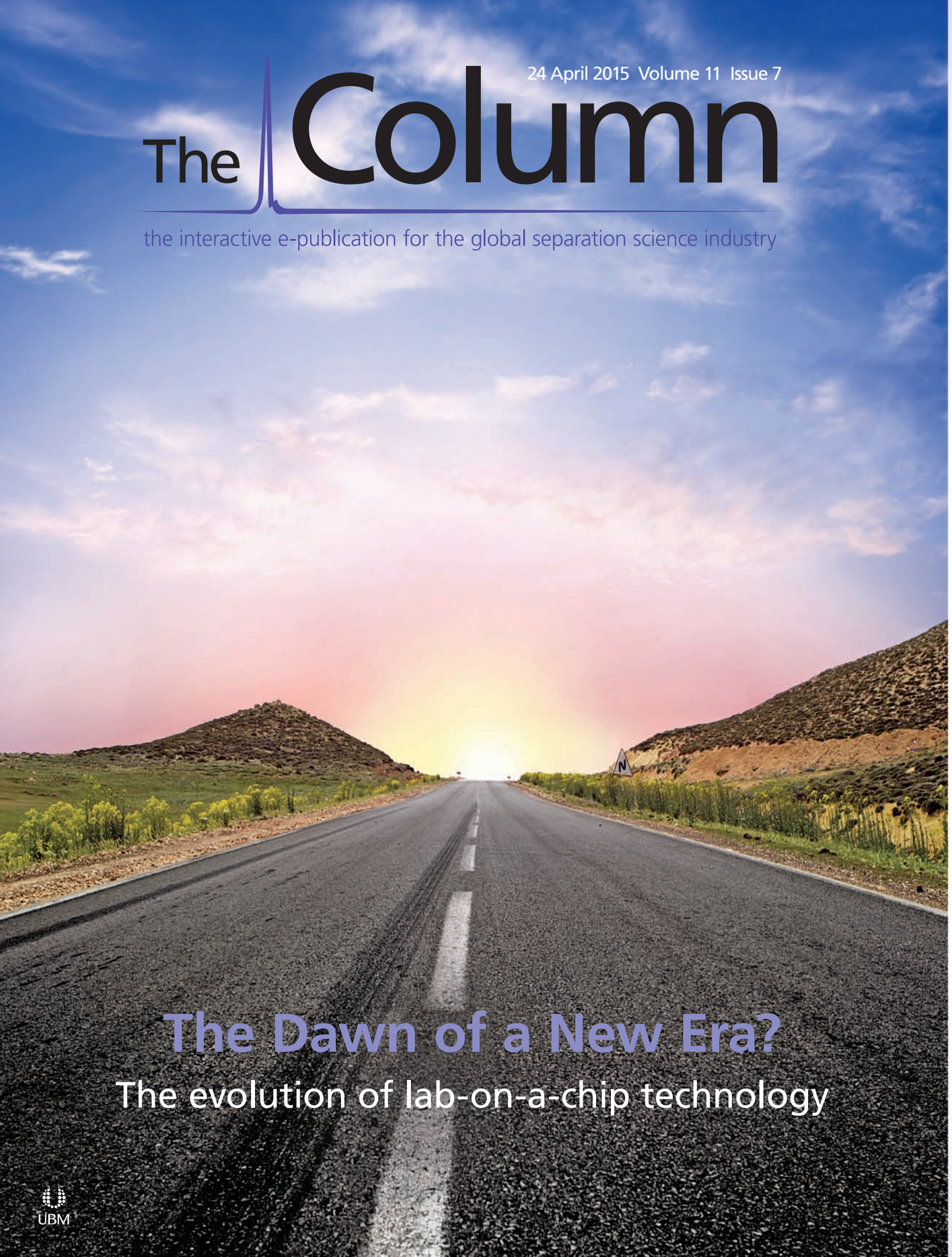
24 April 2015 Volume 11 Issue 7

The Column

the interactive e-publication for the global separation science industry



The Dawn of a New Era?

The evolution of lab-on-a-chip technology

UBM

Cover Story

Features

Regulars


Photo Credit: Stephen Morris/Getty Images

A New Era for Lab-on-a-Chip Technologies?

Holger Becker, microfluidic ChipShop GmbH, Jena, Germany.

This article discusses the evolution and current applications of lab-on-a-chip technology in chromatography and explores the possibility of a new market for microfluidics in separation science.

Miniaturization has been one of the biggest trends in recent years in all areas of technology. In the analytical and the life sciences it has been a strong driver of academic and industrial developments under the headings "microfluidics", "lab-on-a-chip", or "microTAS" (miniaturized total analytical systems). Historically, the separation sciences have played a significant role in the early stages of the development of this field. A fundamental, but often overlooked work, was carried out by Stephen Terry in Stanford in the mid-1970s, when he integrated a complete gas chromatograph on a silicon wafer.[1] Later, the early works of the pioneering groups involved in lab-on-a-chip technologies — including work by Manz[2], Harrison[2], and Ramsey[3] — focused on electrophoretic separations but efforts were also made to transfer chromatographic methods onto a microchip. The reasons for this choice can easily be found in the fundamental equations for diffusion and separation efficiency,[4] indicating a superior performance of chip-based capillary electrophoretic (CE) systems especially with respect to analytical speed. It is therefore not surprising that the first commercial instruments based on lab-on-a-chip technologies were systems which used capillary electrophoretic separation in a microchannel for the analysis of biomolecules (DNA, RNA, proteins), including miniaturized CE instruments from Agilent,[5] Shimadzu,[6] and Bio-Rad.[7]

The promise of all these systems was, besides the decrease in "time-to-result", a significant reduction in manual operating steps, a certain amount of process automation, and a better reproducibility of the results. Chip-based capillary electrophoresis is now established as the standard method for the analysis of biomolecules. For chromatographic

separations however, the transfer to a chip-based method proved to be significantly more challenging for a variety of reasons. First of all, the maturity of high performance liquid chromatography (HPLC) and gas chromatography (GC) methods meant that the performance level with respect to the quality of separations was already very high and difficult to achieve with compact chip-based devices. Secondly, the overall analytical process in chromatography has not been perceived as being overly cumbersome (in comparison to casting gels in electrophoresis for example), therefore reducing the potential advantages of chip-based methods.

Thirdly, the integration of stationary phases into microchannels posed a significant challenge in the manufacturing process (and still does). And finally, the high pressures required made the interfacing of chips typically made out of silicon or glass problematic. Despite these problems, a fair amount of academic work on chip-based chromatographic methods has been performed.[8] The coupling of LC with mass spectrometry (MS) has proven to be a particularly attractive area, pioneered in particular by the group of Karger,[9] because silicon/glass microfabrication technologies allow a very precise and reproducible generation of sharp and narrow structures suitable to generate a stable Taylor-cone configuration for subsequent injection into a mass spectrometer. Furthermore, the integration of sample purification and other functionalities into a single device made for a reduction in size, which allows for a better integration into a coupled system.

On a commercial level, work performed by Killeen[10] at Agilent used laser-ablation as a microfabrication technology to generate an integrated microfluidic chip as a front end for a mass spectrometer. This was developed into a commercial HPLC-Chip–MS system.

Waters Corp later developed a UHPLC nano device which uses a microfabricated ceramic substrate to perform HPLC prior to MS coupling.

In the field of GC, the developments were noticeably fewer. On the academic side, it was work by Yu at Lawrence Livermore Lab[11] as well as the group of Kostiainen at the University of Helsinki[12] that demonstrated feasibility for GC.

On the commercial side, the Dutch company C2V had developed a GC system based on a silicon microchip during the mid-2000s, which was launched in 2010 by Thermo Fisher, however this product was discontinued.

Despite these developments, it should be noted that the progress and uptake of miniaturization technologies in the

chromatographic sciences has been slower than in other disciplines. In my opinion, this is the result of several factors: Firstly, it should be noted that the areas in which microfluidics makes the fastest commercial advances are areas in which complex protocols are commonplace. A typical example of such a field is molecular diagnostics, which is currently experiencing a dramatic increase in the number of integrated, microfluidics-based cartridge solutions. The driver here is that in the conventional workflow, a large number of protocol steps have to be performed, either manually or by complex laboratory robotics. Both approaches increase the time-to-result and increase the risk of errors as well as the need for a suitable infrastructure to perform these protocols. A further example can be found in biology, where complex cellular assays can be performed in an integrated microfluidics device without human interaction.

A second reason is the inherent structure of the analytical tasks addressed by the current analytical laboratories. While many technologies (HPLC, MS etc.) are high-performance methods requiring comparatively complex instrumentation, skilled personnel, and a laboratory infrastructure, the main thrust in the development of microfluidics-based devices is oriented towards a decentralized application at the point-of-interest or point-of-care. In these cases, the ultimate analytical performance might not be needed, or may not be achievable. However, there could be be a need (and a market) for moderately priced, fast "time-to-result" compact systems with limited analytical resolution that are easy to use by moderately skilled personnel to complement the current "high-end" market of analytical instrumentation.

Microfluidics-enabled systems might prove to be the technological answer to these challenges. In particular the ability to integrate complex workflows into a single device is very appealing.

