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The Column

the interactive e-publication for the global separation science industry

Diamond Jubilee

Celebrating 60 years of
The Chromatographic Society

Cover Story

Features

Regulars

UBM

Past, Present, Future

To mark the Chromatographic Society's 60th birthday, *The Column* interviewed the current President of The Chromatographic Society, Dr. Paul Ferguson, and Associate Principal Scientist for Separation Science at AstraZeneca, Macclesfield, UK, on the past, present, and future of the Chromatographic Society — and separation science.

— Interview by Lewis Botcherby

Photo Credit: Royden Jurjansz/Getty Images

Q. When and why did the Chromatographic Society begin?

A: The Chromatographic Society's evolution almost matches the development of instrumental separation science itself. Indeed it was the "buzz" created by the burgeoning influence of gas chromatography (GC) in the petroleum industry that led to the formation of the Chromatographic Society or, as it was called in its first manifestation, the Gas Chromatography Discussion Group. The Society was inspired by an international meeting of gas chromatographers held in 1955 at Ardeer in Scotland; it was formed in 1956 and later that year collaborated with groups from France and Germany to stage the first International Symposium on Chromatography (which also therefore celebrates its 60th anniversary) in London. The ISC conference has actually been running longer than the excellent HPLC conference series.

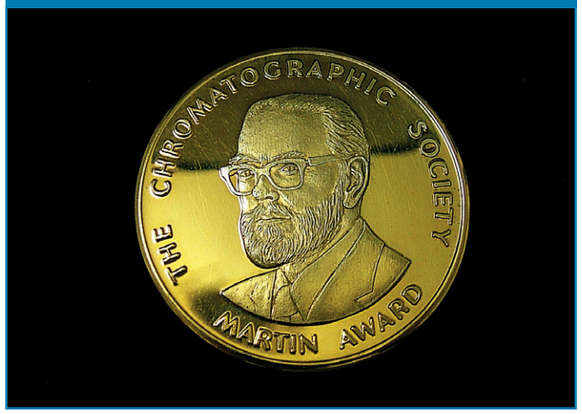
Q. Looking back on the 60-year history of the organization for which you now hold the principal leadership role, what is your own personal view of the impact The Chromatographic Society has had over these years?

A: In the UK the Society has continued to provide topical meetings that reflect the current — and sometimes future — state-of-the art developments in separation science such as the "Advances in Bio-Separations" meeting we held at MedImmune in 2015. Through this we hope to support and develop separation scientists in the UK. Our meetings are also excellent opportunities for networking. I like to think the Society is the "glue" that holds an increasingly disparate and evolving separation science community together and it's great that we are able to showcase some of the best academic and industrial separation science in the UK, which could go unseen if it wasn't for our meetings.

Q. What were the major successes?

A: We believe we are one of the oldest societies dedicated to supporting separation science in the world and the fact we are still around helping to shape the separation science community in the UK is important in itself! Without doubt our successful meeting programme continues to be a key strength for the society and we continue to follow the latest trends and propagate them within

The Chromatographic Society's highest award — The A.J.P "Martin" Medal — conferred annually to scientists who have demonstrated outstanding contributions to separation science.



the separation science community. I think this is reflected by the high-quality speakers we are always able to attract from Europe and further afield to present at our meetings and the positive feedback we receive from attendees to our meetings.

Back in the 1970s and 80s, the Society was at the forefront of separation science teaching in the UK, holding high performance liquid chromatography (HPLC) training courses at the University of Surrey (organized by one of our past Presidents, Derek Stevenson) and also helping to steer approaches for the analysis of chiral compounds when that became a major concern for pharmaceutical companies.

The Chromatographic Society plays an important role on the International Symposium for Chromatography (ISC) organizing committee. Here the then President Chris Bevan awards the Jubilee medal to Professor Ernie Dawes at ISC 2004 in Paris, France.

I also feel we have continued to do our own modest bit to support the academic development of separation science in the UK through our various travel grants and research bursaries.

