interviewed on video with other leading experts at the conference. Look out for these interviews on *LCGC International's* social media channels.

If you have any comments on the issue, or if there is any aspect of separartion science that you would like us to cover in more detail, please let us know!

Enjoy the Summer and happy reading!

Mike Hennessy Jr.,
President and CEO, MJH Life Sciences®

Knauer Sponsors Ghana Sustainability Project

Knauer recently provided an update at its Berlin, Germany headquarters on a project in Ghana for more than two years in the west African country of Ghana (1)..

In Ghanaa's Binduri district bordering Burkina Faso, agricultural economist Tony Rinaudo of Australia—known in the industry as the "forest maker"—is spearheading a method known as FMNR, or farmer-managed natural regeneration. Its goal, instead of planting new trees, is to naturally regenerate trees using the existing underground roots of cleared ones (2). If those roots are developed under the right conditions, according to project partner World Vision, they will sprout again and one day grow into new trees. A 2022 documentary, the trailer of which can be found on YouTube, offers more information about Rinaudo's background and the basis of the FMNR approach (3).

A company officials from Knauer said in a press release that sustainability is "close to the heart" of its approximately 200 employees, and that corporate responsibility has been concentrated on the impact of the environment on society (1).

According to World Vision, the initial two-year project phase has been completed, and the next one is expected to take another two years (2). Knauer, which provided a first round of €400,000 in funding, is assisting with securing an additional €100,000 in donations, as all the stakeholders work together to not only educate locals about sustainable agriculture, but also train them in numerous environmental practices.

It helps that most of the 5,000 people—out of nearly 77,000 in the Binduri district—who figure to be helped most by the project are already farmers, many of whom rely on what they themselves grow to live (2). But they may lack resources, not only financially, but simply in tools and equipment, to keep up with adaptations that must be made with respect to climate change.

A Knauer spokesman stated that the FMNR process has helped improve the lives of farmers to the amount of an average additional family income of $1000 (U.S.) annually, much of that done without the beneficiaries being aware, in many cases, that they are being put on a more sustainable footing (1)

— Patrick Lavery

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2. Knauer Enables a Comprehensive Environmental Project in Ghana. World Vision International. World Vision Germany eV, 2024. https://www.worldvision.de/spenden/unternehmen/kooperation/knauer-fmnr-projekt (accessed 2024-06-03).

Bob Pirok Wins HTC-18 Innovation Award

Left to right: Deirde Cabooter, Bob Pirok, Alasdair Matheson

Bob Pirok from the University of Amsterdam in The Netherlands is the winner of the 2024 HTC Innovation Award, which was presented at the Hyphenated Techniques and Separations Technology (HTC-18) conference in Leuven, Belgium on Friday 31 May 2024. The award is co-sponsored by the HTC conference and LCGC International.

Deirdre Cabooter, the Chair of the HTC-18 Scientific Committee, said, "Bob Pirok has an impressive research output. He has worked on polymer characterization, including pyrolysis-gas chromatography and hydrodynamic chromatography (HDC), and multidimensional liquid chromatography separations, using the interface between separation dimensions as a point of chemical transformation using photolytic or immobilized enzymatic digestion.

For this award, the Scientific Committee and Industry Board of HTC-18 particularly recognize Pirok's research on using cutting-edge machine learning and chemometric approaches to automate method development for both single dimension as well as two-dimensional liquid chromatography separations. Since method development for complex samples, such as synthetic polymers and oligonucleotides medicinal drugs, is currently a true bottleneck, the impact of automating this process on many research fields and industries cannot be underestimated. The same approaches can also be extended to other techniques, such as GCxGC, further broadening the application field to, for example, petrochemicals."

Pirok worked first two years at Shell Global Solutions before he performed his PhD research with Peter Schoenmakers and Ron Peters at the University of Amsterdam. In 2019, he became assistant professor at the University of Amsterdam in the Forensic Analytical Chemistry group of Arian van Asten. Pirok's main research concerns the application of computational techniques such as chemometrics and machine learning to enhance the use of separation technology through better method development, with a particular emphasis on LC and 2D-LC.

His other interests include fundamentals of chromatography and modulation technology. He currently runs the Chemometrics and Advanced Separations Technology group with Andrea Gargano at the University of Amsterdam. He has co-authored more than 75 peer-reviewed publications and book chapters. Currently, he is finishing a textbook together with Peter Schoenmakers entitled Analytical Separation Science. With their textbook, Pirok and Schoenmakers aim to contribute to the teaching of modern separation sciences in higher education.

Pirok is visiting research professor at Gustavus Adolphus College in the group of Dwight Stoll. He received several international recognitions, including a Shimadzu Young-Scientist Award at HPLC2015 Beijing, the Young-Scientist-Award Lecture during the SCM-8 meeting in Amsterdam in 2017, the Csaba Horváth Young-Scientist Award at HPLC2017 Prague, the Journal of Chromatography Award during the ISCC Conference in Riva de Garda in 2018, and the SCM Award at the SCM-9 meeting in Amsterdam in 2019.

Pirok's research team runs several industrial-academic collaborations and projects, mainly with the pharmaceutical and polymer industry. The group is known for bringing its research into education and vice versa.

"I am very honored to be receiving the HTC Innovation Award. I am accepting this as a representative of a great team of people who made this work possible, in particular Tijmen Bos, Stef Molenaar, and Jim Boelrijk," Pirok said. "These achievements are due to our teamwork. I feel blessed with the many academic and industrial collaborations and opportunities that have come on my path with great scientists around the globe. I would like to thank Peter Schoenmakers, Dwight Stoll, and Arian van Asten for their continuous support."

The HTC Innovation Award was launched by LCGC International and the HTC Scientific Committee and Industry Board to celebrate a separation scientist who 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 applications that benefit society.

"LCGC International is very pleased that the HTC Scientific Committee and Industry Board have awarded the prize to Bob Pirok who is widely regarded as a pioneer and innovator in separation science," said Alasdair Matheson, Executive Editor of LCGC International. "We look forward to seeing how he will advance separation science in the future." *—Alasdair Matheson*

Kate Perrault Awarded LECO Pegasus BTX 4D by LECO Corporation

LECO Corporation announced that Kate Perrault Uptmor is the recipient of a brand-new LECO PegasusBTX 4D with Paradigm Flow Modulator.

Each year, the instrument manufacturer LECO, selects one scientist to present a piece of equipment to for their laboratory. This tradition of offering scientists and chemists equipment goes back to 2021, with LECO providing an opportunity for American chemists to obtain a two-dimensional gas chromatography (GCxGC)-enabled Pegasus time of flight mass spectrometer (TOFMS) for their laboratory. Winners are selected based on the application work being performed, the benefit that application area would see from GCxGC instrumentation, and the passion for discovery of the applicant.

"LECO's Pegasus BTX 4D with Paradigm Flow Modulator is a great fit for the kind of work Dr. Perrault Uptmor is doing in her lab," John Hayes, separation science product manager at LECO said in a press release. "Based on the lab's needs, the importance of the work being done, and the genuine passion for GCxGC, we believe she is the most deserving of this state-of-the-art equipment."

GCxGC is an analytical technique that uses two columns of differing phase selectivity connected by a modulation device. According to LECO's website, setting up a GCxGC system improves peak capacity, resolution, and detectability. "On average, a GCxGC analysis has five times the sensitivity and gains three times the number of compound identifications of typical GC-MS runs," the company claims (1).

Perrault Uptmor is an assistant professor of Chemistry at William & Mary, a research university located in Williamsburg, Virginia. Her laboratory typically focuses on nontargeted analysis on highly complex samples, with research studies typically being related to life, health, disease, or death. Current chemical forensic projects from her research laboratory include organic gunshot residue profiling, fingerprint residue characterization, and microbial forensic analysis of decomposing remains for search and recovery/mass disaster applications. Additionally, tandem gas chromatography–time-of-flight mass spectrometry (GCxGC–TOFMS) is used in the laboratory for improving understanding of scent-detection canines as biosensors in operational settings. Currently, Perrault Uptmor's laboratory is also working on creating translation studies between helium and hydrogen as a greener analytical approach, alongside creating post-processing machine learning strategies for batch and longitudinal GCxGC data.

In 2023, LECO presented Petr Vozka, assistant professor in the Department of Chemistry and Biochemistry at California State University with a GCxGC-enabled Pegasus TOFMS system. Vozka's win was based around his elaborate working in using GCxGC to study microplastics while traning the next generation of GCxGC users.

"The goal of this program is to not only empower labs that are doing truly amazing work with innovative tools, but also to partner with them in an effort to push the boundaries of innovation in solving complex chemistry problems using LECO's instrumentation and software," Farai Rukunda, director of Separation Science Customer Success at LECO said in a press release. "These laboratories are gaining much more than an instrument—they are also gaining access to LECO's GCxGC support network, which includes leaders in the field of GCxGC technology, software development, method development, and applications."— *Aaron Avecedo*

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HILIC Advances for Analyzing Therapeutic Oligonucleotides

A recent paper published in Trends in Analytical Chemistry (TrAC) by scientists from the Institute of Pharmaceutical Sciences of Western Switzerland at the University of Geneva (1), presents recent advances, as well as current challenges, relating to hydrophilic interaction chromatography (HILIC) for the analysis of therapeutic oligonucleotides (ONs).

HILIC is a liquid chromatography (LC) technique that uses a polar stationary phase (for example, silica or a polar bonded phase) in conjunction with a mobile phase containing an appreciable quantity of water (usually at least 2.5% by volume) combined with a higher proportion of a less polar solvent (often acetonitrile). Most commonly, separations are carried out using 5–40% water (or aqueous buffers); the technique also is compatible with gradient elution (2).

ON therapeutics have begun to emerge as a strong alternative to treat a variety of diseases. Drug manufacturers view these therapeutics as a rapidly expanding category of products that may evolve into more personalized treatment. However, the chemical synthesis of ON therapeutics has limited use and development, as well as created concerns with stability, purity, and cost of development (3,4)

The TrAC paper says that there have been significant advances in understanding the retention mechanism of ONs in HILIC mode. These advances have resulted in the expansion of analytical methods which are able to effectively resolve ONs mainly based on chain length and are influenced by accessible groups involved in the HILIC retention mechanism; these groups include phosphorothioate groups, as well as base nature or sugar modifications). Furthermore, the susceptibility of adsorption of ONs onto column hardware has resulted in advances in specially adapted columns which were established to lessen this problem. As a result, the use of bioinert HILIC hardware has begun to occur as a necessity in the avoidance of sensitivity loss, and to diminish problems such as peak tailing and broadening, as well as inadequate general execution.