Typical for microfluidics-based solutions, especially in life science or diagnostic applications, is the use of the microfluidic device as a disposable tool, which is in contrast to the model of reusable chips or on-chip columns used in the current commercial systems. The reason for this can be found in the cost structure, because it is cheaper to use a new device each time rather than to develop and apply a validated cleaning procedure.

Although challenging, a business model based on disposable consumables could also provide an alternative to the current situation, providing a revenue stream

that is not so heavily dependent on instrumentation. This would certainly require a significant system and business model development but again could enable new approaches and markets.

In conclusion, the advances in technology and commercialization of microfluidics-enabled products do currently happen more readily in fields other than the separation sciences, despite the fact that much of the early ground-breaking work took place in this field.

Lab-on-a-chip technology could enable new markets in addition to the currently served analytical markets. The science, knowledge, and supplier infrastructure are all available, it only takes a bold move to open up new opportunities. Microfluidics is moving into the mainstream in many scientific and technological fields[13] and it would be a shame if separation science misses out on this opportunity.

References

1. S.C. Terry, J.H Jermann, and J.B. Angell, *IEEE Trans. Electron. Devices* **ED-26**, 1880–1886 (1979).
2. D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, and A. Manz, *Science* **261**(5123), 895–897 (1993).
3. Stephen C. Jacobson, Roland Hergenroder, Lance B. Koutny, and J. Michael Ramsey, *Anal. Chem.* **66**(7), 1114–1118 (1994).
4. C.S. Effenhauser, in *Microsystem Technology in Chemistry and Life Science* A. Manz and H. Becker, Eds. (Topics in Current Chemistry Vol. 194, Springer-Verlag, Heidelberg, Germany, 1998), pp. 51–82.
5. A. Masotti and T. Preckel, *Nature Methods* August 2006 (http://www.nature.com/nmeth/journal/v3/n8/pdf/nmeth908.pdf)
6. H. Nagata, M. Tabuchi, K. Hirnao, and Y. Baba, *Chromatography* **26**(1), 23–28 (2005).
7. http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_5286.pdf
8. J.P. Kutter, *Journal of Chromatography A* **1221**, 72–82 (2012).
9. Iulia M. Lazar, Lijuan Li, Yu Yang, and Barry L Karger, *Electrophoresis* **24**(21), 3655–62 (2003).
10. H. Yin, K. Killeen, R. Brennen, D. Sobek, M. Werlich, and T. van de Goor, *Anal Chem.* **77**(2), 527–33 (2005).
11. C.M. Yu, Hand-held multiple system gas chromatograph, US Patent 6306200 B1, (2001).
12. M. Haapala, L. Luosujärvi, V. Saarela, T. Kotiaho, R.A. Ketola, S. Franssila, and R. Kostiainen, *Anal Chem.* **79**(13), 4994–9 (2007).
13. H Becker, *Lab on a Chip* **9**(15), 2119–2122 (2009).

Holger Becker, PhD, FRSC, is co-founder and Chief Scientific Officer of microfluidic ChipShop GmbH. He obtained his PhD in physics from Heidelberg University, Heidelberg, Germany, in 1995 with a paper on surface acoustic wave chemical sensors. He then worked with Andreas Manz as a research associate at Imperial College, London, UK. He has held several positions in the microsystem industry before founding microfluidic ChipShop in 2002. His research interests lie in the application of micro- and nanotechnologies in chemistry and the life sciences.

E-mail: **hb@microfluidic-chipshop.com**
Website: **www.microfluidic-chipshop.com**

News

Using Proteomics to Determine Key to Embryo Implantation Success

There are many couples worldwide that are affected by infertility and require medical intervention to achieve a successful pregnancy. *In vitro* fertilization–embryo fertilization (IVF–ET) is one procedure that can be undergone; however, it can be limited by the low rate of embryo implantation. A new study published in the *Journal of Proteome Research* presents a proteomics approach to investigate changes at the molecular scale that could control whether or not implantation using IVF is successful.[1]

The authors state that around two-thirds of implantation failures in IVF are attributed to the receptivity of the membrane lining the uterus, the endometrium, which is a complex tissue that undergoes changes in response to hormone levels in the body. To detect the changes governing receptivity, the authors took a proteomics approach to screen for changes in proteins expressed in the tissue when receptive versus proliferative phase (non-receptive).

Clinical biopsy tissue samples were taken from 12 women undergoing IVF treatment and analyzed using high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS–MS). More than 2000 proteins were identified and quantified — 300 proteins were found to vary between the receptive and proliferative phase. According to the authors, the method developed could be performed in future studies looking at variations in protein expression in other endometrium-related diseases. — B.D.

Reference

1. Q. Chen *et al.*, *Journal of Proteome Research* **14**, 1831–1842 (2015).

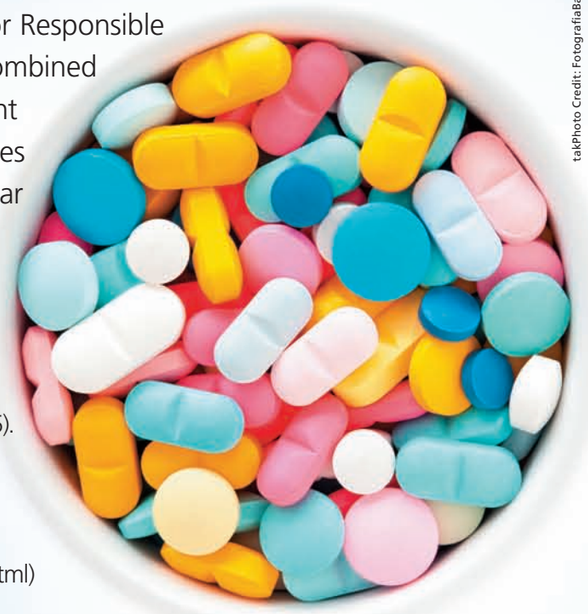
Dietary Supplements Tainted with Amphetamine Isomer BMPEA

Selected dietary supplements labelled to contain *Acacia rigidula* have been found to be adulterated with the amphetamine isomer β-methylphenylethylamine (BMPEA).[1] This is not, however, a new finding. In 2014 the U.S. Food and Drug Administration (FDA) published on the detection of BMPEA in *Acacia rigidula* dietary supplements, but failed to warn consumers of the potential danger from the adulterant.[2]

The compound BMPEA was first synthesized in the early 1930s as a substitute for amphetamines, but, until recently, was treated as a research chemical. It is not found naturally in *Acacia rigidula*, but is added as an additional (often unlisted) ingredient for its physiological effects even though it has not been evaluated for safety in humans. Dr. Pieter Cohen of Harvard Medical School in Massachusetts, USA, told *The Column*: "We were aware of the FDA findings and mystified that they had not warned consumers or clarified the legality of these spiked products. We were interested to know what would manufacturers do once the FDA had discovered the stimulant."

The authors purchased 21 dietary supplements labelled as containing *Acacia rigidula* online in the USA in 2014, and performed liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–QTOF-MS) analyses. The paper reports that more than half of the samples were adulterated with BMPEA ranging from 8.4% to 31.8% per serving.

Although only a small number of supplements were analyzed, making it impossible to know if the BMPEA adulteration is a limited issue, it is concerning that the FDA have not exercised their regulatory power to force manufacturers to remove BMPEA. In a press release published in response to the study by the Council for Responsible Nutrition,[3] Steve Mister, president & CEO, said: "Given FDA's earlier findings combined with this new study by Dr. Cohen, we urge FDA to take immediate enforcement action against these adulterated products containing BMPEA and the companies illegally spiking these products with this synthetic drug. BMPEA does not appear to be a legitimate dietary ingredient, and therefore its inclusion in a product labelled as a dietary supplement makes the product adulterated under the Dietary Supplement Health & Education Act (DSHEA)." — B.D.



takPhoto Credit: FotografiaBasica/Getty Images

References

1. P.A. Cohen, C. Bloszies, and R. Gerona, *Drug Testing and Analysis* DOI 10.1002/dta.1793 (2015).
2. R.S. Pawar, E. Grundel, A.R. Fardin-Kia, and J.I. Rader, *Journal of Pharmaceutical and Biomedical Analysis* **88**, 457–466 (2014) doi:10.1016/j.jpba.2013.09.012.
3. Council for Responsible Nutrition: Press Release "CRN Asks FDA to Enforce the Law" (Last Accessed 10 April 2015: http://www.crnusa.org/CRNPR15-AsksFDAtoEnforcetheLaw040715.html)

Sustainable Reuse of Fracking Water

Hydraulic fracturing — also referred to as "fracking" — is a technique used for the extraction of natural gas that has been widely adopted to exploit natural gas resources in the USA. However, there are growing concerns about the potential impact of fracking activity on the environment and human health, one of which is the question of how to dispose of or recycle the large volume of wastewater generated. Researchers from the University of Colorado have published a study in the journal *Science of the Total Environment* to characterize flow-back water generated from fracking activities, using a combination of analytical techniques.[1]

Fracking describes the process of producing fractures in rock formations by pumping large quantities of hydraulic fracturing fluid at high pressure down a drilled wellbore.[2] Hydraulic fracturing fluid contains a mixture of water and chemical additives that expand fractures and, once the fracturing process is complete, is returned to the surface. If not disposed of safely or treated effectively, the wastewater can contaminate water sources in the surrounding area.