Q. Has the nature and role of the Society changed over the years?

A: I don't believe fundamentally the Society has changed over the last 60 years. At our core we are still dedicated to supporting the development of chromatography and chromatographers with a principal activity of organizing meetings addressing the latest developments in the field. We still

Past ChromSoc Presidents Ted Adlard and Derek Stevenson chatting with Professors Peter Myers and Dave Perret at a ChromSoc meeting at Shimadzu, Milton Keynes, UK, in May 2005.

have an international reach but hopefully we have moved with the times through the development of our website, the use of social media channels such as LinkedIn, and through our publishing activities like our members-only publication *ChromCom*. In terms of our membership base, and while we are actively growing this, it has never been large even historically, and I see the principal activity of the Society as a meeting society where we have a strong track record.

Q. Does the Pye 104 club still exist and has its collection developed?

A: Ha! The Pye 104 club unfortunately doesn't really exist within the Society any more. I suspect you need to be of a certain age to even know what a Pye 104 is (a GC instrument prevalent some 30 years ago)! One of our previous Presidents, Prof. Ian Wilson, started this group many years ago and still even has one of these instruments in his basement of his home along with many other historical analytical instruments. This year we will be reflecting on the history of some of Ian's collection through various publications and on our website. While the Pye 104 club may not exist, gas chromatography is still a key focus for the Society and is reflected through our biennial "Advances in GC" meeting and our support for academic research using GC.

Our first meeting of 2016 in March revisited the heritage of our first ever meeting location (the Institute of Engineering and Technology in London) and a strong component of this meeting focused on GC with contributions from Prof. Pat Sandra of the University of Ghent and Tom Lynch of BP, alongside other star performers from the world of separation science like Prof. Peter Schoenmakers and Prof. David McCalley.

As a direct result of the excellent speaker programme assembled by Alan Handley (our past President), this was a very popular event, which has received very positive feedback regarding the quality and

relevance of the presentations, the venue, and logistics. As ever, the event also provided a great opportunity for networking, with delegates from industry and academia, the vendors, and of course the speakers.

Q. What were the challenges you faced as President in steering the Society through these final few years towards the 60th anniversary?

A: As I mentioned earlier, the Society's key avenue of interaction with UK-based separation science is through our meeting programme where we face a number of interrelated challenges. We are only able to fund these meetings (and our support of academic researchers) through the strong support of exhibitor organizations, and we are aware that we must maximize their investment through interaction with our attendees. Alongside this, it is becoming increasingly difficult to attract scientists to our meetings (a topic other learned society's are also having issues addressing), which may be a result of a combination of meeting location, scientists having to focus solely on their day jobs and not having the time for external personal development activities, or maybe we need to consider more closely the topics for our meetings.

It should be noted that we are a charitable organization and all our meetings are organized by our committee in addition to their day jobs. Over the last couple of years, we have observed a significant increase from other organizations external to the UK organizing meetings with an increasing focus on separation science and related topic meetings. This obviously provides significant competition to our meetings so we want to highlight the value of our meetings to potential attendees. The ChromSoc conferences only cost around £250 for a two-day meeting, which is incredibly cheap, and we need to publicize our meetings much more widely, emphasizing the networking opportunities and the opportunities to speak with key separation scientists in academia and industry. In 2016 we are focusing more effort on this and will be more active on LinkedIn. We have also set up a Twitter account (@chromsoc) to reach the various groups involved with separation science through that medium.

The final challenge we face is that our experience base is very strong in the pharmaceutical industry, but chromatography is increasingly being used by many industries such as the food, agrochemical, and environmental sectors. Going forwards we need to focus more attention on these sectors because there are key challenges in these areas where I think

we can bring key opinion leaders together to move their separation science forward.

Q. So, what are the Society's current strengths, weaknesses, opportunities, and threats, and how does your Executive plan deal with them?

A: This could be a long question to respond to! I think the Society has a number of strengths, not least the dedication of our committee. We have a number of key meetings, which are either firmly established, for example, the Reid Bioforum and our "Advances in GC" meeting, or are establishing themselves, such as the "Advances in Clinical Analysis", which we co-organize with the RSC's separation sciences group (RSC SSG).

I think our support base is very strong and we work with all the key scientists and companies when organizing our meetings. I also think we are well connected with academic groups in the UK, including institutions that are not necessarily recognized for separation science, which we often support through our summer studentships programme. Finally, I think we have very strong relationships with other learned societies such as the British Mass Spectrometry Society (BMSS) and the RSC SSG and we often co-organize or promote each other's meetings, which is good for science generally.