The paper notes there has been positive developments in improving the performance of HILIC–MS performance. Among these developments are the significant enhancement of the technique's effectiveness uisng the systematic optimization of chromatographic conditions to favor negative ionization of ONs, along with fine tuning of MS nebulization parameters. The authors said that this analytical strategy may represent an intriguing substitute for ion-pair reversed-phase mass spectroscopy (IP-RPLC-MS), especially in cases where when ion-pair free LC-MS methods are more favorable, if not mandatory. While the performance of IP-RPLC–MS appears to still prevail over that of HILIC–MS, especially in terms of MS sensitivity, adopting of new technological strategies, such as miniaturization, for example, may help in overcoming these challenges, which would then allow for greater flexibility in the selection of the most suitable method for the characterization of ONs. — *John Chasse*

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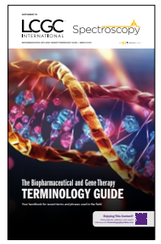
Peaks of the Month

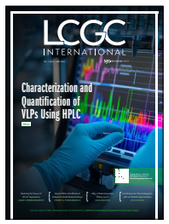
- **The LCGC Blog: Celebrating Women in Separation Chemistry at Pittcon 2024**—In this edition of The LCGC Blog, Emanuela Gionfriddo discusses the two world-class scientists and trailblazer women in separation chemistry and the awards they received at Pittcon 2024. **Read Here>>**

- **CHROMtalks 2024: Mastering Method Development: The Science Behind Successful Results**—Mastering method development is a crucial skill for analytical scientists to develop and achieve the results they want in the laboratory and be successful in their careers. This special two-day virtual symposium organized by *LCGC International* in collaboration with CHROMacademy will focus on liquid chromatography (LC) on and gas chromatography (GC). **Watch Here>>**

- **For the Love of Separation Science**—In this episode, podcast co-hosts Dr. Dwight Stoll and Dr. James Grinias talk with Dr. Andre de Villiers, Professor of Chemistry at Stellenbosch University in South Africa. Dr. de Villiers is an expert in multi-dimensional chromatography and leads a research program mainly focused on the use of two-dimensional liquid chromatography coupled with mass spectrometric detection for the deep characterization of natural products such as tea, wine, wood, and cocoa. Some of his recent work has explored the potential for ion mobility separations to increase the utility of mass spectrometric detection coupled with two-dimensional liquid chromatography separations. **Listen Here>>**



- **Recent Developments in HPLC**—In the world of liquid chromatography (LC), innovative strides in column technology continue to take place. We are also reminded that there is always more to learn about "well-known" methodologies, and our craft is continuously influenced by important social concerns. **Read Here>>**



- **June 2024 Issue of *LCGC International***— In this issue of *LCGC International,* Dwight Stoll discusses how the application of two-dimensional liquid chromatography (2D-LC) is expanding due to its remarkable flexibility and ability to address complex analytical challenges, and candidly addresses the common hurdles and uncertainties faced by users, offering practical solutions that will undoubtedly empower you to leverage 2D-LC to its full potential. This issue also features an article examineing the synergy between gas chromatography (GC) and ion mobility spectrometry (IMS). **Read Here>>**

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News In Brief

Determining Cardiovascular Risks Resulting from Xylitol Use with LC-MS/MS

Researchers from the Lerner Research Institute at the Cleveland Clinic are using isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) to determine if the sugar substitute xylitol has an association with incidents of major adverse cardiovascular event (MACE) risk, according to a study published in the *European Heart Journal*. Xylitol, is a natural sugar alcohol found in plants, including many fruits and vegetables. Xylitol tastes sweet but, unlike sugar, it does not cause tooth decay and is commonly used in sugar free candies and gums.

The scientists performed untargeted metabolomics studies on overnight fasting plasma samples in a discovery cohort of 1157 sequential stable patients undergoing elective diagnostic cardiac evaluations. Subsequent isotope dilution LC-MS/MS analyses were performed on an independent validation cohort comprising 2149 participants. The effect of xylitol on platelet responsiveness and thrombus formation in vivo was examined in complementary isolated human platelet, platelet-rich plasma, whole blood, and animal model studies. Finally, the effects of xylitol consumption were examined on platelet function in 10 healthy volunteers.

The researchers found that circulating levels of a polyol tentatively assigned as xylitol were associated with incident major adverse cardiac event (MACE) risk in the initial untargeted metabolomics study. The association of xylitol with incident MACE risk was confirmed in stable isotope dilution LC-MS/MS analyses (third versus first tertile adjusted hazard ratio, 1.57). Xylitol-enhanced multiple indices of platelet reactivity and in vivo thrombosis formation at levels observed in fasting plasma were seen in complementary mechanistic studies. Xylitol-sweetened drinks markedly raised plasma levels in interventional studies and enhanced multiple functional measures of platelet responsiveness in all individuals. - *John Chasse*
Read more here.

Moving To A Chromatography-Free World?

Alasdair Matheson, Executive Editor, *LCGC International*

Bruker's new mass spectrometry manufacturing site was officially opened in Bremen in May 20024. *LCGC International* spoke to Jeffrey Zonderman, Senior Vice President of Bruker Applied MS, about the opening of the new facility, current trends in mass spectometry, and moving to a "chromatography-free" world.

What is the rationale behind Bruker setting up the new mass spectrometry facility in Bremen?
The new MS facility was established to deal with the expected growth of both the company and MS technologies, primarily driven by the success of trapped ion mobility spectrometry (TIMS). We expect big things from this, as well as technologies that we are launching at upcoming conferences.

How long has it taken from the initial idea to build the site to the official opening day?
I have been with the company two years and this was well underway when I started. It was at least four or five years from the initial idea to the actual execution.

Is there any particular reason why Bremen was chosen as a location?
Bruker is historically located here. The manufacturing and primary research facility for mass spectrometry was already here. And there is also an employee base of 800 people. If it was moved somwhere else we would not retain the expertise, or the quality of employees that we have built. It is amazing that you can build a complex MS system and ship it to anywhere in the world, install it and start doing research or running samples, but it is the people that actually know how to do this that make this possible. The

expertise of the Bremen team ensures that we do not skip a beat.

What type of MS instruments are you building in Bremen?

We build the whole range of MS instruments in Bremen: Triple quadrupole, (QqQ), quadrupole time-of-flight (QTOF), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), trapped ion mobility spectometry time-of-flight (timsTOF) and Fourier transform (FT).

What are the main trends in mass spectrometry in the analytical chemistry community at the moment.

There is a continual push for increased performance in areas where Bruker is very strong. For example, in life science research and that is the Bruker sweet spot really and where we have grown, particularly with the timsTOF instrument – and that is not changing. There is a need to dive deeper, and there is more dynamic range and sensitivity and selectivity that need to be achieved. And certainly, timsTOF came along and changed the game and Bruker became a major player in proteomics research because of it – and there is more of that to come.

Researchers and scientists are just scratching the surface with the tools they have today. So there are more tools coming, and Bruker is in a really strong position with high ultimate resolution technology. Ion mobility technology is here to stay. It is the future. High end mass spectrometry is not going away.

So it is going to continue to grow in the research areas, but there is a move into more applied markets. I won't call them non-research because the applications people do research as well. We are taking the technologies that have been developed for core research and moving them over into the more routine markets.

This level of user wants a simpler system, a more cost-effective system and a more sustainable system. And that is really a lot of what my team is looking at: How do we take ion mobility spectrometry (IMS) and DART technologies and develop solutions where we can perhaps reduce the need for chromatography to separate in other ways — or not at all, if they are not needed. We are getting a lot of positive response from the market. We explore how we can enter a particular sector and ask: "What if we can move the analysis from minutes to seconds for each sample?" and "What if we could

remove all your chromatography solvents in organic waste?" Maybe not the sample extraction, but at least when you are running the samples. "What if we can remove about 95% of your organic waste in that whole assay overall and make it more sustainable?" If you are running ten triple quadrupoles in an LC–MS sytem you are generating a massive amount of organic waste.

There are definitely more segments where you can minimize chromatography or do separations a different way that are more sustainable and have better return on investment (ROI).

We have also built a site that is more sustainable. The site will operate with 100% green energy in the near future. It has been built according to LEED standards and is ISO 50001 and ISO 14001 saccredited for Energy Management and Environmental Management Systems.

I think the real differentiation amongst vendors is to produce products that are more sustainable so that customers can be more sustainable – and they are asking for that in a big way.

What applications is chromatography-free MS being used in?

I came from an industry that typically used a chromatography-free method for a type of targeted screening. A good example of this was using direct analysis in real time mass spectometry (DART-MS) for an application for the National Institute of Standards and Technology (NIST). NIST has implemented this method across the United States. For drug seizures analysis where you can have a powder, a pill, or a liquid, no sample preparation is required, and you can do it in a very high throughput manner for qualitative analysis. Drug analysis is a big application. That's historically what ambient ionization and DART–MS were used for initially.

ThIs is changing. Bruker is looking at actually moving into quantitative analysis, which is different because if you want to do quantitation the common thought is you need a peak and you need chromatography, or you cannot do quantitation.

This is what Bruker is exploring. We are looking at areas where chromatography-free exists today, which is using DART with a very powerful triple quadrupole mass spectrometer. Simple, fast sample preparation is where can remove chromatography.

In Massachusetts, our method development group is working on

moving assays from chromatography for drug screening to chromatography-free analysis, and that's really good. We have a lot of expertise from working with NIST moving from just analysing the pills to looking at the drugs and metabolites in urine, blood and oral fluids.

We are also looking into food authentication and contaminants. Anything where there is a high volume requirement, where the chromatography is short and really used more as a cleanup, we can possibly replace that.

We are also doing chromatography-free in forensics and toxicology applications with simple drug assays, where we are looking at benzos, opiates and other stimulants. These can all be done quantitatively dried, and we can move the analysis from minutes for each analysis to seconds and get the same high-quality results

A classic example is the drug crisis in the United States. It is difficult to keep up with the drug analyses that are required. A LC–MS system can possibly get through 100 samples. We have shown that for the same application, instead of achieving an analysis of 90–100 samples a day, we are doing 1500 samples a day.

Can you expand on what "chromatography-free" methods are being used outside life science.

The biggest market currently is forensic toxicology workflows, and we're looking into contaminant analysis, including microplastics and per- and polyfluoroalkyl substances (PFAS) for environmental monitoring. Ion mobility is a useful separation tool for very complex and challenging separations, such as dioxins, PFAS and other persistent organic pollutants (POPS). DART has also been used for polymer and additive analysis.

Bruker is very strong in polymer analysis using MALDI technology, so it's very complementary for lot of polymer work. A lot of chromatography-free applications use MALDI or DART.

I want to move "chromatography-free" into the LC–MS and GC–MS markets, to offer robustness, ease of use, a better ROI, and sustainability. Those are really the key factors.

The utimate question: Is this the end of chromatography?

No, absolutely not! Don't forget sample preparation is a form of chromatography. There are application areas, however, where chromatography can be replaced that we are looking into. In laboratories using triple quads to run many routine analyses, many of the important assays do not need chromatography anymore. In these cases we are talking about analysis times in seconds rather than minutes. Chromatography is always going to be important. There will be new versions of chromatography coming out, I'm sure for untargeted screening applications where you need a separation for the mass spectrometer to work properly. For example, a 500 residue pesticide screen will still need the fast separation of chromatography. But today, I think chromatography has a place alongside chromatography-free.

Will Bruker still be focusing on the instruments that can be hyphenated to chromatography systems.

Yes, absolutely. My group is looking at chromatography systems today and evaluating them to see what they do with our instrumentation for the best separations. I think chromatography is what most of the market uses today, so Bruker will continue to support this as it transitions over to chromatography-free for many applications.