Corresponding author Karl. G. Linden from the University of Colorado told *The Column*: "This study is part of a larger effort by our group funded by the National Science Foundation to evaluate sustainable options for water reuse in the oil and gas industry. The first step is to know what is in the flowback and produced waters from hydraulic fracturing, [so] then we can design effective treatment processes to meet goals of reusing the water — either for more fracturing to save other freshwater sources or for safe reuse or distribution into the environment."

Flowback water was sampled from a well in the Denver-Julesberg basin and assessed for general quality. Volatile fatty acids and inorganic anions were measured using ion chromatography (IC), and gas chromatography coupled to mass spectrometry (GC–MS) was performed to determine volatile and semi-volatile organic compounds. Linden said: "The specific chemicals and stabilizers that are used in fracturing fluids, mixed with the deep-bed saline formation waters, under pressure and temperature, may form compounds we do not expect to see. To be able to detect unknowns, we need to apply advanced analytical methods such as time-of-flight liquid chromatography–mass spectrometry, with accurate mass analysis."

According to the paper, the flowback from the Denver-Julesberg basin contained salts, metals, and high levels of organic matter composed of fracturing fluid additives and degradation products. This led the authors to conclude that using biological processing would be the best approach to treating flowback water in this location.

When asked about future work, Linden told *The Column*: "We are currently studying the evolution of water quality that flows back from the start of a hydraulic fracturing job to the time that the well is fully producing oil and gas. This study will be a first-of-its-kind time course study to evaluate how water quality evolves during a hydraulic fracturing job and will help inform the treatment strategies that will allow for effective reuse of this water from day 1. Ideally we want to keep the water safely in the hydraulic cycle and not have to rely on deep well injection for disposal, which has its environmental costs." — B.D.

References

1. Y. Lester, I. Ferrer, E.M. Thurman, K.A. Sitterley, J.A. Korak, G. Aiken, and K.G. Linden, *Science of the Total Environment* DOI:10.1016/j.scitotenv.2015.01.043 (2015).
2. United States Environmental Protection Agency [Last accessed 13 April 2015: http://www2.epa.gov/hydraulicfracturing/process-hydraulic-fracturing]

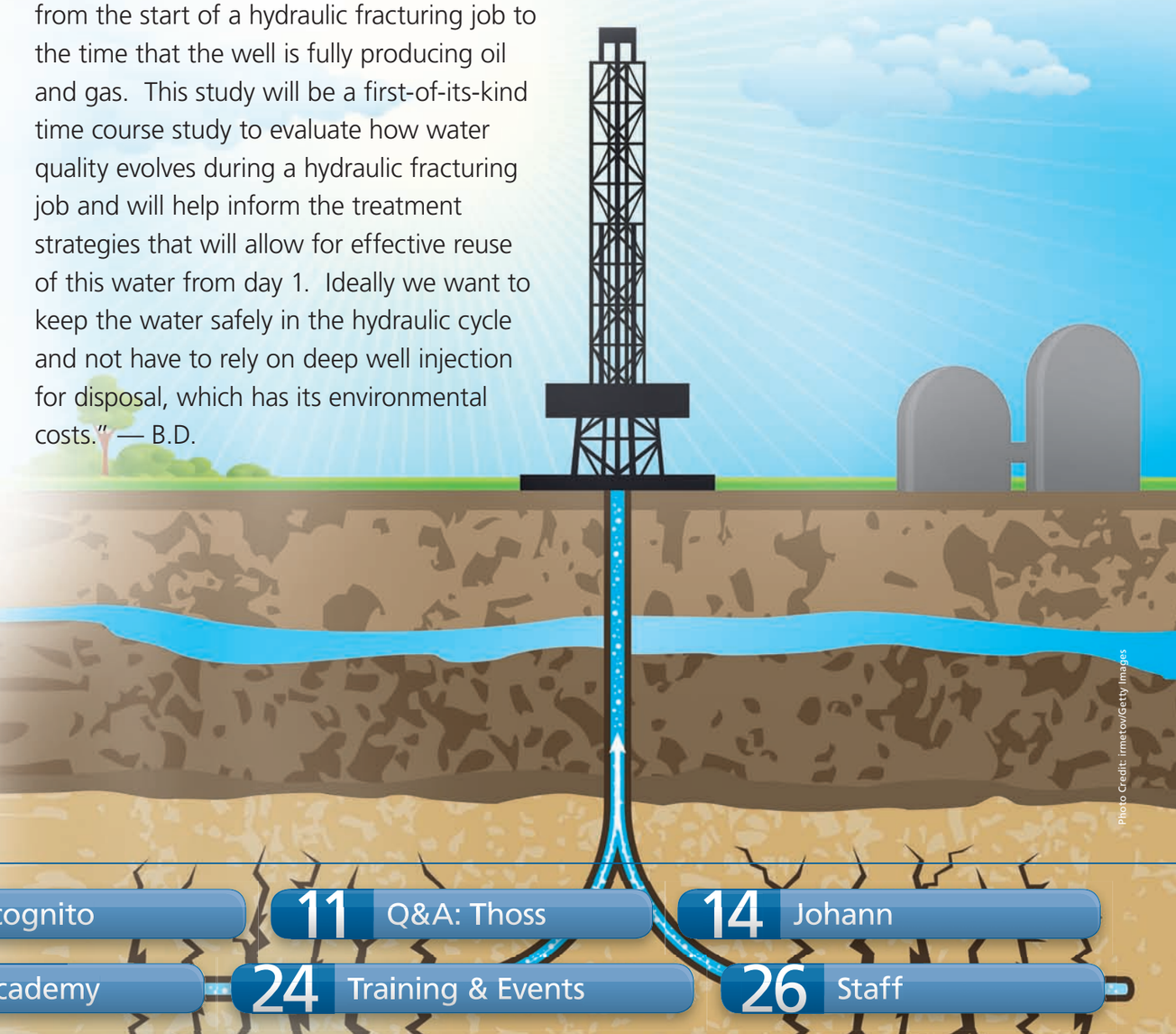


Photo Credit: irmetov/Getty Images

Photo Credit: Epoxydude/Getty Images

The Post-Pittcon Post-Mortem

Incognito offers his views on Pittcon 2015. What were your experiences like and did you have any arguments?

It was warm and sunny as we emerged into New Orleans for Pittcon — 12 h later than we should have arrived and following a tortuous 34 h of travel (including one missed flight connection). I know the frequent travellers out there will mock and consider this a trifling journey, but I'm guessing your boarding group isn't somewhere after the cargo and livestock and your flying position is horizontal rather than semi-vertical! I have also often wondered what cartel operates to retain the Pittsburgh Conference (Pittcon) at a range of locations that are wholly inaccessible, or highly inclement at the time of year of Pittcon. It used to be said that there were only certain convention centres large enough to accommodate the meeting, but that was in Pittcon's heyday and I'm really not sure that this is true anymore. All of that being said, I do realize that to be able to attend the meeting at all puts me in a very privileged position for which I'm very grateful.

In previous years, I have often reported on my adventures at Pittcon with a general summary of the meeting, but this time I've decided to change things up a little. Before I start that, I do want to say that this year both the technical programme and exhibition were very good and that we saw the return of some notable large vendors following a period of absence in recent years (One or two of the big guns do continue to shun the meeting). We also seem to have reduced the number of oral sessions whose titles are "pimped" compared to the actual content, which saved me quite a lot of "lecture hopping" this year. Once again, the poster sessions were excellent and anyone who is a regular reader of this column will know that I'm a big fan. It's a superb way to distil the highlights of new product introductions and the technical programme into manageable chunks so that nothing of significance is missed. It's also a great opportunity to speak with folks that you wouldn't get the chance to in the oral sessions, where there is often not enough time for lengthy discussion.

So, to the new Pittcon review approach that I am calling "Arguments I had at

Pittcon". Obviously these are scientific arguments, not the type I had with a TSA official who, after an hour of queueing, told me that the boarding card I had was "For yesterday and sir you will need to return to your airline desk" — it really isn't all Hurricanes at Pat O'Brien's and Jazz at Generations Hall.

Argument 1: Microfabricated Paper-Based Analytical Device

While reading a poster I hadn't intended to visit titled "Colorimetric pH Paper with a Scale Bar" (Yeongbeom Cho, Hanyang University, Korea), I overheard one passerby mumble to another some derogatory comments regarding a degree of wheel reinvention. The device was a five-layer, wax-paper-based microfluidic device that resembles a mercury thermometer, onto which a sample is spotted and the hydrophilic coating causes the sample to rise past 14 colorimetric indicators. Result — an almost instantaneous measurement of pH (if eight indicators change colour on the scale then the pH is around 8) without having to wait for three pH sensitive pads to change colour and then compare them to the colour scale on the packaging (that you may or may not be able to find), or having to calibrate and use a pH meter. In my opinion, an excellent example of lateral thinking.