As I eluded to earlier, membership is certainly a weaknesses for the society, simply because the larger our supporters, the more people we can promote our meetings to, the greater funding we will have to support the development of separation science, and the greater influence we could exert with for example, research funding bodies. While we are taking positive action to increase our membership, I suspect we will never be a huge society. We are also looking at opportunities to increase our overseas membership. While we have some foreign members, these numbers are low — so we would like to increase these to further improve our visibility and reach overseas too. Another issue we have is one of perception. While we occasionally partner with the RSC for some of our meetings, we are a completely separate charity and we need to do a better job promoting the Chromatographic Society brand, particularly in the UK.

In terms of opportunities, as the use and versatility of separation science continues to grow, particularly in the area of biomedical sciences, there are obviously more opportunities for the Society to move into different areas that we haven't worked in before. Behind the scenes, another opportunity we are working very hard on is supporting efforts to bring a major international separation science conference to the UK. As these are allocated for a number of years in the future, this wouldn't be in the near term, but this should be in the next six or seven years hopefully. By bringing a major meeting to the UK, hopefully we can rally partner organizations and industry to come together and generate momentum to focus on the development of separation science in the UK.

There are a number of threats we need to be aware of in terms of chromatography generally, some more of which we will touch on later I suspect, which may impact on Society activities. Working in analytical science, I see spectroscopic techniques being applied more and more to areas where chromatography was traditionally used, including synthetic reaction monitoring with off-line LC being replaced by on-line spectroscopic and spectrometric approaches; and off-line dissolution measurements with LC being replaced with on-line UV probes — so chromatography may be less prevalent in the future, which could have a corresponding impact on chromatography research.

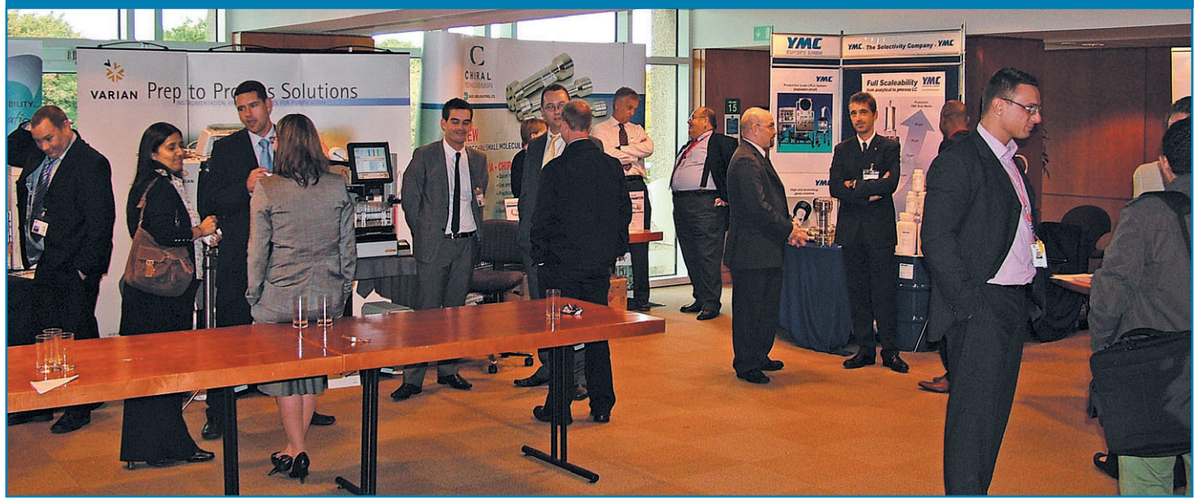
The final threat may arise from the teaching of separation sciences at an undergraduate level. Separation science is becoming increasingly marginalized as a subject and is often taught to undergraduates by non-experts with little background in chromatography. If this key science is taught poorly then it is unlikely to encourage students to pursue it as a career and the Society will have fewer students to support and help develop. The Society will obviously do all we can to prevent this scenario, but it's a big challenge and not one we can solve by ourselves.

Q. Is the Society partly responsible for the perceived weakness in UK separation science?

A: A very interesting question! I think it is fair to say that separation science in the UK isn't as strong as it was 20 years ago. In the 1990s there were at least 12 high-profile academic institutions undertaking separation science and some of the best industrial chromatographers in the industry. Currently there are probably around five or six Universities recognized for their separation science research in UK and industrial chromatography groups have been significantly reduced because of cuts in company size.