Biography

Jeffrey Zonderman serves as the Senior Vice President of Bruker Applied Mass Spectrometry, a new division of the company focused on providing mass spectrometry-based solutions for the applied market segments such as forensics, food, environmental, industrial/polymer, and clinical research. Zonderman graduated from Northeastern University with a bachelor's in biology and has been involved in mass spectrometry and chromatography for over 30 years. Before joining Bruker, he worked in applications, sales/marketing and general management roles. His previous position was CEO of IonSense Inc, a company focusing on ambient ionization technology and commercialized DART. Zonderman looks to expand the applications of DART technology, amongst others, for chromatography-free mass spectrometry-based workflows.

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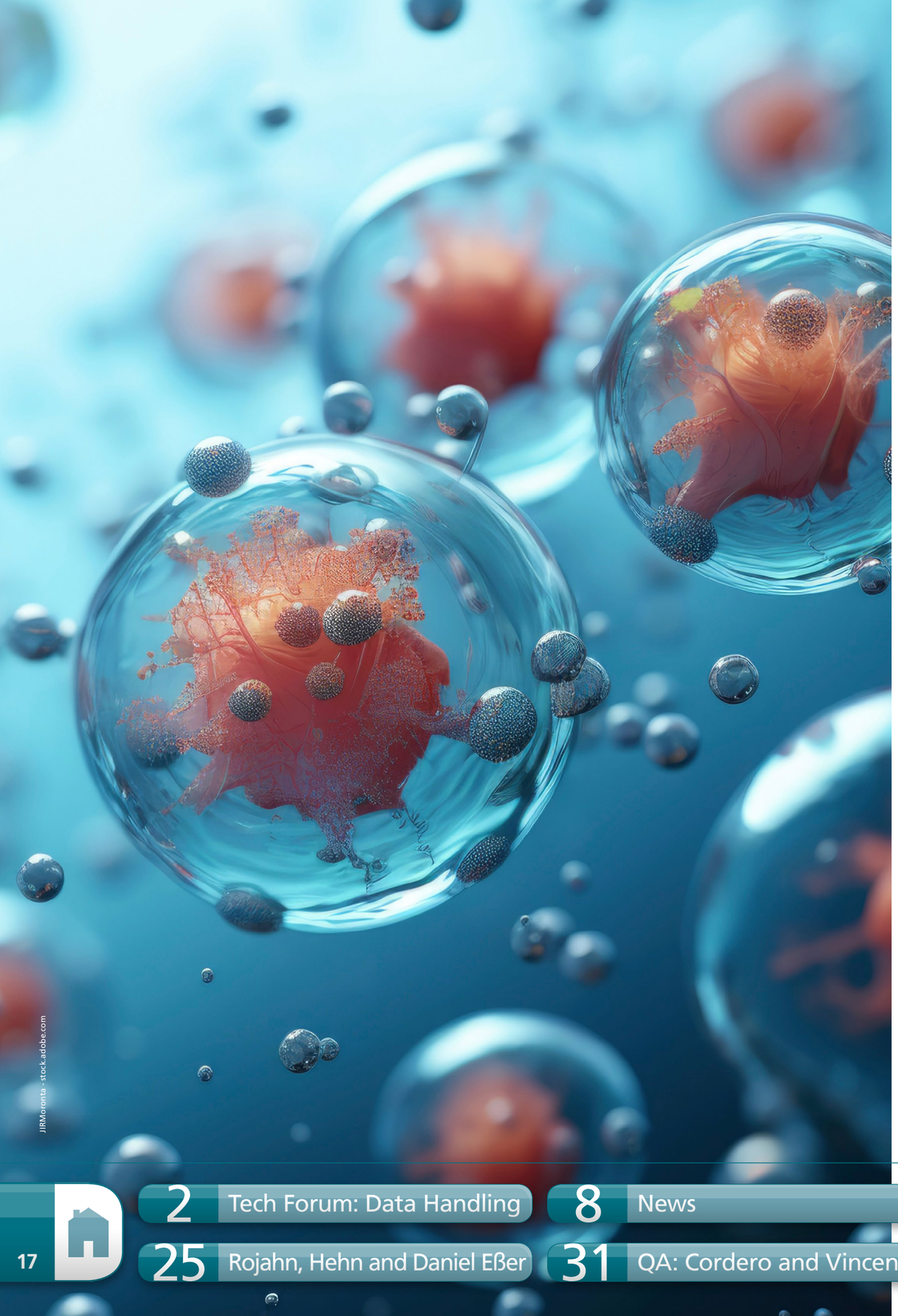

Advantages of Sequential or Bracketed Injection Methods to Improve the Chromatographic Analysis of Biotherapeutics

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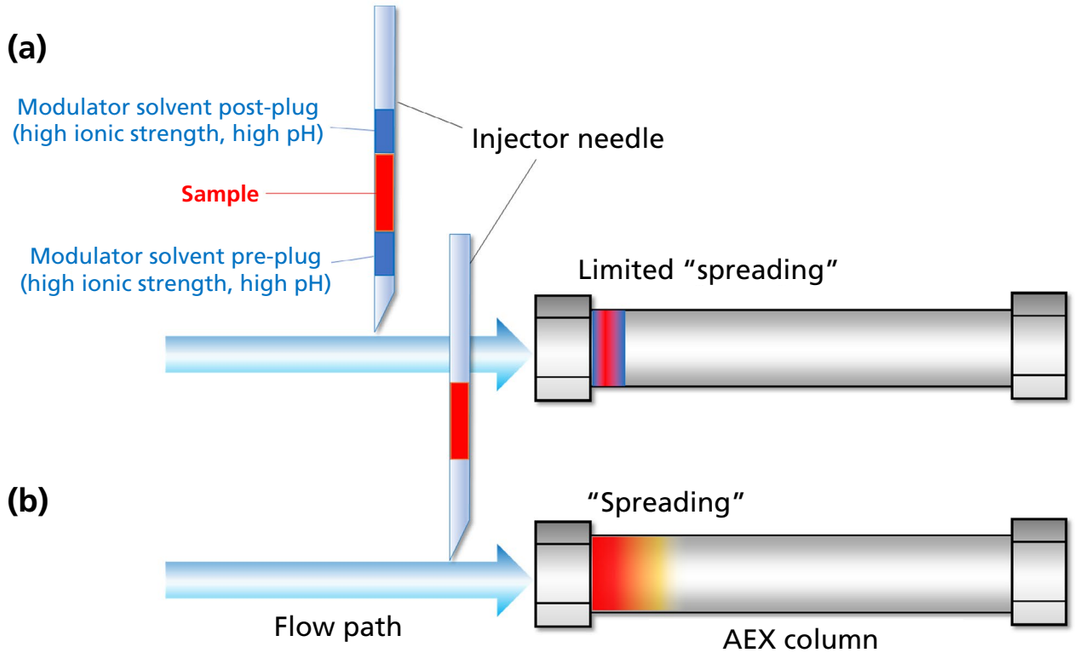
High-performance liquid chromatography (HPLC) analysis of biotherapeutics introduces new challenges because large molecules may require distinct injection modes or sample handling protocols to improve the analysis. Some challenges can be addressed by using autosampler methods that allow an analyst to program specific injection sequences. This article explores three case studies where autosampler injection programs help to (i) reduce the carry-over observed for large nucleic acids during anion-exchange chromatography (AEX), (ii) reduce peak distortion and breakthrough for monoclonal antibodies (mAbs) during hydrophilic-interaction chromatography (HILIC), and (iii) facilitate dissolution of lipid nanoparticles (LNPs) so that a size-exclusion chromatography (SEC) method can be applied for ribonucleic acid (RNA) payload analysis. The appropriate use of autosampler capabilities offers unique benefits to analyze biotherapeutics.

Modern autosamplers provide precise, accurate, and fast sample introduction into the mobile-phase flow path, and can also perform additional liquid handling steps such as sample dilution, mixing, internal standard addition, and precolumn derivatization (1,2). Significant savings in terms of reduced labor hours can be

Figure 1: Schematic view of an injection sequence (a) where the drawn sample is bracketed with high ionic strength and high pH modulator plugs, and (b) a traditional injection.



found in many different example projects an it is the dilution-related tasks that are most valuable. In addition to sample manipulation options, so-called "sequential injection" methods can also be applied. Sequential injections are most commonly used for two purposes. They can limit solvent mismatch problems, and they can also limit extra-column band broadening effects (3,4). A sequential injection method is often referred to as "bracketed injection" or "performance optimizing injection sequence" (POISe) when it is applied to improve band focusing.

The POISe technique involves injecting a defined volume of weak solvent (usually weaker than the mobile phase) along with the sample to increase solute retention during sample loading. This will result in a better match between the sample solvent and the mobile phase. Such a technique is particularly effective when gradient elution is applied, because it improves the peak shape and width of early eluting peaks. In isocratic elution, it can also reduce the detrimental effects of pre-column band broadening (especially when the solute retention is low) (5).

A number of investigators have previously reported the benefits of sequential injection methods (3–9). However, it is still rarely used in routine analysis.

The purpose of this article is to demonstrate the capabilities of sequential injection methods for the analysis of large molecule biotherapeutics. Three methods are discussed: (i) a recently introduced "salt plug injection method" to improve the carryover and recovery of messenger ribonucleic acids (mRNAs) in anion-exchange (AEX) separations, (ii) an "acetonitrile (MeCN) pre-plug injection method" to avoid peak deformation and breakthrough of monoclonal antibodies (mAbs) and related species in hydrophilic-interaction chromatography (HILIC), and (iii) an automated injection program to denature lipid nanoparticles (LNPs) so that their nucleic acid payloads can be analyzed by size-exclusion chromatography (SEC).

Salt Plug Injection Method for Improved AEX Analyses of Large Nucleic Acids

Although large-scale AEX separations (purification) are regularly performed for oligonucleotides and nucleic acids, very few analytical-scale AEX separation methods have been published. The reason for the limited use of AEX for analytical purposes is probably the lack of method robustness, poor recovery and high carryover effects, which are especially problematic for larger nucleic acids (10–12). Very long equilibration times between two sample injections may be required to reduce carryover (10). In addition to time-consuming equilibration steps, specific additives have also been used to suppress unwanted intermolecular interactions. Some cations have also been found to be beneficial because they stabilize the higher order structure of nucleic acids, and therefore improve recovery (12).

With the above considerations in mind, we recently proposed a new and efficient technique to significantly reduce carryover occurring in AEX separations (13). The phenomenon behind the high carryover effects observed with biomolecules (and macromolecules in general) is mostly related to non-desired secondary (or non-specific) interactions with surfaces and to both inter- and intramolecular interactions occurring in a macromolecular system.

Macromolecules are surface-active systems, which undergo non-specific adsorption when they come into contact with different types of surfaces (14). During an adsorption step, macromolecules undergo shape

changes (that is, unfolding). The area on the adsorbent surface that is occupied by the macromolecules is often referred to as the "footprint" (15). The size of a footprint usually increases with the time available for adsorption. The increase of footprint size is sometimes referred to as "spreading." If the solute concentration is high enough, the surface is occupied faster and therefore there is less time available for the spreading to occur. This results in a smaller average footprint. In addition to concentration and available time (residence time), other parameters such as solvent pH, ionic strength and temperature may also impact the spreading of the footprint and thus the recovery and carry-over observed in liquid chromatographic separations.