Needless to say I had to interject between the derisory comments to inform my fellow Pittcon-eers that they should step out of the woods to see the trees. I asked them to Google "Microfabricated paper-based analytical devices" and see some of the excellent work being carried out at several centres around the world, not least of which includes Richard Crook's group in the Center for Nano- and Molecular Science and Technology at the University of Texas at Austin (USA) and the group from Hanyang University (Korea), who do some wonderful basic research into lab-on-a-chip technology. I also remarked that if one were really to future gaze, there are several paper-based microchannel devices in research that measure critical properties of peptides and proteins and which may, one day, do us out of our jobs. That would be ironic as the reinvented wheel rolls right over us all!

Argument 2: Relative Merits or Demerits of Chiral Core–Shell Particles

I still can't find anyone who produces the common (widely used) phases commercially and I still don't know why.

I heard many arguments why one wouldn't consider this approach during Pittcon week including the following: a) Limited loadability — doesn't really wash with me because it didn't when folks used this argument against the technology at the analytical-scale from the mid-1970s onwards. b) No requirement for faster or higher efficiency chiral separations — give me a break. c) The main commercial market for chiral separations is in preparative columns and who would use a core–shell material for preparative separations? Er, me — I'll take a narrower band (less solvent) with 70% comparative mass loading any day of the week! d) Manufacturers are enjoying the spoils of producing large diameter columns packed with grams of highly expensive materials and wouldn't actually want to reduce this alongside their profits? Requirement to optimize the core-to-shell ratio — well get on with it then!

I've been told the bonding process is very similar whether on core–shell or fully porous substrates, so I'm still struggling to see why I don't see these columns in catalogues. I would use them! Maybe it's just a matter of time or that I'm not looking in the right places, after all there is at least one high profile research group working on the technology that I know of...

Argument 3: Supercritical Fluid Flash Chromatography

Several groups are working on the technology to make this happen and I've read two or three key papers that outline the challenges to making this technology a reality; however, I keep being told that even when perfected, the technique will have limited applicability. Really?

I'm not a synthetic or medicinal chemist, but I've seen plenty of flash purifications in my time and almost without fail these end up with solvent having to be got rid of in some way (to preconcentrate to achieve analytical sensitivity, to recover product). Would it not be a significant step forwards if someone could produce a system that recycles the CO_2 (money-saving technology that already exists at the analytical scale) and elutes the fractions with the minimum amount of polar organic modifier? I know that someone out there will explain why this doesn't make great sense —please get in touch if you are that person at admin@chromatographyonline.com

Argument 4: Accessibility of Simple Statistical Methods for Method Robustness Determination or Estimation of Analytical Error

Again, whilst reading a poster from Leah Buhler of Merck entitled "Utilization of Design of Experiments to Characterize HPLC Method Precision and Robustness in Early Pharmaceutical Development" I

overheard a conversation regarding the statistical validity (actually the statistical power) of the approach which had been taken. The poster demonstrated that with a combination of reasonably simple statistical methods (a simple design of experiment (DoE) and results treatment) allied with good knowledge of the chromatography, one can relatively easily arrive at a single number assessment of the error contribution of the analytical determination to the accuracy of the result when determining, for example, the impurity profile resulting from active pharmaceutical ingredient (API) manufacture.

It was great to see a large pharmaceutical company taking a practical and usable approach to such problems. Believe me, I talk to a large number of folks in laboratories around the world who find quality-by-design (QbD) or statistical methods simply inaccessible, because of the complexity or a lack of experience in the software or methodologies.

I really don't care if methods have enough statistical power to be bullet-proof. What I think we should all care about is the fact that, when combined with sensible decision-making regarding the range and scope of critical variables in the analytical process and the significance of the results when these variables are tested, such approaches can be accessible and usable by more people to improve their methods and save valuable time in the laboratory!

Well done to Leah and coworkers on this very nice approach and boo to the statistical purists — I'm not even sure they were correct about the power of the statistics anyway.

These are just some of the arguments I had last week, I'd love to hear your contributions to these discussions — a virtual Pittcon brainstorm. I didn't even have space to cover the discussions on the need to improve the speed of printing 3D components for laboratory use or the relative merits of sub-1-µm particles for high performance liquid chromatography (HPLC). Maybe another time.

I'm sure you will all be glad to know that my travel home was very uneventful and that for once the flight gods were smiling on me and my seat row was completely empty resulting in a horizontal flight home, and all for the price of an economy ticket. Next year Pittcon will be in Atlanta and will be a direct flight, so I'll have to think up something else to be grumpy about.

Contact author:**Incognito**
E-mail: **admin@chromatographyonline.com**

Photo Credit: Fuse/Getty Images

Back to Nature with Flash Chromatography

Dr Vera Thoss from Bangor University (Bangor, Wales) spoke to Bethany Degg of *The Column* about her work investigating the chemistry of plants and the value of flash chromatography in this area of research.

Q. Your research activities are focused on the investigation of plant secondary metabolites. How did you become interested in this field and why is this area of research important?

A: Plants can't run away so they either need to defend themselves chemically from predators or they need to attract pollinators. This results in some fascinating, mostly organic, compounds that have evolved for specific biological activities. Our work has shown that a herb *Acinos suaveolens* (thyme basil) produces more pulegone, which is a liver toxin, when exposed to grazing by herbivores compared to plants that evolved without herbivory pressure. This is known as the evolutionary arms race.

I have always been interested in ecology because of the abundance of smells and colours in nature in addition to it being a joy just lying in the sun and observing animals. I keep bees and generally we do taste plants when out and about. My background in chemistry is ideal to probe whether specific compounds have ecological roles. In the future this may lead to new medicines, for example bluebells contain iminosugars that are anti-diabetic.

Q. What are your main research interests involving plants?

A: I am working on biorefining high-value chemicals from plants, and we are looking at three species particularly closely: bluebells, bracken, and an African tree *Trichillea emetica*. I am also working closely with industry on quality control and process monitoring as part of the WISE Network. In the WISE Network we support Welsh companies, some of which work on plant chemistry, for example in producing essential oil from lavender or assessing a specific plant-derived metabolite for its use as a nutritional supplement.

I also apply chromatography to investigate the smell of plants, particularly pines and lavender, and isolation work. Plants produce an array of metabolites, often with variation

in the glycosylation pattern. In order to assess this variety, we ideally need to assess every metabolite on its own.

Q. You use flash chromatography in your work. What is flash chromatography and why do you use it in your field?

A: Flash chromatography is an instrumental technique that uses a column with varying amounts of filling material, from 12 g to 1.5 kg. The stationary phase is comparable to those used in high performance liquid chromatography (HPLC) or solid-phase extraction (SPE). The component mixture is applied either in solution or adsorbed onto silica. Elution is achieved via a pumped solvent or solvent mixtures, again similar to HPLC. In flash chromatography the eluent is monitored, our system uses 2 UV–vis wavelengths and an evaporative light scattering detector (ELSD), and when a peak occurs above a set threshold, the peak is automatically collected.

Flash chromatography is relatively cheap and can be a great workhorse in a natural product laboratory. We use flash chromatography to prepare standard materials for quantitative analysis. Working in plant chemistry often requires separating mixtures of very similar components, such as glycosides with the same aglycon, and we need to isolate these compounds to undertake structure elucidation.

In the current climate, the consumer market prefers "natural" materials. They often perceive a synthetic chemical as "bad" and seek a natural alternative. Consequently, working with plant extracts is becoming more common and there is a need to analyze the plant extract for the molecular composition and this is research we undertake in our laboratory.

Q. What are the advantages of flash chromatography versus column chromatography?

A: Traditional column chromatography requires more user attention, does not offer automated peak collection, and is less flexible in relation to stationary phases than flash chromatography. We usually use C18 cartridges in flash, which allows us to go from analytical HPLC to flash. Another important consideration is the detector. A lot of organic compounds are non UV-active, requiring aggressive spray reagents in thin layer chromatography (TLC) to make them visible, for example carbohydrates with 10% H_2SO_4. Having an ELSD detector on the flash systems allows the detection of anything, which is a big advantage. ELSD is also a universal detector and is really handy, particularly as the maintenance requirements are low.

Q. Do you have any advice to offer to analytical chemists considering using flash chromatography for plant extracts?

A: Make your own standards! Flash chromatography allows us to keep the cost lower by producing our own standards, and making isolates for biological screening. Even with flash chromatography the isolation of standards is not trivial and usually requires a number of chromatographic stages. While the thought of being able to inject a crude extract to obtain a chemically pure compound is attractive, usually we need to undertake a number of fractionation steps to achieve this. A good tip is to match your analytical column with the flash system, which means running the same stationary phase to allow for direct translation between the two systems.

Q. You have previously published work on the analysis of British bluebell (*Hyacinthoides non-scripta*) seed oil. What was the motivation behind this study? Can you discuss the analytical methods that you used and what were your main findings?

A: The work on bluebells is driven by a conservation interest of mine. If you have been involved in organizing conservation work, such as bracken clearing, there are generally costs involved even when working with volunteers and there is no financial incentive. I was hoping to find metabolites in bluebells that could be of commercial interest and hence potentially pay for the conservation effort.