I think it's a collective responsibility how this situation has arisen and a number of groups need to come together to address this situation. The Royal Society of Chemistry (RSC) who accredit University degree courses need to focus more on

Exhibitors provide an important contribution through financial support and their scientific presentations at ChromSoc meetings. Scientists and exhibitors interacting at the "Big Prep 5" meeting at GSK, Stevenage, UK, in September 2009.



the analytical content of chemistry degree courses, and in particular, the separation sciences; the Engineering and Physical Sciences Research Council (EPSRC) who fund UK academic research need to focus on supporting separation science research, which is currently woefully underfunded (an area we are actively influencing); we need Universities to attract and grow separation scientists, and undertake key research in this area; we need vendor organizations to help support academic research more in the UK; industry needs to invest more on supporting academic separation science research and invest more in their own staff by developing their chromatography skills.

It's a huge undertaking, but we feel the Society can play a role in redeveloping UK separation science through our meeting programme and providing financial support for research within our means, but we need to align all these parties to refocus separation science as a core discipline in the UK.

Q. Given the ever-increasing rate of change in society, science, and the environment in which we conduct science, do you envisage that there might still be a Chromatographic Society in another 60 years time?

A: That's a difficult question to answer and one I suspect I'll not be around to find out the answer to! I sincerely hope the Society is around in 60 years because I feel we are an important part of the UK separation science landscape. However, I recognize these are very challenging times for a society like ours. We have a small membership base that we are keen to grow, but this is difficult in a climate of dwindling separation scientists. In 2014, the pharmaceutical analytical science group (PASG) folded through — essentially — a lack of interest in their activities. Having attended and presented at some of their meetings, this is a great shame as the ones I attended were always vibrant both scientifically and socially. While we are currently on a secure financial footing and still have the support of vendor companies and a significant body of separation scientists attending our meetings,

Some of the awardees of The Chromatographic Society's Jubilee and Martin medals who were present at the Society's "Advances in Microcolumn Separations" meeting in London earlier this year. Back row (l–r): Dr. Norman Smith (Jubilee medal 1995); Dr. Tom Lynch (Jubilee medal 2003); Prof. Pat Sandra (Martin medal 1994). Front row (l–r): Ian Mutton (Jubilee medal 2003); Prof. Keith Bartle (Jubilee medal 1990); Dr. Rodger Smith (Jubilee medal 1998); Prof. David Perrett (Jubilee medal 2001); Prof. David Goodall (Jubilee medal 1991); Prof. David McCalley (Jubilee medal 2011); Prof. Ian Wilson (Jubilee medal 1994, Martin medal, 2016); Prof. Peter Myers (Jubilee medal 2006, Martin medal, 2016); Prof. Peter Schoenmakers (Jubilee medal 1989, Martin medal, 2011).

a lot of effort is required by the committee to ensure successful meetings. We need to be cognizant of the possibility of a change in our circumstances and not be lax about our current position. In the future we may need to consider aligning more closely with other learned societies to continue our activities, which may also allow us to influence on a wider scale too.

Q. Ten years ago during the 50th anniversary of The Chromatographic Society some predictions were made by one of your members. Briefly they were: built in injectors/detectors in the column; smart chips inside columns; and the end of analytical separations due to dynamic field gradient focusing. Firstly, how close to the truth were these predictions and secondly what are your predictions for advances in the field of separation science in the next 10 years?

A: This sounds very much like comments from the good Professor Myers at the University of Liverpool, who was a previous member of our committee and continues to be a keen supporter of our activities! I think that he was right in a couple of areas in that there is an increased interest in lab-on-a-chip technology that have integrated injection systems, but detectors are still largely separate from the fluidic system. Smart chips haven't reached a level where they are embedded in the column yet, but as many of your readers will know, some UHPLC columns have smart chip cords that contain limited data regarding the use of the column, for example, the number of injections and highest pressure observed.

Giddings first discussed dynamic field gradient focussing in the early 1970s — so it's not that new a technique. There have been a number of articles discussing its application over the last 10 years, but it hasn't been widely adopted as an analytical technique. One area that I do see more uptake of, particularly in the field I work in, is the use of electrophoresis for analysis of biomolecules. All major pharmaceutical companies are developing novel biotherapies, be these mAbs, oligonucleotides, peptide therapies etc, and I believe electrophoresis will play an increasingly important role in the next 10 years. In terms of what I expect to see in the next decade, I think we will see development of more portable or point-of-analysis chromatography systems (including microfluidic systems) and also the development of simpler and more robust UHPLC systems, which may be dedicated for specific analysis types.