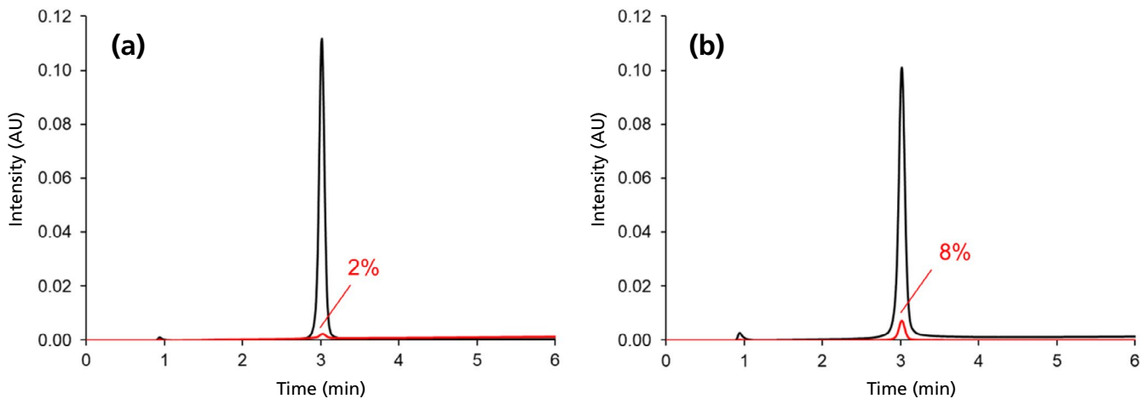
Nucleic acids in AEX follow a so-called on–off (bind and elute)-like elution mechanism (16). Therefore, it can be assumed that large nucleic acids, such as mRNAs, are bound at the head of the column and remain motionless until they experience the eluting mobile-phase composition. Hence, a certain time is available for the nucleic acid to spread and to establish multipoint interactions with the stationary phase. It is reasonable to assume that the binding interaction between nucleic acids and AEX ligands is inherently very strong (and increases as the residence time at the column inlet increases).

To limit the strength of the initial binding interaction, we propose that a program to inject the sample together with a strong solvent/eluent plug is created. To do this, we recently proposed bracketing nucleic acid samples with injection plugs containing a high concentration of salt (counterion). It is expected that the counterions—if injected in excess—will bind to the strong binding sites of the stationary phase and compete with the nucleic acid analytes.

If a large fraction of the stationary phase ligands is occupied by the counterions (at the time of the sample injection), then the nucleic acid spreading process will be inhibited and therefore a weaker binding interaction is expected to occur at the column inlet. In addition to high ionic strength, the pH of the solvent plug can also be adjusted to be close to the pKa of the stationary phase functional groups, that is, pH 10 in the case of a weak anion exchanger. The combined effect of salts and high pH is intended to limit the strength of the initial solute adsorption and therefore improve recovery and reduce carryover.

It was found that the effect of the salt plug injection method is sample dependent. Therefore, the following variables are worth studying and optimizing: (i) the volume of a salt pre-plug, (ii) the volume of a salt post-plug, (iii) the total volume of the bracketing salt plugs, (iv) the type of salt employed

Figure 2: Comparison of (a) bracketed versus (b) traditional sample injection. A weak AEX column (100 × 4.6 mm) was operated in salt gradient mode (6 min linear gradient, 0.6 mL/min flow rate and ambient temperature were set). Black traces: Cas9 mRNA sample injection; red traces: first blank injection following the sample injection.



(NaCl, $(NH_4)_2SO_4$, guanidine-HCl or NaBr are the most efficient salts) and (v) the pH of the salt plug. In general, the most effective injection procedure was found to be an injection sequence where the sample plug is bracketed with pre- and post-plugs of 2 M NaBr solution (at pH ~10).

Figure 1 shows a schematic view of a bracketed versus traditional injection. Please note that at pH higher than 10.5–11.0, nucleic acids (mRNAs) might be denatured, and the base-pairing and base-stacking interactions might be disrupted. At high pH, the intact RNA can thereby be linearized which can lead to significant spreading, a larger binding footprint, and stronger adsorption. Furthermore, some nucleobases can be deprotonated above pH 10, which would add additional negative charge to the nucleic acid analyte, and increase the retention time even further.

Based on our observations, carryover decreases, and recovery significantly improves with increasing ratio of modulator to sample volumes (V_m/V_s), until the ratio reaches a value of ~1.2–1.3. Above this "limit," a fraction of the injected sample is taken by the salt plug and a partial breakthrough peak appears on the chromatogram. If value is greater than 2, then the entire mRNA peak is eluted at the column's dead time (total breakthrough). When setting a $(V_m/V_s) \approx 1–1.2$, carryover values as low as 1–3% can be reached instead of 8–20% which is often observed without salt plug modulation. Setting $(V_m/V_s) \approx 1$ seems to be a good starting point. As an example, if a 2 µL volume of mRNA sample is going to be injected, then a good start is to program a sequence with a 1 µL modulator pre-plug + 2 µL sample + 1 µL modulator post-plug.

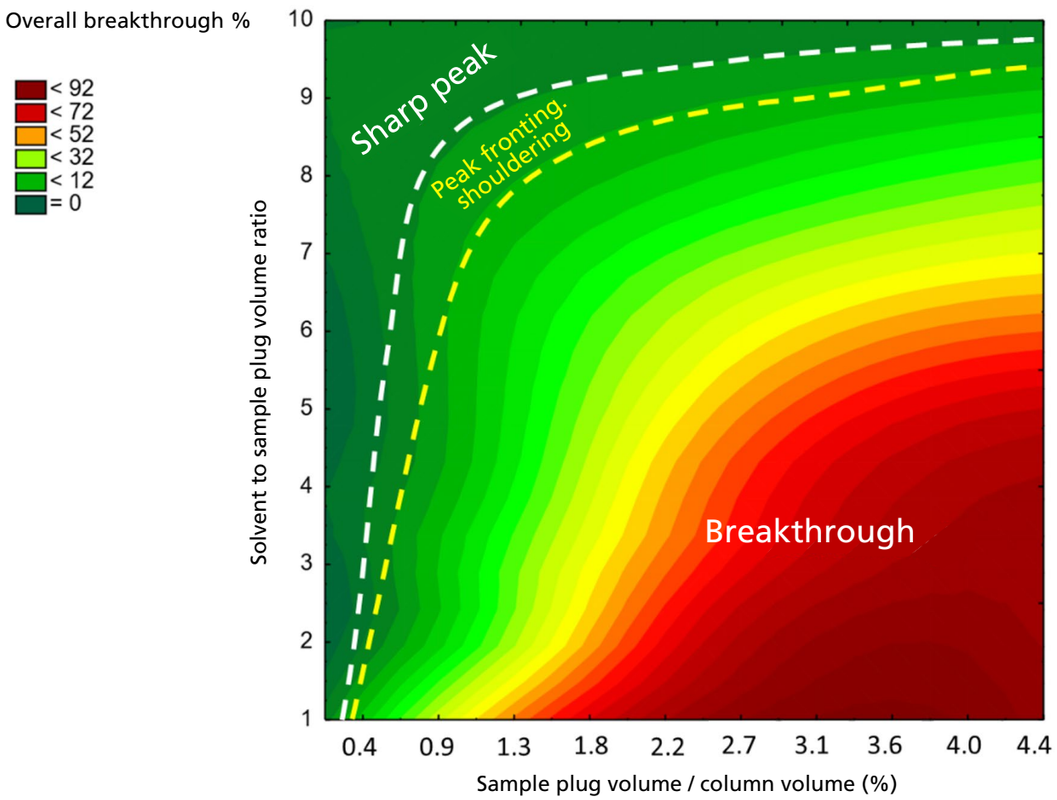
Figure 2 shows an example of a bracketed versus traditional injection for modified Cas9 mRNA (length: 4521 nucleotides). A weak AEX column was used with salt gradient elution. The 2 µL sample plug was bracketed with pre and post 1 µL 2 M NaBr (pH = 10.2) modulator solvent plugs. The red traces show the first blank injection after the sample. A carryover value of ~2% was observed with the bracketed injection, while carryover amounted to ~8–9% with the traditional injection.

Our proposed approach helps to reduce the strength of solute (mRNA) binding at the head of the column and thus improves recovery and carryover in AEX. By using this bracketed injection method, the carryover of large mRNAs can be reduced to ~2%, in contrast to the 6–20% carryover which is often observed with conventional AEX methods.

Acetonitrile Plug Injection for Improved HILIC Analyses of mAbs and Therapeutic Proteins

When water-soluble biopharmaceuticals, such as mAbs, are analyzed in HILIC, there is an inherent mismatch between the sample diluent (aqueous) and the mobile phase, which is usually composed of a high proportion of an aprotic organic solvent such as acetonitrile (MeCN). A difference in eluent strength between the sample diluent and mobile phase can result in severe solute breakthrough and peak distortion effects. Adjustment of the sample diluent can minimize this mismatch, that is, increasing the ratio of MeCN in the sample diluent. However, this adjustment is very often impossible because of the nature of the sample. The use of diluents with a high proportion of MeCN might be counterproductive as denaturation phenomena or even precipitation might occur. Since the breakthrough effect is related to the injection volume, the term "critical injection volume" (V_{crit}) is often used to express the maximum injectable volume that will not result in breakthrough (7,8). This critical volume directly depends on the column volume and the retention factor of the solute in the injection solvent (sample diluent). Due to the abovementioned on-off elution mechanism of large molecules, it can be expected that only a very low sample volume can be injected safely (typically V_{crit} = 0.1–0.3 µL aqueous mAb sample into a 2.1 × 150 mm HILIC column).

Figure 3: Contour plot of overall breakthrough (expressed in %) as a function of a pre-plug solvent (MeCN) to sample plug volume ratio (vertical axis) and of sample volume to column volume ratio (%) (horizontal axis). F = 0.3 mL/min, column: 20 × 2.1 mm ultra-short wide-pore HILIC. Adapted and modified with permission from (17).



To avoid the complications discussed above, we propose introducing aqueous mAb samples together with a MeCN solvent plug onto the column (17). Various injection programs were tested, (pre-, post- or bracketed injection plug). Interestingly, introducing the sample with a solvent pre-plug was found to be the most effective approach. Several sample to solvent plug ratios from 1:1 up to 1:10 were tested. Figure 3 shows a plot of "solvent to sample plug ratio" as a function of "sample plug volume to column volume ratio %". The plot suggests that the required solvent plug volume depends on the sample volume injected. It has been found that the 1:10 ratio helps avoid breakthrough and is a valuable strategy for injecting relatively large volumes of therapeutic proteins, for example, mAb samples. As large as 2–5% of the column volume can be injected without any breakthrough effect. However, it should be noted that slight peak broadening may be observed when the sample to solvent plug ratio is high (greater than 5). Therefore, if a smaller sample volume injection is sufficient, the solvent/sample plug ratio can be reduced. Various flow rates were also investigated, and it was found that flow rate had no effect on the required solvent to sample plug volume ratio.