Our work on the seed oil composition showed that bluebells are an untapped resource in Britain because they do not grow anywhere else in the densities observed in the UK. This is purely a result of the mild winter climate because bluebells grow mostly during the winter with flowering taking place in spring. It is not right that we import palm or soya oil if we have potential sources in the UK. For me this is a sustainability issue and worth pursuing further.

To analyze the oil, we undertook a method comparison using different stationary phases for gas chromatography coupled with mass spectrometry (GC–MS) of fatty acid methyl esters (FAMEs). We also used high temperature GC for the triglycerides. One of our foci was the location of the double bond in the unsaturated fatty acids; hence we included ozonolysis to assess this, in addition to nuclear magnetic resonance (NMR). So it was quite an analytical paper. As far as the results go, oils do not vary in composition from year to year.

Q. Is there anything that you would like to add?

A: Natural products chemistry is an old branch of chemistry, after all scientists have worked on this for centuries. New developments, like flash chromatography, aid in our research. In addition, we seem to have passed the high use of petroleum and are shifting back to renewable resource use. It is surprising that in the UK nearly half of native plants traditionally used for medicines are still in need of chemical exploration. I think the future for natural products chemistry, for which chromatography is essential, is bright.

Vera Thoss is a lecturer in chemistry and project manages, on behalf of the School of Chemistry, the ERDF funded pan-Wales projects WISE Network (www.wisenetwork.org) and BEACON (www.beaconwales.org). Vera's scientific interests are in natural products chemistry, chemical analysis and chemical ecology. She has a thorough understanding of analyzing materials, whether it is for elemental composition or targeting distinct compounds. With a remit for industrial liaison, Vera frequently interacts with commercial partners to identify their challenges and finds analytical approaches that yield relevant information. This is supported by access to state-of-the-art facilities either in-house or collaborating with other partners. Vera also has a strong interest in sustainability, particularly with respect to utilizing underexplored native plants, particularly bluebells.

E-mail: **admin@chromatographyonline.com**
Website: **www.bangor.ac.uk**

Nanoparticle Toxicity: Responding to Analytical and Regulatory Challenges with Field-Flow Fractionation

Christoph Johann, Wyatt Technology Europe GmbH, Dernbach, Germany.

This article illustrates how the fate and modification of nanoparticles in real life matrices can be investigated by the combination of field-flow fractionation (FFF) with inductively coupled plasma mass spectrometry (ICP–MS).

Photo Credit: bgblue/Getty Images

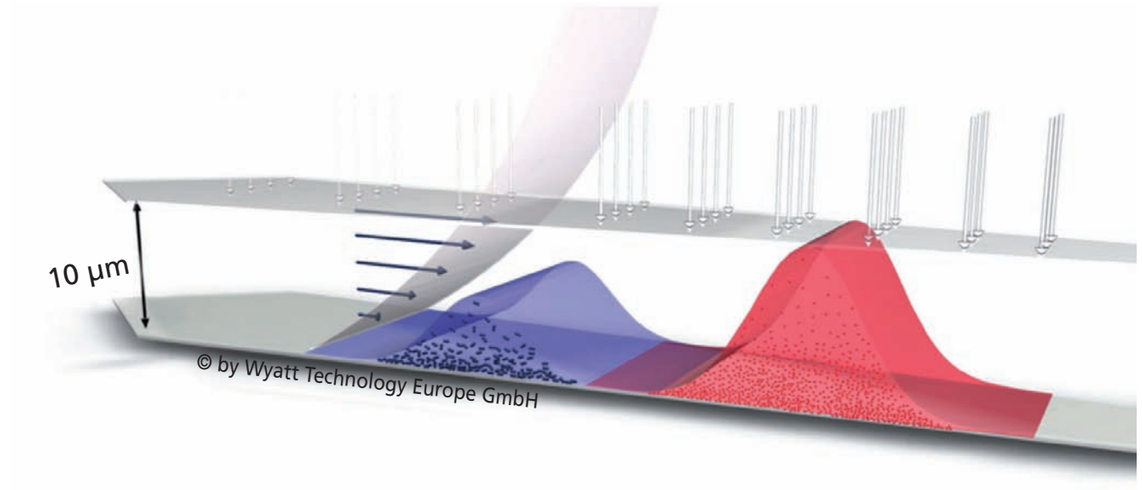
Engineered nanoparticles (ENPs) play an important role in a range of industrial and commercial products; however, there are concerns over their potential toxicity. The regulations governing the use of ENPs are not fully defined or harmonized — current guidance by the US Environmental Protection Agency (US EPA) requires "*manufacturers of new (nanoparticle) chemical substances to provide specific information to the Agency for review prior to manufacturing chemicals or introducing them into commerce*".[1] This requires a comprehensive approach to analysis, particularly with regards to factors that contribute to the rate of diffusion or uptake by an organism such as particle size, size distribution, and concentration.

Analysis of nanoparticle suspensions presents a unique analytical challenge, because NPs can be sensitive to even slight variations in media and can readily change size by dissolution or aggregation. NP suspensions therefore have to be analyzed in matrices that are as close as possible to expected environmental conditions (surface water, sea water, or sewage sludge), and at realistic concentrations (low ppb). Field-flow fractionation (FFF) is a powerful separation method for proteins and colloidal particles that (when combined with multiple particle-sizing techniques) can provide high resolution and high dynamic range in terms of size, which is not possible with column-based chromatography.

An Introduction to Field-Flow Fractionation (FFF)

FFF separation takes place inside a thin-fluid layer within a flat channel or hollow fibre. A liquid force-field within the channel causes

Figure 1: A laminar flow within the fractionation column separates particles into discrete size fractions without inducing particle shearing or column interaction.



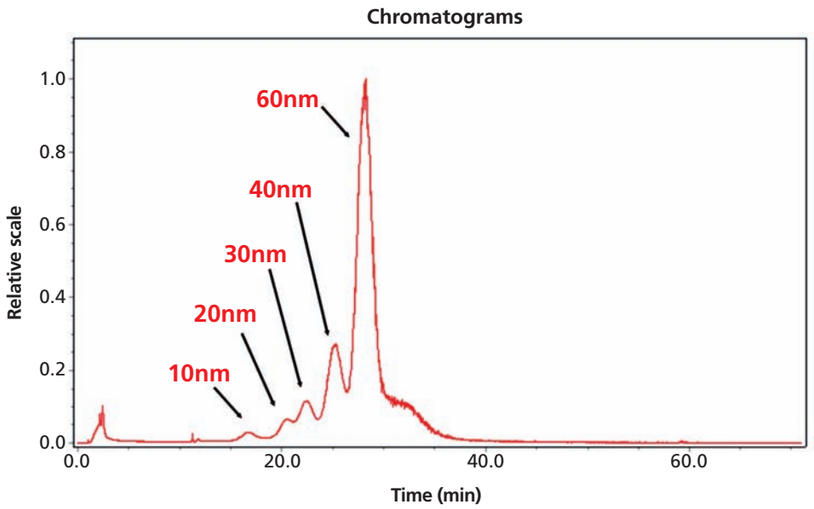
the sample to concentrate along the bottom wall, which is lined with a semi-permeable ultrafiltration membrane. Particles diffuse back into the channel because of Brownian motion, with smaller particles travelling higher than larger ones. These are collected by a laminar flow that separates particles according to size (see Figure 1). As elution progresses, the sample components separate further to form either discrete or more broadly dispersed peaks, with smaller particles emerging before larger ones. FFF is a remarkably simple technique that delivers several advantages including:

- Ability to use a range of complex sample media.
- Wide separation range from one nanometre to several micrometre.
- Minimal shearing.
- Minimal column surface interaction.

The Stokes radius of the particles may be estimated from the FFF retention equation, but it is only through combination with particle characterization that the full analytical productivity of FFF is truly achieved.

Light scattering is widely considered the gold standard method for particle size analysis and most modern FFF instruments are compatible with either dynamic light-scattering (DLS) or multi-angle light

Figure 2: Fractionation and characterization of gold nanoparticles in aqueous solution with FFF and DLS.



scattering (MALS) systems. Figure 2 shows a chromatogram for a polyethylene glycol suspension of gold nanoparticles (GNP). Individual GNPs, nominally 10 nm, 20 nm, 30 nm, 40 nm, and 60 nm, were fractionated with FFF. Each fraction is successfully separated and clearly resolved.[2] However, the reliability of light scattering is limited at ultra-low concentrations and often requires validation with an additional particle sizing technique. An alternative method is to combine FFF with inductively coupled plasma mass spectrometry (ICP–MS).

The following study shows how FFF linked to ICP–MS can be performed to monitor changes in particle size distribution and recovery caused by variation in ionic strength of the carrier solution. Single-particle ICP–MS (sp-ICP–MS) is applied over a complete FFF separation run, demonstrating it to be an efficient tool to obtain size information where other methods (such as light scattering) cannot be applied.

Case Study: Characterization of Surface-Modified Gold Nanoparticles (GNPs)

Sample Preparation: Two GNP samples with diameters of 15 nm (INM) and 60 nm (NIST 8013) were citrate-reduced in aqueous

Figure 3: Fractogram of two nanoparticles with 15 nm and 60 nm diameter.

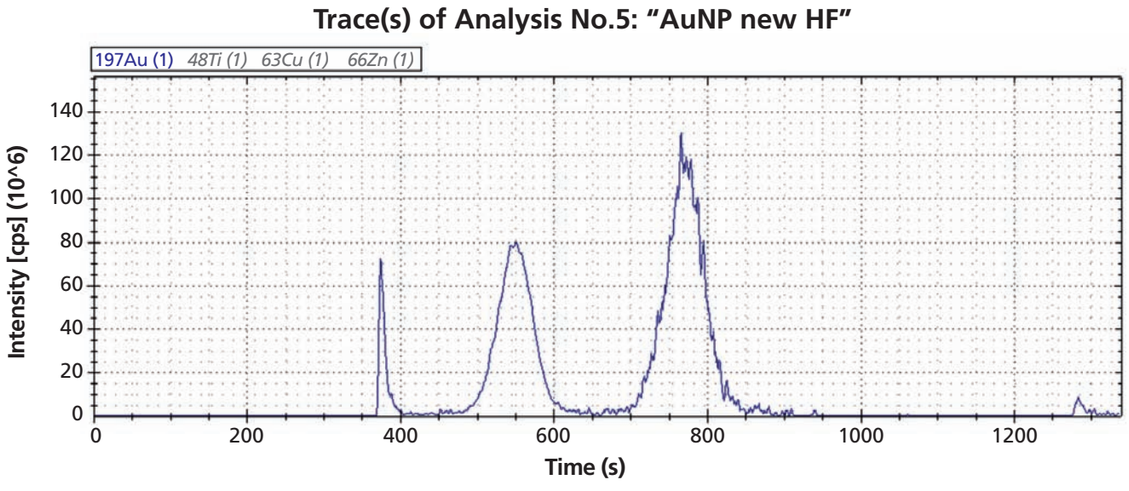
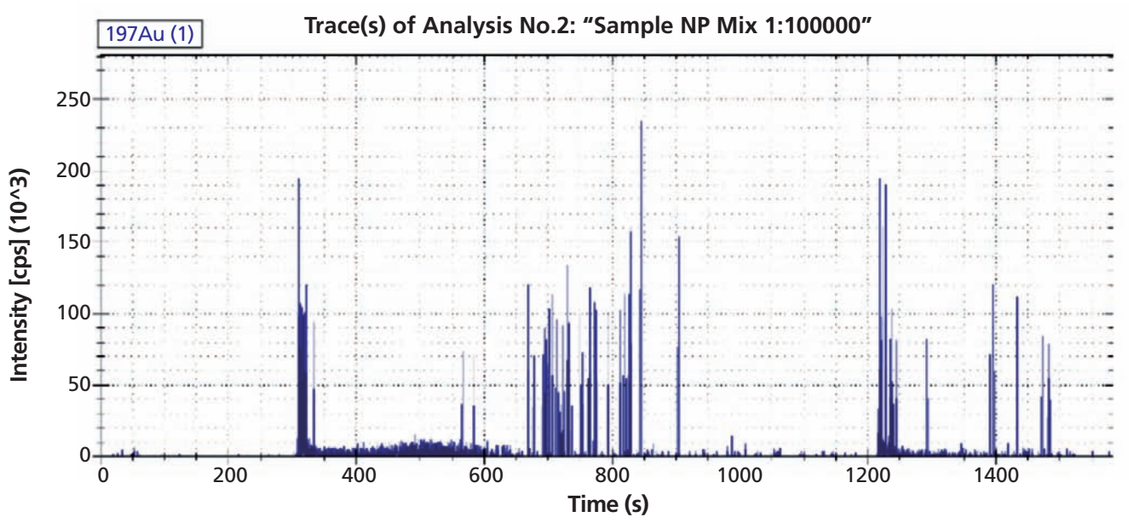


Figure 4: sp-ICP–MS data upon separation of the two GNPs in 0.02% SDS.



solution (0.01 % $HAuCl_4$). The 15-nm particle solutions were then modified with (11-mercaptoundecyl)-tetra(ethylene glycol), bonded covalently by the thiol group to the particle surface. Three different carrier solutions for the FFF separation were prepared: 0.02% sodium-dodecylsulphate (SDS), and the same SDS with the addition of 0.1% and 0.5% NaCl.

Instrument Set-Up: The data presented in this study were obtained using an Eclipse DUALTEC Flow FFF (Wyatt Technology Corp.) equipped with a hollow-fibre channel (HF5). The mobile phase was delivered using an ICS-5000 quaternary, metal-free HPLC system (Thermo Scientific) and injections were performed using a DS-DP ICS-5000 autosampler (Thermo Scientific). The FFF unit was coupled to an iCAP Qc ICP–MS system (Thermo Scientific). A PFA-ST nebulizer (Thermo Scientific) was used for all ICP–MS and sp-ICP–MS determinations. The Eclipse chassis splits the flow appropriately with a series of specially configured valves and creates the inject flow and cross-flow using needle valves and flowmeters. Separation was achieved on a single hollow fibre with 800 µm internal diameter (polyethersulphone, 10 kDa cutoff) mounted in a cartridge, which creates a cylindrical channel. This disposable unit has a 90-µL volume resulting in low sample dilution. Flow conditions were selected to achieve baseline resolution for the two peaks. Dwell time was 100 ms for ICP–MS and 0.005 ms for sp-ICP–MS. For the sp-ICP–MS mode, the injected sample was diluted by a factor of approximately 1000.

Qtegra Intelligent Scientific Data System software (Thermo Scientific) was used throughout for iCAP Q control and data acquisition. Chromeleon software (Thermo Scientific) with the Eclipse plug-in was used to drive the Wyatt Eclipse and ICS-5000 in a single method. ISIS software (Wyatt Technology) was used to calculate the theoretical retention time of the particles under different flow conditions to optimize the FFF separation conditions.

Results: Figure 3 shows the fractogram for GNP in 0.02% SDS. Four distinct peaks can be observed in the ICP–MS signal, which correspond to the following:

- The void peak with a retention time corresponding to the channel volume at 320 s.
- Two successive peaks corresponding to the 15-nm and the 60-nm particles.
- A fourth peak eluting after activation of the flow through the injector at the end of the run at 1200 s.

ISIS software calculated retention times as 220 s and 510 s for 15 nm and 60 nm

particles, respectively, after the void peak. This compares to 190 s and 420 s for the experimental values. The 60 nm particle elutes earlier as expected, which can be explained by the contribution of electrostatic repulsion between the particle and the membrane. Within the void, peak particle size is unpredictable with FFF alone because it may consist of oversized particles from steric elution as well as small particles.

The effect of changing conditions was monitored to establish the impact of variations in ionic strength on GNP stability. NaCl was added to the carrier solution to produce 0.1% and 0.5% concentrations, and the modified solutions analyzed using FFF.

The recovery of both particles drops by approximately 50% with the addition of 0.1% NaCl. Increasing NaCl concentration to 0.5% further reduces recovery by 50% for the 15-nm particle and results in almost complete loss of the 60-nm signal. sp-ICP–MS was then used to better understand how particle size changes with increased ionic strength. The sample load was reduced by a factor of 100 to reach a dilution high enough to detect single particle events. The results from sp-ICP–MS analysis can be seen in Figure 4.

The height of the spikes provides an indication of the corresponding particle size. Assuming a spherical shape, the spikes from 15-nm particles are expected to be lower than those of the 60-nm particles, which are around 100 k counts. Significant further dilution would be required to resolve single 15-nm particles and signal-to-noise would probably not be sufficient for reliable detection. These results indicate that 60-nm particles are present in the void peak alongside oversized aggregates.

The results following addition of 0.5% NaCl show that the recovery of 60-nm particles is so low that the sample does not need to be diluted, while 15-nm particles are still too concentrated to give single particle events. Spikes of 100 k counts can still be found in the elution time range of 600 to 800 s, which correspond to the small fraction of particles which were not aggregated. Interestingly, there are larger spikes in the elution and injection peaks compared to the void peak. Flow-injection into higher ionic strength conditions leads to aggregation, but not to adsorption, because the particles are not concentrated at the membrane.

The results indicate that sample loss in higher ionic strength solutions is caused by formation of aggregates and then adsorption of those aggregates on the membrane or other surfaces. This phenomenon is independent of the different surface coating of the two Au particles. Retention time of the 60 nm GNPs is considerably shorter than predicted by theory, indicating non-ideal effects not influenced by the ionic strength. Therefore, sp-ICP–MS is a useful tool to provide additional size information where it cannot be obtained from FFF retention time, and it can be used to detect aggregates in the void as well as the release peaks.

Summary

When used with other detection techniques such as MALS, DLS, and ICP–MS, FFF allows comprehensive characterization of nanoparticles. The demand for a reliable technique for nanoparticle separation has led to recognition of the capabilities of FFF and the potential of sp-ICP–MS to quantify and validate separation results at trace levels. However, the detection limit of ICP–MS depends heavily on size and varies dramatically depending on the metal of interest. In unfavourable cases where the detection limit is above the critical size of 100 nm it is often challenging to find a suitable dilution range to enable detection of a broader size range. This highlights the importance of using complementary analysis with direct sizing detectors, such as light scattering instruments, during ENP characterization.