This strategy has already been applied to a wide range of therapeutic mAb products of different physicochemical properties (17). In all cases, relatively large volumes could be successfully injected onto small-volume HILIC columns (ultra-short columns) using purely aqueous samples. Note that this approach is not limited to protein injections; the same rules apply to other macromolecules, such as nucleic acids.

Programmed Injection Method To Analyze Nucleic Acids Released From Denatured LNPs

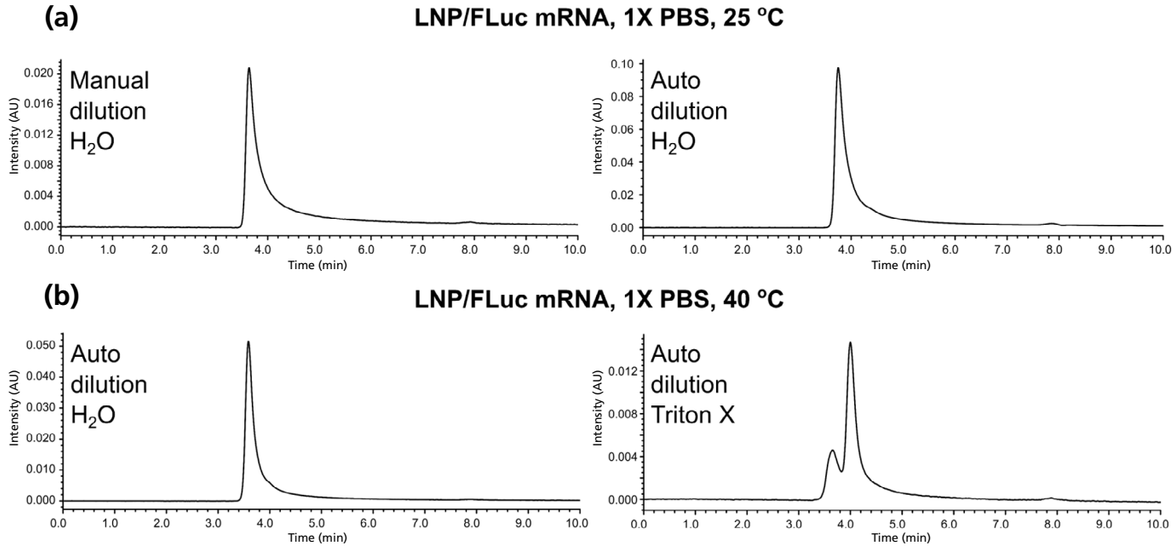
Large molecule biotherapeutics can also benefit from programmed injection methods to facilitate sample preparation. Apart from increased throughput and labor-savings, automation is also beneficial to handle sensitive or hazardous samples, for example infectious viral vectors, reducing the possibility of sample handling artifacts and exposure to hazards. There is a growing body of evidence that autosampler liquid handling functions can be successfully applied to more complex biopharmaceuticals products, as it was used to prepare dilutions of adeno-associated virus (AAV) samples that resulted in highly accurate calibration curves (18).

Nucleic acid-loaded LNPs are an example of highly delicate species that can show changed physical properties upon experiencing relatively low mechanical stress (19). For this reason, we investigated the potential of programmed injections to prepare sample dilutions for SEC separations that are becoming increasingly common for such samples (Figure 4) (20).

Firstly, we compared an SEC profile obtained from a direct injection of manually water-diluted intact LNPs (1 µL of 0.05 mg/mL loaded with FLuc mRNA) or auto-sampler diluted solutions (5 µL of 0.1 mg/mL and 5 µL of water) (Figure 4[a]). The applied non-denaturing conditions led to comparable fractionation of both samples, indicating the suitability of this method for handling of LNPs.

Moreover, to show that such solutions can be efficiently manipulated, the LNP sample was diluted with a detergent—Triton X-100

Figure 4: SEC 260 nm chromatograms for LNP/FLuc mRNA samples showing (a) lack of impact of automated injection dilution on an intact LNP fractionation profile, (b) denaturing effect of sequential injection of detergent, causing the release of encapsulated mRNA. F = 0.25 mL/min, column: 150 × 4.6 mm, 450 Å SEC. Author's own data.



Detergent (Figure 4[b]) which is known to cause deformulation of the lipid nanoparticles and the release of their payload (Note: Triton X-100 Detergent itself is a hazardous chemical, because it is a phenol-based surfactant. Its impurities and degradants can act as endocrine disruptors, and exposure to analysts and the environment should be minimized. Alternatively, the use of a more ecologically friendly non-ionic surfactant, such as Brij 58 Detergent, can be explored.). In this work, Triton X-100 Detergent was indeed applied, and an injection of LNP/FLuc mRNA (1 µL of 0.1 mg/mL) sample was combined with a plug of aqueous Triton X-100 solution (1 µL of 0.4% v/v). This resulted in the disruption of the particle and allowed the detection of the encapsulated nucleic acid, which was observed as a peak eluting at a later elution time. This non-optimized experiment indicated that the denaturing action of the surfactant can be achieved. This is a proof-of-concept experiment; as shown in Figure 4(b) it can be seen that the extent of denaturation of the LNP is incomplete. Further optimization of the technique might be possible by adjusting the number of mixing cycles, injection delay, and volume/concentration ratios.

Conclusion

Programmed (multi-step or multi-vial) injections are most commonly used for sample preparation (dilution, internal standard addition, mixing, derivatization). However, sequential (plug, bracketed) injection methods may offer more advantages than initially realized. These types of injection modes can be applied to improve recovery and reduce breakthrough effects related to mismatched diluent to initial condition mobile phase compositions. It is large molecule analytes that can benefit most from sequenced injections.

In AEX, the recovery of mRNA can be significantly improved by performing a bracketed injection. By introducing the sample between pre- and post- plugs of high pH salt solution (that is, 2 M NaBr at pH ~10), carryover values can be reduced to 1–3%. Without bracketed injections, carryover of intact mRNAs can be as high as 10–20%.

In HILIC, injecting the sample along with an acetonitrile pre-plug resulted in symmetrical and sharp peaks. No breakthrough was observed. When applying a relatively large solvent plug volume (that is, 5–10 times larger than the sample volume), the overall loaded sample volume can be significantly increased. As much as 2–5% of the column volume can be injected without any breakthrough effect.

In SEC, LNP samples can be efficiently manipulated via automated sample preparation. This allows development of protocols for automated dilutions or disruptions of the intact LNP sample, potentially increasing reproducibility of the results and reducing impact of human induced errors.

Overall, we aimed to show that programmed injections can not only facilitate biotherapeutics sample preparation, but they can also be advantageously applied to address new types of chromatographic challenges.

Acknowledgement

The authors would like to acknowledge Christian Reidy (Waters Corporation) for reviewing the manuscript.

Declaration of Competing Interest

The authors are employees of Waters (Milford, MA, USA), a manufacturer of chromatography systems and consumables.

Cas9 mRNA and FLuc mRNA were purchased from TriLink BioTechnologies. The Fluc mRNA/LNP was purchased from PackGene Biotech – Cap 1+N1meψUTP+LNP (Firefly Luciferase-mRNA-SM-102). Triton is a trademark of Union Carbide Corporation. Brij is a trademark of Croda Americas LLC.

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A Study on the Optimization of the Ion-Pair Reversed Phase Liquid Chromatography Analysis of Protected And Unprotected DNA Oligonucleotides

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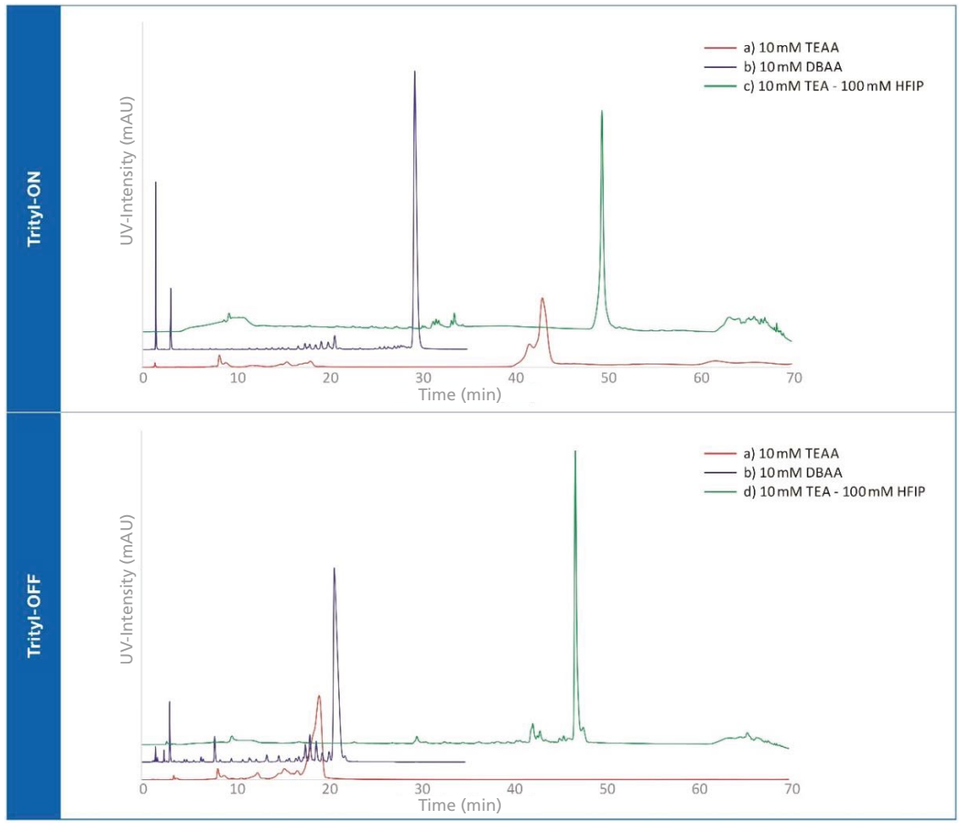
This article describes a comprehensive study on optimizing ion-pair reversed-phase liquid chromatography for analysing protected and unprotected single-stranded DNA oligonucleotides. The study identifies optimal conditions for improved chromatographic resolution and discusses the advantages of using bioinert column hardware and specific temperature settings to enhance the recovery and peak shapes of oligonucleotides, providing a detailed guide for similar analytical setups in the field.

Oligonucleotides have become more and more important in terms of things like polymerase chain reaction and successful treatment of diseases such as viral infections or cancer (1,2). Because of oligonucleotides' very polar properties and electron-rich backbone, high-performance liquid chromatography (HPLC) has become the

Figure 1: Chromatograms for Trityl-ON and Trityl-OFF using different eluents with optimized gradients.



standard separation technique. Typically, ion-pair reversed-phase high performance liquid chromatography (IP-RP-HPLC) or anion-exchange (AEX) chromatography are used, but hydrophilic interaction chromatography (HILIC) and size-exclusion chromatography (SEC) have also been applied to an increasing extent (3–5). However, the electron-rich phosphate backbone of DNA causes retention on common hydrophobic stationary phases to be low. Ion-pairing agents such as n-alkylamines can help to overcome this challenge. The ion pairs formed between N-alkylamines and oligonucleotides can be separated according to their hydrophobicity using a reversed-phase column.