References

1. U.S. Environmental Protection Agency, *Control of Nanoscale Materials under the Toxic Substances Control Act,* http://www.epa.gov/oppt/nano/ (Last accessed: 26/03/2015)
2. Tae Joon Cho and Vincent A.Hacley, *Annual Bioanal Chem* **398**(5), 2003–18 (2010).

Christoph Johann is managing director and founder of Wyatt Technology Europe (WTE) GmbH. Dr. Johann has been active in polymer and biopolymer analysis for over 30 years and has several peer-reviewed publications in the field of macromolecular characterization and field-flow fractionation (FFF). He earned his PhD in 1985 in physical chemistry at the University of Mainz. In the same year he was the co-founder of PSS Polymer Standards Service GmbH, a start-up company that offered model polymers for research purposes and developed SEC columns. In 1993 he left PSS and founded a second company, Wyatt Technology Deutschland, now called Wyatt Technology Europe.

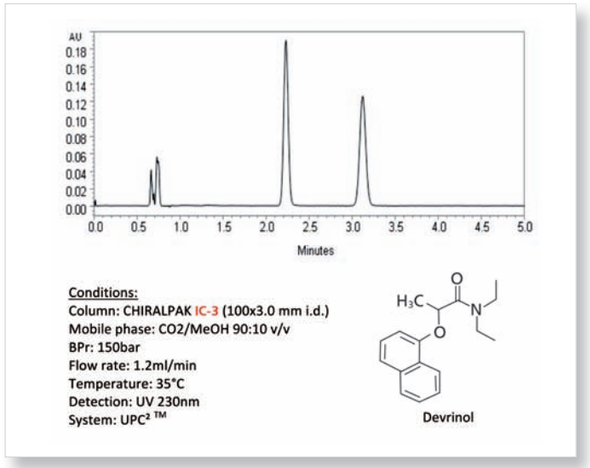
E-mail: **christoph.johann@wyatt.eu**
Website: **www.wyatt.com**

Pittcon Product Profiles

Selected highlights of products presented at Pittcon 2015.

SFC Chiral Columns

SFC chiral columns from Chiral Technologies feature Daicel 3 µm immobilized and coated chiral stationary phases (CSPs) packed into a 3-mm internal diameter column. According to the company, the columns ensure a high success rate in supercritical fluid chromatography (SFC) method development for enantiomer separations by combining fast analysis time and high resolution, while also matching the needs of SFC instruments. These properties can be attributed to:



- High-enantiorecognition ability of the CSPs
- Fast mass transfer kinetics
- Favourable van Deemter velocity profile
- Column packing stability and the selection of column diameter

The columns are available in: 100 × 3.0 mm, 150 × 3.0 mm, and 50 × 3.0 mm dimensions, and are suitable for most demanding SFC separations.

www.chiraltech.com

Nitrogen Solutions for Laboratories

Engineered around PSA technology, i-FlowLab from Peak Scientific is available in various pre-configured specifications to suit specific flow and purity demands. According to the company, a single i-FlowLab generator installation can provide nitrogen at flow rates ranging from 40 LPM @ 99.999% to 3402 LPM @ 95%. Purities or residual oxygen are specified at time of system design to meet the needs of the applications and range from 98–99.999%.

Thanks to the expandable design, additional CMS column banks can be added to each generator after installation to increase maximum flow rate.

According to the company, the streamlined, compact footprint design of the system allows for an efficient use of available space.

Applications include:

- Providing high-purity nitrogen for multiple LC–MS instruments
- High-flow nitrogen supply for sample evaporators
- Other laboratory instruments requiring nitrogen — eg. FT-IR, TOC, NMR, and more
- Meets instrumentation manufacturers specifications (IQ/OQ certifications available on request)

www.peakscientific.com

Improving Productivity in Your Lab

Trajan Scientific and Medical unveiled new SGE Analytical Science products at Pittcon 2015 to make things easier in the laboratory, according to the company.

These include:

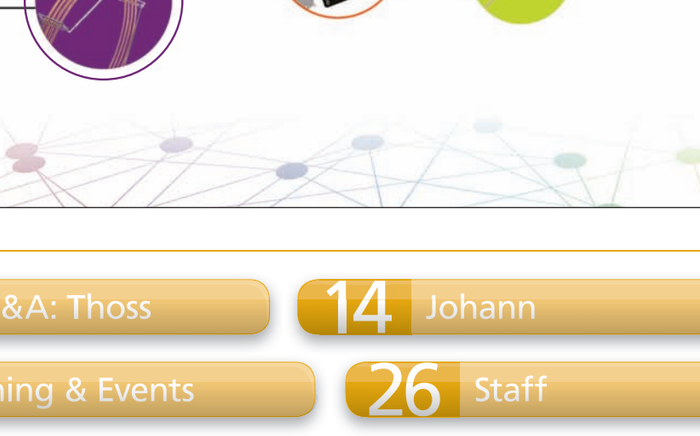
MyCapLC™ Kit for Life Sciences:
Customize CapLC for Your Application
• DIY capillary column packing, no special instrumentation required.

PEEKTite™ Ti-Lok EXP UHPLC Fittings:
Uncomplicated and Reliable UHPLC Connections
• Finger-tight nut and ferrule system for PEEKsil® tubing up to 18,000 psi.

ProteCol® BioInert UHPLC Column Hardware:
Smooth and Inert Glass Lining for Optimal UHPLC Bio Applications
• Improved performance for bio applications with your phase at 20,000 psi.

5-mL Diamond Headspace Syringes:
Higher Limits in Headspace Analysis
• A gas-tight seal providing low carryover and performance up to 150 °C.

www.sge.com



Versatile (U)HPLC Columns

YMC has introduced YMC-Triart for versatility, robustness, and scalability from ultrahigh-performance liquid chromatography (UHPLC) to prep, according to the company. The latest YMC column family is based on a "hybrid-style" support with a wide range of stability towards pH, temperature, and even 100% aqueous eluents. High versatility is achieved through 6 different modifications: C18, C18 ExRS, C8, Phenyl, PFP, and Diol-HILIC. YMC-Triart is available in particle sizes from 1.9–20 µm, and is reportedly scalable from UHPLC to analytical to process scale! Preparative bulk material (10-, 15-, and 20-µm) is available and can be supplied in large quantities with a short lead time. Request a brochure or a test column now!

http://ymc.de/ymc-triart.html

Comprehensive Solutions

Zoex Europe is a solution provider in the field of multidimensional comprehensive chromatography! Samples with very complex matrices can be run with GC×GC or LC×LC for maximum separation and identification. Zoex supplies thermal modulators, with or without liquefied nitrogen. Thermal modulation gives the best focus and also a substantial gain in sensitivity. According to the company, thermal modulation also provides the best separation in the second dimension. This modulation can be used in combination with all conventional detectors, but will give the best results with mass spectrometry. As a partner of Agilent Technologies, Zoex provides solutions with the High End QTOF, a quadrupole TOF with high mass accuracy and high mass resolution (HRMS). Zoex also supplies capillary flow technology (CFT) modulators, without the need for liquid nitrogen.

- Thermal modulation with LN2: C2+ modulation, very sensitive, combines with QTOF HRMS systems, complete separation and compound elucidation.
- Thermal modulation without LN2: C6+ modulation, same specifications as with LN2.
- Flow modulation: C2+ modulation, use conventional detectors, less sensitive because of (high) flow dilution, group type separations.

www.zoex.eu

LC GC's **CHROMacademy**
powered by crawford scientific

HPLC GC MS SPE IR

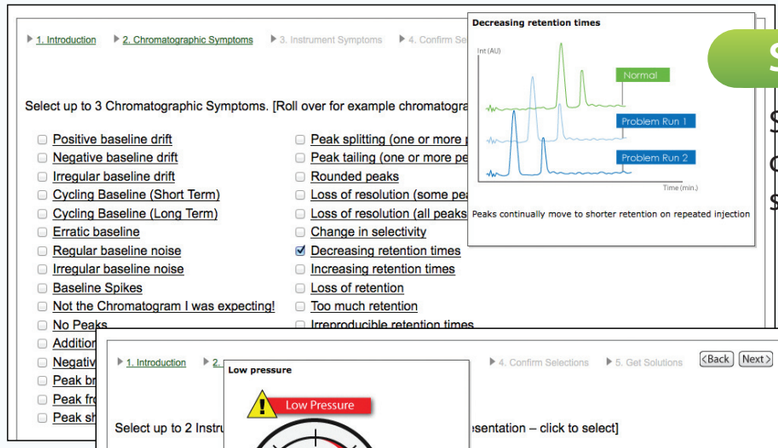
Try our Interactive HPLC Troubleshooter

Get answers fast, reduce downtime and improve efficiency

We developed the CHROMacademy Troubleshooters with busy chromatographers in mind.
In 3 simple steps we can help you overcome your instrument, separation, and quantitation issues.