Short oligonucleotides are usually produced using solid-phase synthesis (6). To achieve a controlled sequence, the 5′ terminus is protected with a protecting group such as dimethoxytrityl (DMT). After successful synthesis, respectively after every synthesis step, the protecting group is removed.

In this article, the optimization of the analysis of two single-stranded DNA samples (20mer) using IP-RP-HPLC is described. Both samples consist of the same sequence but differ in the presence (Trityl-ON) or absence (Trityl-OFF) of the DMT protecting group. Parameters such as ion-pairing agents and their concentration, gradient, column hardware and temperature were optimized for both samples.

Experimental

Two single-stranded DNAs (20mer), one with the DMT protecting group on (Trityl-ON) and one with the protecting group already removed (Trityl-OFF), with the following sequence:

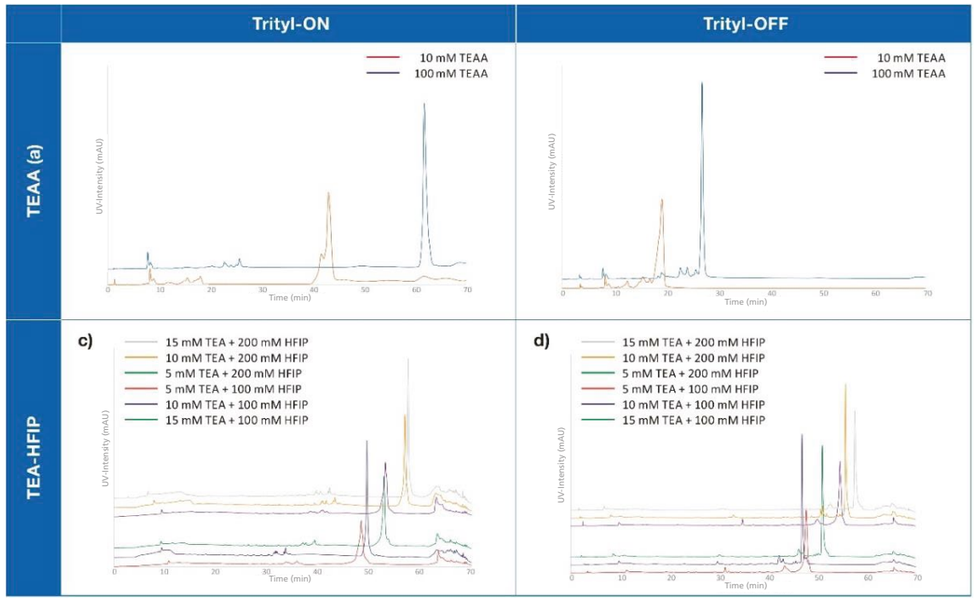
5′-ATACCGATTAAGCGAAGTTT-3′

were used in this study. The general conditions were kept as follows: flow rate 0.2 mL/min, UV detection at 260 nm, injection of 5 µL. The screening gradient was 15–35 %B in 30 min with mobile phase B being acetonitrile and using dibutyl ammonium acetate (DBAA), triethyl ammonium acetate (TEAA) or triethylamine (TEA) with 1,1,1,3,3,3-hexafluoride-2-propanol (HFIP) as mobile phase A at concentrations of 10 mM and 100 mM for HFIP (pH not adjusted). During optimization, different concentrations of the ion-pairing agents were used. In selected cases, methanol instead of acetonitrile was applied as mobile phase B. The optimised gradient slope is 0.42 %B/min–0.58 %B/min depending on the ion-pairing agent used. Two different ultrahigh-pressure (UHPLC) column hardware options packed with an organic/inorganic hybrid-silica C18 phase (12 nm, 1.9 µm) were tested: stainless steel (100 × 2.0 mm i.d, 1.9 µm YMC-Triart C18) and abioinert coated stainless steel (100 × 2.1 mm i.d, YMC Accura Triart C18). The column temperature was kept at 25 °C for screening of the ion-pairing agents and column hardware options. A temperature screening was performed using temperatures between 15–90 °C, therefore the flow rate was increased to 0.4 mL/min in order to shorten the run time.

Influence of the Type and Concentration of Ion-Pairing Agent:

DBAA, TEAA and TEA with HFIP were used for the initial screening at concentrations of 10 mM and 100 mM for HFIP (pH not adjusted). Acetonitrile was chosen as eluent B and a gradient from 15–35 %B in 30 min was applied (not shown, [7]). When using

Figure 2: Screening of the optimum ion pairing agent concentration.



TEAA, the retention time of the sample is very short. In addition, multiple peaks are eluted, indicating the ion-pairing is incomplete. The chromatogram of the Trityl-ON sample shows that there is no elution when using DBAA. A peak is obtained for Trityl-OFF but its resolution and peak shape are not sufficient. When using TEA-HFIP, both samples are eluted directly from the column, indicating that the initial conditions are too strong.

The gradient and starting conditions are adjusted in the next step. For TEAA, the gradient slope was reduced to 0.5 %B/min so that a gradient of 15–45 %B in 60 min is obtained. The starting conditions for DBAA were set to 20 %B and the acetonitrile ratio was increased with the initial gradient slope (0.67 %B/min) to 40 %B in 30 min. Regarding TEA-HFIP, two distinct gradients were applied for the DNA samples. A reduction in the initial gradient ratio was necessary to attain retention. For Trityl-ON, a gradient of 0–35 %B in 60 min (0.58 %B/min) was applied, while for Trityl-OFF, a shallower gradient of 0–25 %B (0.42 %B/min) was used. The organic solvent was changed from acetonitrile (ACN) to methanol (MeOH) using TEA-HFIP as eluent A, as HFIP is not soluble in acetonitrile. Methanol was also used for the separation using TEAA as ion-pairing agent. TEA-HFIP provides the highest resolution and best separation for both samples. The chromatograms with optimized conditions can be seen in Figure 1.

The optimum concentration of the ion-pairing agent depends largely on the sample. Therefore, both lower and higher concentrations were tested (Figure 2). The exception was TEAA, which was only tested at a higher concentration of 100 mM because the sample was not fully paired at a concentration of 10 mM. In addition, different TEA concentrations were tested in combination with 100 mM as well as 200 mM HFIP.

Using 100 mM TEAA instead of 10 mM increased the retention time for both samples. The peak for unpaired DNA is reduced, showing complete pairing had not been achieved.

With the lowest DBAA concentration of 5 mM, the samples are not fully paired. The main peak broadens. At the highest concentration of 25 mM DBAA, the retention time increases but the peaks become broader, too (not shown). TEA concentrations of 5 mM, 10 mM and 15 mM were tested in combination with 100 mM and 200 mM HFIP, respectively. Increasing the TEA concentration led to a significant increase in separation efficiency. Doubling the HFIP concentration had only a slight effect when using a concentration of 5 mM TEA or 10 mM TEA. One reason could be that the thermodynamic equilibrium for the protonation of TEA has already been reached with 100 mM HFIP. At a concentration of 15 mM TEA, increasing the HFIP concentration improves resolution and separation efficiency more significantly.

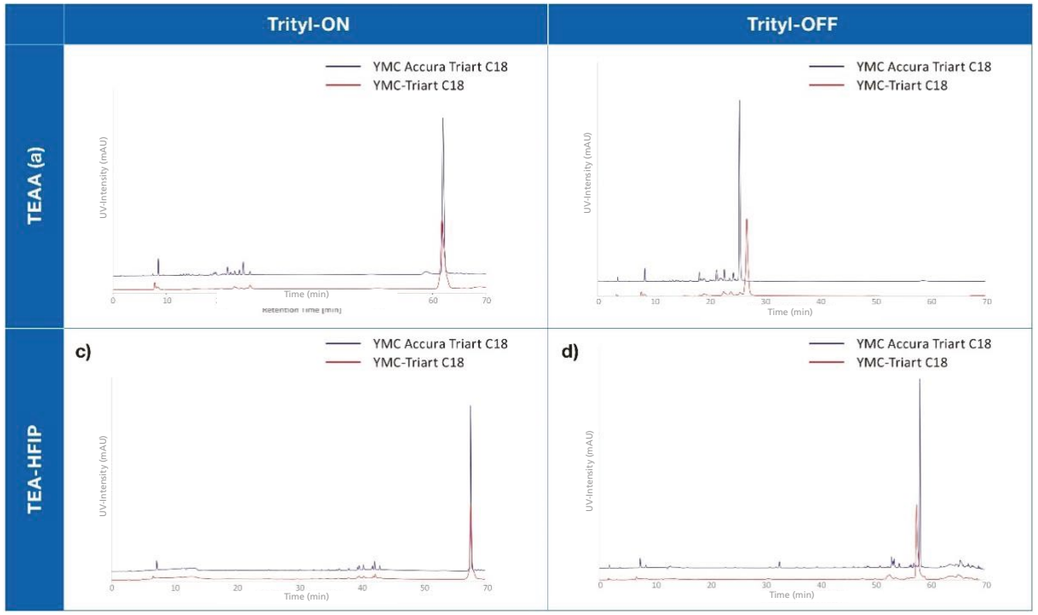
The following concentrations provided the best resolution and peak shape and were therefore selected for further testing:

- 100 mM TEAA
- 10 mM DBAA
- 15 mM TEA + 200 mM HFIP.

The Effect of Bioinert Column Hardware

Oligonucleotides can be irreversibly adsorbed on metal surfaces, including the wetted parts of conventional (U)HPLC columns. This nonspecific adsorption significantly disrupts recovery and peak shape. The effect is even greater when working at low to neutral pH, as metals are more electropositive under these conditions. To solve this problem, HPLC columns and systems can be passivated with strong acids or pre-conditioned with a similar sample (8). However, these procedures are time-consuming and a recurring task. In addition,

Figure 3: Comparison of a stainless-steel and bioinert coated column used for Trityl-ON and Trityl-OFF with different ion pairing agents.



nonspecific adsorption can occur again when the sample is changed.

A simpler and much more robust and reliable solution is to use fully bioinert columns. Different bioinert concepts are available: a bioinert coating of the stainless steel column body and frits, PEEK-lined stainless steel columns in combination with PEEK frits, and columns made of titanium. In this study, a column with a bioinert polymer coating of the column and frits was used. A direct comparison between regular stainless steel hardware and bioinert coated column hardware shows the clear advantages of the bioinert column: improved resolution and recovery (Figure 3). The previously optimized ion-pairing agent concentrations and gradients were applied. For the further investigations, only the stainless steel column was used.

Optimum Column Temperature

Temperature can have a significant impact on the retention in IP-RP-HPLC because the electrostatic interactions with the ion-pairing agents are usually enhanced as the temperature is increased (9). Meanwhile, the hydrophobic adsorption strength of

Figure 4: Temperature screening using a flow rate of 0.4 mL/min and a bioinert coated column.

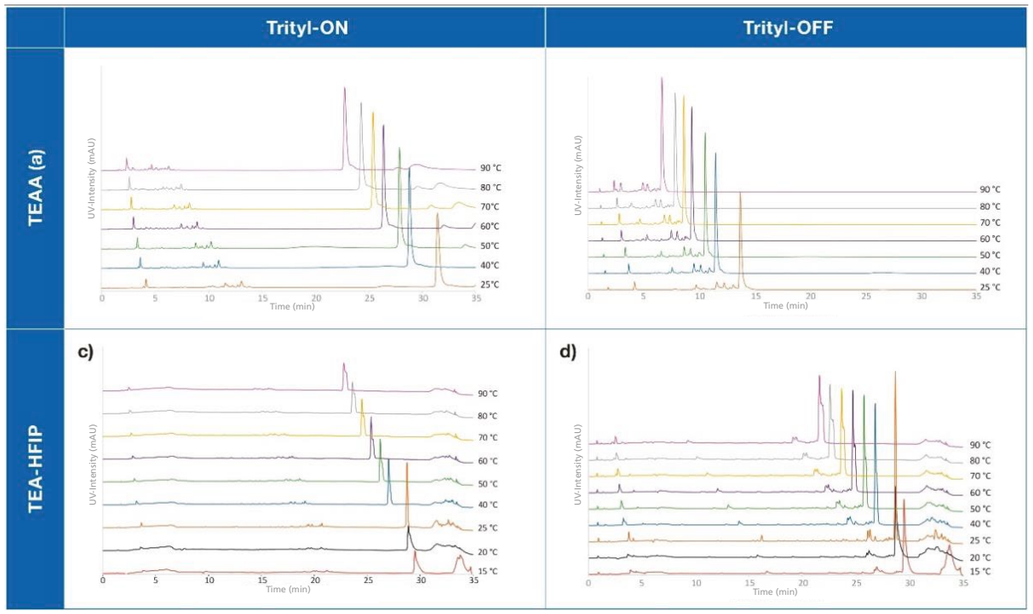
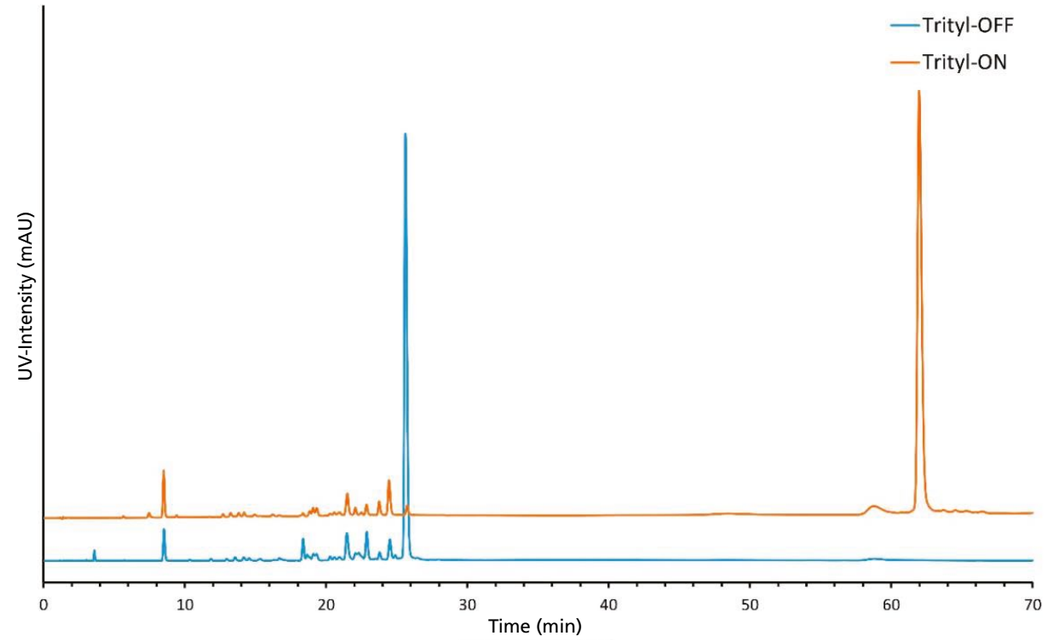


Figure 5: Optimum chromatographic results for the separation of Trityl-ON (orange) and Trityl-OFF (blue) using a bioinert coated column and TEAA as the ion pairing agent, with 15–45 %B in 60 min as the gradient and a flow rate of 0.2 mL/min.



the oligonucleotides and ion-pairing agents on the stationary phase decreases. For this reason, the temperature optima are highly dependent on the ion-pairing agents, organic solvents and the samples themselves and require experimental determination of the temperature optimum. To determine the optimal temperatures, samples were analysed at different temperatures between 15 °C (25 °C) and 90 °C. In order to shorten the run time for the different methods, the flow rate was previously doubled to 0.4 mL/min. In all cases, doubling the flow rate resulted in a lower separation efficiency than for the previous flow rate. The effect of temperature on the separation of Trityl-ON and Trityl-OFF DNA samples is shown in Figure 4.

With a temperature change, the separation efficiency for TEAA and DBAA was improved, which in turn exceeds the separation efficiency at a flow of 0.2 mL/min. The optimum temperature for both samples is 60 °C for TEAA and DBAA. For TEA-HFIP the separation efficiency and resolution decrease with increasing temperature. Therefore, lower temperatures of 15 °C and 20 °C were also investigated. At lower temperatures significant tailing is seen for both samples. Therefore, the optimum temperature for both samples was 25 °C.

Conclusions

In this study, several key parameters of the IP-RP analysis of oligonucleotides were examined. For the analysis of the single-stranded DNA samples in this study, Trityl-ON and Trityl-OFF, 15 mM TEA and 200 mm HFIP with two distinct gradients for both samples provided the best results at a lower temperature of 25 °C (Figure 4). Using TEA-HFIP as the ion-pairing agent entails the advantage of mass spectrometry (MS) compatibility. However, the high costs of HFIP and the environmental concerns regarding polyfluorinated substances are a disadvantage. A TEAA buffer is also MS-compatible and offers very good resolution as a cost-effective alternative, especially

for high-throughput analyses. Additionally, for the separation of DNA samples both protected and unprotected, the ion-pairing agent TEAA provided the best resolution at a concentration of 100 mM with a gradient slope of 0.5 %B/min over 60 min (see Figure 5). In addition, bioinert column hardware is crucial for ideal and reliable results, ideally in combination with a bioinert (U)HPLC system. This boosts recoveries and ideal peak shapes are achieved. Nevertheless, the analysis of DNA using IP-RP-HPLC must always be individually optimised to the properties of the respective DNA.

Acknowledgements

We gratefully acknowledge Jessica Ulke (University of Münster) for the experimental contribution to this work during her internship at YMC Europe GmbH.

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Advances in Chromatography using Artificial Intelligence and Machine Learning

Artificial intelligence (AI) is "the theory and development of computer systems able to perform tasks normally requiring human intelligence, such as visual perception, speech recognition, decision-making, and translation between languages" (1). Foodomic domains, including food metabolomics, sensomics, nutrimetabolomics, and food volatilomics, require great analytical efforts to comprehensively capture sample composition and connect it with biological phenomena or functional properties (2–5). Effective workflows start with the pre-processing of raw data generated by multidimensional analytical systems and proceed with dedicated processing to identify or "visualize" chemical patterns linked to, or predictive of, key properties, such as like nutritional quality, sensory profile, authenticity. AI, by definition, supports researchers in this process that translates chemistry into properties or functions. Chiara Cordero and Marco Vincenti from the University of Turin, Italy have learned how to combine their complementary competencies in analytical chemistry and big data analytics to achieve significant advances in food science and health.

Chiara Cordero and **Marco Vincenti,** University of Turin, Torino, Italy

Q: AI and machine learning (ML) are increasingly becoming "hot topics" in separation science. Is there a difference between the role of AI and machine learning in relation to chromatography?

MARCO VINCENTI: AI and ML are increasingly becoming essential in separation

science because the large, multidimensional, high-resolution data generated in the chromatographic process can no longer be efficiently handled with classic intuitive representations based on two-dimensional (2D) features selection. There is no clear distinction in the literature between AI and ML except that ML can be regarded as a subchapter of AI. In relation to chromatography, ML generally operates on large, internally-produced experimental data by means of prearranged programming, methods, and instructions, to generate compositional information not obtainable by classic data processing. Beyond these tasks, AI uses multi-source data including external depositories and databases, to achieve complex objectives by means of flexible strategies, for example, to highlight otherwise undetectable data patterns or to optimize specific analytes' class separations under multiple and simultaneous constraints. In particular, AI procedures involve—to a variable degree—autonomous decision-making steps leading to the final output.

Why is AI or ML necessary in chromatography, generally? Do you think it will become increasingly used in two-dimensional chromatography?

VINCENTI: AI and ML strategies are becoming essential because at the moment because chromatography is consistently combined with 2D chromatography itself, or spectroscopic techinques, or tandem- or high-resolution mass spectrometry. These hyphenations generate high-density, multi-dimensional data for each of the samples that should be compared to one another, as for example in metabolomics. Extracting minimal differences from these data with similar patterns is a task not feasible by human senses, even if helped by selective data plotting. Two-dimensional chromatography adds further complication to this frame, appending the need of accurate data pre-treatment for peak alignment among different samples or injections to correct potential retention times shifts along a two-dimensional plane. Notably, these shifts are not necessarily linear with retention time. Intelligent automated peak recognition algorithms are crucial to operate these corrections.

Chiara, you recently reviewed the AI tools and concepts adopted to exploit the informative potential of comprehensive two-dimensional gas chromatography (GC×GC) in foodomics. Can you tell us more, and what problems AI solves?

CHIARA CORDERO: Comprehensive two-dimensional chromatography, that is, GC×GC or LC×LC, offers unique opportunities to investigate in greater detail the sample's compositional complexity. When samples are characterized by a high chemical "dimensionality," as defined by Giddings, their comprehensive investigation should be approached by suitably designed multidimensional systems (6). In particular, when comprehensive 2D-GC or 2D-LC are used, they highlight ordered structures in retention data, for example, homolog series or chemical classes, and support efficient separation of isomers or isobars with great benefits for confident identification and accurate quantification.

If we can connect the detailed chemical profile with functional and nutritional properties or quality of food, as in many foodomic domains, we advance our knowledge and develop more accurate and precise predictive models (2). Volatiles and semi-volatiles have, in GC×GC–MS, the analytical platform of choice for detailed profiling and effective fingerprinting, investigation strategies that are currently realized with the aid of AI tools (7). Image pattern recognition (PR) is efficiently used to track and realign features across many chromatograms and samples, while computer vision (CV) exploits the full data array of chromatographic images to highlight compositional differences even in the presence of unresolved mixtures or confounding phenomena.

What do these tools solve?

CORDERO: In the context of comprehensive 2D chromatography, image PR by template-matching algorithms enables effective and confident tracking of features across many samples even in case of temporal misalignments due to oven temperature (un)stability or carrier gas flow and pressure inconsistencies (8,9). The use of different modulation technologies, such as thermal modulation or differential-flow modulation or changes in column configuration can be also effectively tackled with this AI tool to enable metadata transfer between applications or analytical campaigns (8,10). This process or realignment is particularly challenging for untargeted components where classical approaches of peak tracking fail or require extensive computational time. Moreover, untargeted features could add further knowledge to the interpretation of many phenomena, their reliable mapping using the information provided in full by the analytical system, such as retention times in two dimensions, retention index, detector response, spectral signature.to support effective re-investigation of samples.