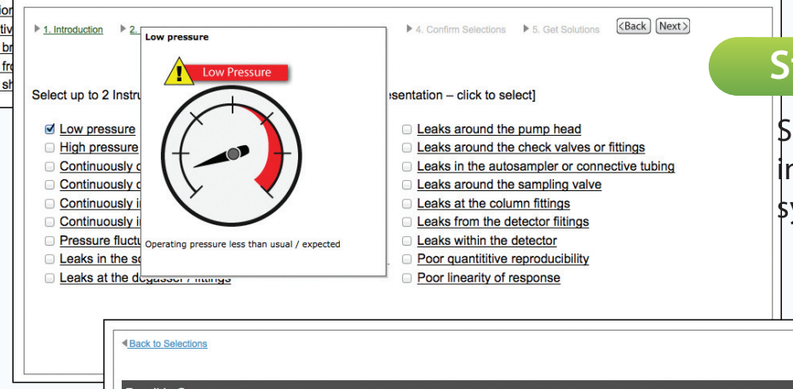
1. Select your chromatographic symptoms.
2. Select your instrument symptoms.
3. The troubleshooter returns a list of possible causes. Each cause has a concise summary of the problem and recommended solutions.
   These solutions are supported by over 1000 references, feature articles, and CHROMacademy content written by our experts.

HPLC Troubleshooter



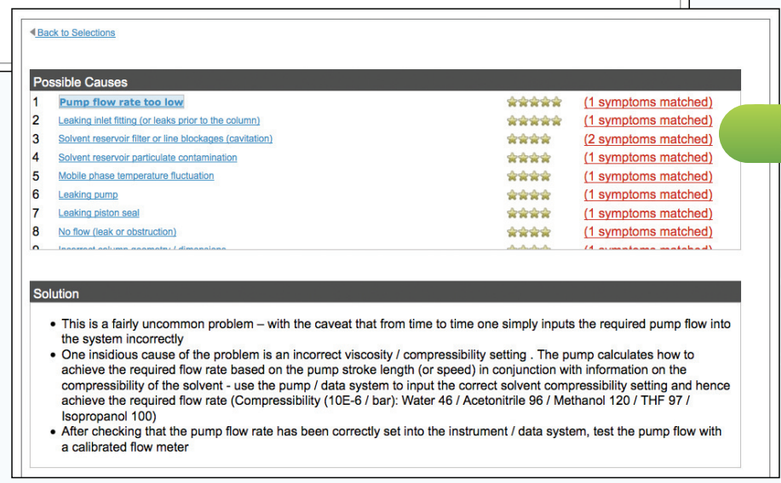
Step 1

Select your chromatographic symptoms.



Step 2

Select your instrument symptoms.



Step 3

See a list of probable causes and the recommended solutions.

Still got a problem? Ask our experts.

If you have a specific enquiry, or just need more information, one of our technical experts will contact you within 24 hours and will work with you until your problem has been resolved.
"Ask the Expert" is available only to Premier Members.

For CHROMacademy Premier membership:
Glen Murry on +1 732 - 346 - 3056 | e-mail: gmurry@advanstar.com

The CHROMacademy troubleshooters are an on-line interactive version of the much-loved troubleshooting poster you see on lab walls all over the world.
Our troubleshooters are available to Lite and Premier members and are used globally as a resource for analytical scientists who wish to more accurately diagnose issues with equipment and separations.

For more on CHROMacademy Premier membership contact:
Glen Murry on +1 732 - 346 - 3056 | e-mail: gmurry@advanstar.com

Training Courses

GC

Hands-on GC–MS Theory and Methods
3 June 2015
The Open University, Milton Keynes, UK
Website: http://anthias.co.uk/training-courses/handson-GC-MS-theory-methods

Hands-on GC & GC–MS Troubleshooting
5 June 2015
The Open University, Milton Keynes, UK
Website: http://anthias.co.uk/training-courses/handson-GC-GCMS-troubleshooting

Practical GC–MS
9 June 2015
Caledonian University, Glasgow, UK
Website: http://www.crawfordscientific.com/training-online-calendar.asp

Gas Chromatography: Fundamentals, Troubleshooting, and Method Development
8–11 September 2015
Axion Analytical Laboratories, Chicago, Illinois, USA
Website: http://proed.acs.org/course-catalog/courses/gas-chromatography-fundamentals-troubleshooting-and-method-development/

HPLC/LC–MS

HPLC Method Development
13 May 2015
Manchester, UK
Website: www.hichrom.co.uk

Practical HPLC Method Development
19 May 2015
Strathclyde University, Glasgow, UK
Website: http://www.crawfordscientific.com/training-online-calendar.asp

Exploiting LC Selectivity & Exploring Eluent pH (2 Short Seminars)
20 May 2015
Birmingham, UK
Website: www.hichrom.co.uk

How to Develop Validated HPLC Methods: Rational Design with Practical Statistics and Troubleshooting
14–15 October 2015
MicroTek, Edison, New Jersey, USA
Website: http://proed.acs.org/course-catalog/courses/essentials-of-modern-hplc-uhplc-1-fundamentals-and-applications-online-short-course/

The Theory of HPLC
On-line training from CHROMacademy
Website: http://www.chromacademy.com/hplc-training.html

Fundamental LC–MS
On-line training from CHROMacademy
Website: http://www.chromacademy.com/mass-spec-training.html

HPLC Troubleshooter
On-line training from CHROMacademy
Website: http://www.chromacademy.com/hplc_troubleshooting.html

METHOD VALIDATION

Validation and Transfer of Methods for Pharmaceutical Analysis
27–29 May 2015
Metro Hotel Dublin Airport, Dublin, Ireland
Website: http://www.mournetrainingservices.co.uk/course_list.html#vampa

SAMPLE PREPARATION

Solid-Phase Extraction
On-line training from CHROMacademy
Website: http://www.chromacademy.com/sample-prep-training.html

MISCELLANEOUS

Light Scattering Training
18–19 June 2015
Santa Barbara, California, USA
Website: http://www.wyatt.com/training/training/light-scattering-training.html

Light Scattering and Viscometry Hands-On Training
29–30 June 2015
Mainz, Germany
Website: www.pss-polymer.com

Please send your event and training course information to Kate Mosford kmosford@advanstar.com

Event News

21–25 June 2015

42nd International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2015)
International Conference Centre, Geneva, Switzerland
Tel: +41 22 839 84 84
E-mail: chairman@hplc2015.org
Website: www.hplc2015-Geneva.org

---

30 June–3 July 2015

21st International Symposium on Separation Sciences (ISSS 2015)
Grand Hotel Union, Ljubljana, Slovenia
Tel: +386 1 477 0265
E-mail: info@isss2015.si
Website: www.isss2015.si

23–28 August 2015

35th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2015)
Hotel Maksoud Plaza, Sao Paulo, Brazil
Tel: +55 11 3056 6000
E-mail: dioxin2015@acquacon.com.br
Website: www.dioxin2015.org

3–6 November 2015

Recent Advances in Food Analysis (RAFA)
Clarion Congress Hotel, Prague, Czech Republic
Tel: +386 1 4760 265
E-mail: rafa2015@vscht.cz.
Website: www.rafa2015.eu

Contact Information

Europe

Group Publisher
Michael J. Tessalone
mtessalone@advanstar.com

Sales Manager
Gareth Pickering
gpickering@advanstar.com

Sales Executive
Liz Mclean
emclean@advanstar.com

Sales Operations Executive
Sarah Darcy
sdarcy@advanstar.com

Editor-in-Chief
Alasdair Matheson
amatheson@advanstar.com

Managing Editor
Kate Mosford
kmosford@advanstar.com

Associate Editor
Bethany Degg
bdegg@advanstar.com

UBM Life Sciences
Honeycomb West,
Chester Business Park,
Wrexham Road,
Chester, CH4 9QH, UK
Tel: +44 (0) 1244 629 300
Fax: +44 (0) 1244 678 00

North America

Group Publisher
Michael J. Tessalone
mtessalone@advanstar.com

Associate Publisher
Edward Fantuzzi
efantuzzi@advanstar.com

East Coast Sales Manager
Stephanie Shaffer
sshaffer@advanstar.com

Account Executive
Lizzy Thomas
ethomas@advanstar.com

Editorial Director, Analytical Sciences
Laura Bush
lbush@advanstar.com

Group Technical Editor
Stephen A. Brown
sbrown@advanstar.com

Managing Editor
Megan L'Heureux
mlheureux@advanstar.com

Administation and Sales Offices
Woodbridge Corporate Plaza,
485 Route 1 South,
Building F, First floor, Iselin,
NJ 08830, USA
Tel: +1 732 596 0276
Fax: +1 732 225 0211

Corporate Office,
641 Lexington Ave., 8th Floor,
New York, NY 10022-4503, USA

Mission Statement
The Column (ISSN 2050-280X) is the analytical chemist's companion within the dynamic world of chromatography. Interactive and accessible, it provides a broad understanding of technical applications and products while engaging, stimulating and challenging the global community with thought-provoking commentary that connects its members to each other and the industries they serve.

Custom Reprints: Contact Brian Kolb at Wright's Media, 2407 Timberloch Place, The Woodlands, TX 77380. Telephone: 877-652-5295 ext. 121. Email: bkolb@wrightsmedia.com.