Computer vision is implemented in

commercial software platforms with tools using simplified grids or tiles or peak regions that explore the 2D or 3D array of data and facilitate the identification of compositional differences between samples and samples' classes CV, when supported by image PR, enables augmented visualization and gives access to the chemical information encrypted in the data array (11).

Does AI help in the "translation between languages" when applied to foodomics applications?

CORDERO: The intriguing application of AI as a sensomics-based expert system (SEBES) capable of predicting key aroma signatures of food without human olfaction has been successfully realized for many foods (12–14). It is referred to as an AI smelling machine because it captures key food odorant patterns, and by their accurate quantification resembles the aroma identity of food in an objective and unbiased way. GC×GC is the core of the analytical platform since it enables efficient separation of odorants from the bulk of interfering volatiles and provides suitable method sensitivity to achieve sub-parts per billion (ppb) levels for the most potent aroma active compounds. The approach of AI smelling truly enables "translation between languages" by means of translating chemical patterns in olfactory qualities.

Any advice to chromatographers who want to incorporate AI to improve their methods?

VINCENTI: Several ML softwares are freely available on the internet at increasingly "user-friendly levels". These softwares may be very useful for the elaboration of chromatographic data produced at laboratory level, provided that the user has basic knowledge on handling R, Phyton, or MATLAB languages and a deeper knowledge on the theory of the different ML methods and their pertinence to the problems to be solved. The ability to exploit these powerful ML methods rapidly increases with practice. More dedicated AI softwares for 2D chromatography, possibly combined with mass spectrometry, are generally proprietary and relatively expensive, but are recommended when the queries' complexity of chromatographic analyses increase substantially.

Can you summarize the role of AI and ML in chromatograpahy?

CORDERO and VINCENTI: From our viewpoint, AI and ML appear to be mandatory to make significant progress in the comprehension of complex phenomena; in other words, to profitably investigate analytes at the the boundries between chemistry and biology (15). Currently AI tools are mostly accessible to specialized computer scientists and bioinformatics, and more user-friendly software platforms should be made available to chemists and biologists— and even provided with sufficient ML knowledge—to boost rapid progresses in crucial applications, such as food science and health. Once validated by a wider community of differently specialized scientists, these manageable AI tools will be more easily accepted in a variety of research and industrial laboratories, where the analytical data frequently trigger the decisions and drive the strategies.

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Chiara Cordero is a full professor of food chemistry at the University of Turin (Torino, Italy). Her passion is gas chromatography (1D-2D) thanks to the unique opportunities it offers in foodomics. Research interests focus on the development of instrumental configurations and data processing tools for comprehensive two-dimensional GC in high-resolution profiling and fingerprinting of complex samples. Application domains include food metabolomics and volatilomics, nutrimetabolomics, and sensomics. The goal is to go beyond the current knowledge and explore the chemistry behind biological phenomena. She received the "Leslie S. Ettre Award" in 2008 as a young scientist for "presenting original research in capillary gas chromatography with an emphasis on environmental and food safety", the "John B. Phillips Award" in 2014 for her research activity in the GC×GC field, and the Scientific Achievement Award in 2022 for her commitment in the GC×GC research community.

Marco Vincenti is a full professor of analytical chemistry at the University of Turin (Torino, Italy). His 40-year career has continuously evolved around organic mass spectrometry and hyphenated chromatographic techniques in different applications. His most recent research interests focus on the development of targeted and untargeted methods for metabolomic studies in clinical, forensic, and agricultural chemistry combined with machine learning data elaboration algorithms. He has authored or coauthored more than 200 peer-reviewed publications and 10 book chapter.

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34th International Symposium on Pharmaceutical and Biomedical Analysis (PBA 2024)

The 34th International Symposium on Pharmaceutical and Biomedical Analysis (PBA 2024) will be held in Geneva, Switzerland from 9–12 September 2024.

PBA 2024 Organizing Commitee

The 34th International Symposium on Pharmaceutical and Biomedical Analysis (PBA 2024) will be held in Geneva, Switzerland from 9–12 September 2024. One of the main objectives of the symposium is to provide a forum for high-level scientific exchange between scientists from around the world in a friendly atmosphere.

Scientific Programme

The symposium will cover all aspects of pharmaceutical and biomedical analysis, including new analytical technologies and methods for (bio)pharmaceuticals, biomarkers, and omics. Plenary and keynote lectures will be delivered by internationally renowned leaders on cutting-edge topics, including Coral Barbas (Spain), Hugo Duminil- Copin (Switzerland), Gert Desmet (Belgium), Michal Holcapek (Czech Republic), Valérie Pichon (France), Koen Sandra (Belgium), Tim Causon (Austria), Marianne Fillet (Belgium), Sandra Furlanetto (Italy), Ana Garcia-Campaña (Spain), Kenji Hamase (Japan), Zhengjin Jiang (China), Jingwu Kang (China), Michal Markuszewski (Poland), Gabriella Massolini (Italy), Gabriella Massolini (Italy), Sibel Ozkan (Turkey), Pablo Sinues (Switzerland), Myriam Taverna (France), Myriam Taverna (France), Myriam Taverna (France), Jean-Luc Wolfender (Switzerland), Nicola Zamboni (Switzerland).

A wide range of contributed talks and poster presentations will also stimulate interdisciplinary discussions. The participation of young researchers from both industry and academia, will be strongly encouraged through a dedicated young scientist session, awards of the best oral presentation and poster, and a reduced registration fee.

PBA 2024 will also offer stimulating and highly informative workshops by recognized experts in their field; which will be held in parallel on Monday 9th September 2024.

Finally, a special attention will be paid to the use of simulation tools in teaching and research.

Young Scientists Sessions and Prizes

There will also be special sessions and opportunities for young scientists. The program features a dedicated session («*Beyond the PhD: What's Next?)*, offering invaluable insights into your career trajectory. Additionally, mark your calendars for our exclusive Job Fair session on Wednesday September 13 2024, where participating sponsors and exhibitors will be conducting interviews with PhD candidates. Plus, don't miss the chance to win exciting prizes for oral and poster presentations, generously sponsored by JB, Bracco, and ccCTA.

Women in Analytics Award

PBA 2024 will also offer a " Women in Analytics Award" to empower early-career women in analytical chemistry and related fields. This award aims to support professional development by allocating funds for receiving specialized training, laboratory visits to acquire new skills or attending workshops/courses.

Geneva

Geneva is a picturesque and vibrant international city located between the Alps and the Jura mountains on Lake Geneva in the heart of Europe. It has an international airport with direct flights to most continents and is easily accessible by air or rail. The congress will be held in the university building "Uni Mail," which is centrally located in the city. We look forward to welcoming analytical scientists from all over the world and hope to enrich our knowledge with their lectures and presentations.

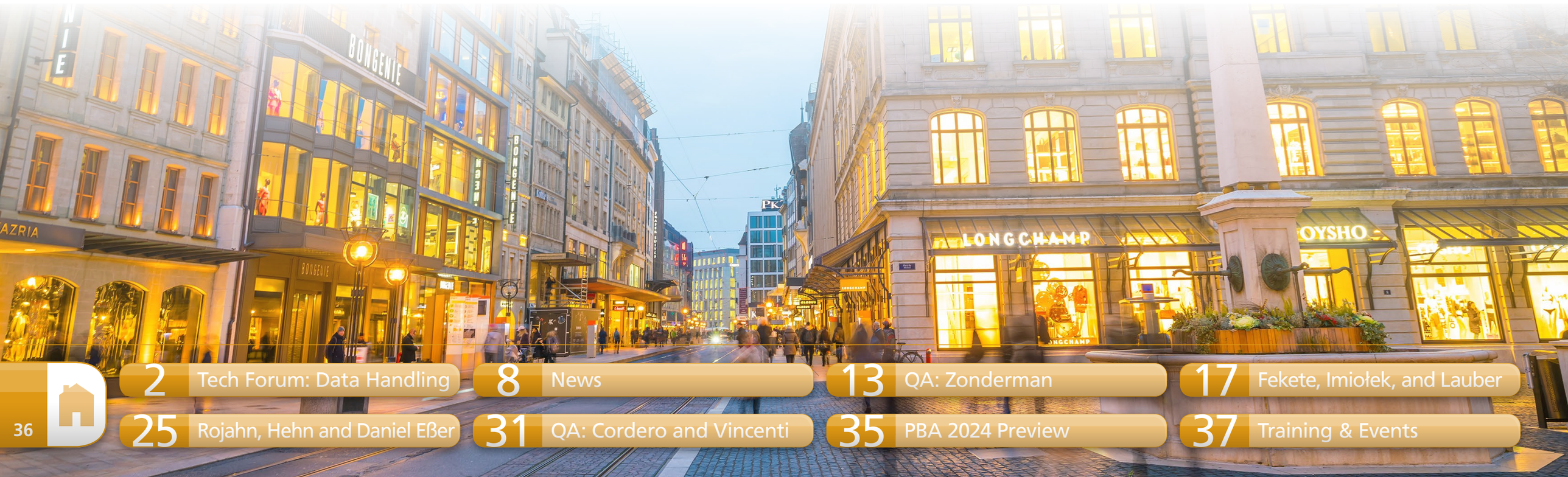
Social Events

In addition to the scientific programme, an attractive social programme will also be arranged during and after the Symposium. The conference festivities commence on Tuesday evening with welcome cocktails at the congress venue, encouraging connections and networking opportunities. On Wednesday, join us for the Congress Dinner, where attendees can further engage and interact.

Throughout the conference, attendees have the opportunity to explore Geneva through guided walking tours, immersing themselves in the city's rich culture. Following the conclusion of the congress on Friday, an excursion to Chamonix awaits, providing a picturesque escape to complement the conference experience.

We look forward to your participation in PBA 2024 in September and to welcome you to Geneva.

E-mail: **contact@pba2024.org**
Website: **www.pba2024.org**



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Website: www.crawfordscientific.com/training-consultancy/gc-training/operating-and-understanding-gc

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HPLC/LC–MS

Development in Chromatographic Methods in DSP
16 September 2024
Website: https://ymc.eu/customer-training-and-education.html

Method Development in Chiral Chromatogrpahy
18 November 2024
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HPLC Troubleshooter
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Online webcast from *CHROMacademy*
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Please send your event and training course information to Alasdair Matheson AMatheson@mjhlifesciences.com

Event News

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HPLC 2024

Denver, Colorado, USA

Email: olesik.1@osu.edu

Website: https://hplc2024-symposium.org/

15–18 September 2024

3rd Sample Preparation Conference/2nd Green and Sustainable Analytical Chemistry Conference

Crete, Greece

Email: epsillakis@tuc.gr

Website: https://www.eusp-gsac2024.tuc.gr/en/home

6-10 October 2024

34th International Symposium on Chromatography

Liverpool, UK

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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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