I also think we'll see much more focus on multidimensional chromatography and 3D chromatography, which Peter Schoenmaker's group at the University of Amsterdam is working on — they could become a commercial product in this time. This will also require further developments in informatic solutions to deconvolute the complex data sets associated with multidimensional separations in order to speed-up the conversion of data into knowledge. I also believe that with the recent advances in supercritical fluid chromatography (SFC) technology by multiple vendors, this will become a more widely adopted technology.

One technology that I find particularly exciting is the paper diagnostic tests that Georges Whiteside's group at Harvard University are pioneering. Being able to produce simple diagnostic tests fabricated from paper for use in the developing world could be a game changer for global healthcare. While I'm probably stretching this as a chromatograph technique (it's principally driven by capillary forces), why couldn't we start thinking about making separation systems from simple disposable materials or using 3D printers? One thing I can say, I definitely don't want to see higher-pressure UHPLC pumps or smaller particle stationary phases. I think we've reached the practical limits to that approach in separation science. Making higher pressure pumps will almost certainly lead to even more robustness issues with these types of systems.

Q. Returning to the present, how exactly does the Chromatographic Society plan to celebrate its 60th birthday?

A: This year we have a very busy set of meetings and events. As mentioned earlier, we revisited our history by holding our first meeting at the Institute for Engineering and Technology in London, which had an exceptional speaker line-up.

The second meeting of the year we are supporting is the ISC2016 meeting in Cork (Ireland — 28 August–1 September) where we are inviting a number of speakers to present on behalf of the Society. This will be the largest chromatography meeting of the year in Europe and a "must attend" event for separation scientists.

Our third meeting of the year will be held on 25 October at the agrochemical company Syngenta (Bracknell, UK). This meeting will be focused on separation science and automation in industry. We have unfortunately had to rearrange this meeting because of the the closure of the MSD pharmaceutical site at Hoddesdon, which is where we were originally hoping to hold this event in Spring.

As mentioned earlier, we will continue to work with partner organizations and to this end once again we will be working with the RSC separation science group on the popular "Advances in Clinical Analysis" meeting, which will be held in the RSC's Burlington House, London on the 30th November.

We propose our final "meeting" of our five planned events of 2016 will be held on the 14–17 October, which will be an educational event focusing on the development of postgraduate researchers and novice chromatographers. We are holding this meeting in the Lake District, and the programme will be a mixture of tutorials, lectures, and poster presentations alongside a social programme taking in some of the attractions of this part of the world and offering excellent networking opportunities. Certainly in my time with the Chromatographic Society, we have focused on delivering meetings based on invited speaker lectures. An event such as this is an ambitious step for the current committee and one which we hope will become another important fixture in our calendar.

So we have lots to focus on this year and we hope your readers, particularly in the UK, will help support these events and help us to reinvigorate separation science in the UK.

Paul Ferguson is a separation science specialist at AstraZeneca in the UK, and leads the separation science strategy for their product development division working on both small- and medium-size novel therapies. He has worked in the pharmaceutical industry since 1999 (previously at Pfizer) following a post-doc at Imperial College London on capillary electrochromatography (CEC) with Dr Norman Smith. Paul has particular interests in UHPLC, SFC, CE, chiral separations, formulated drug sample preparation, green analytical chemistry, and method development. He is a past winner of the Desty Memorial lecture prize (2002), a Fellow and Chartered Chemist in the RSC, and is a visiting lecturer at Kings College London where he has lectured on the MSc Analytical Science for Industry course since its inception in 2009.

Paul is also the President of The Chromatographic Society in the UK. He has organized or co-organized several successful UK symposia for the Society since 2007 and is involved with the organization of two meetings as part of the Society's Diamond Jubilee celebrations in 2016.

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Website: **www.chromsoc.com**

News

LGC Awarded Ethical Status

LGC Standards (Middlesex, UK) has been awarded a Responsible Sourcing status by Sedex for its Standards division in Germany. A non-profit membership organization, Sedex is dedicated to improving responsible and ethical business practices in global supply chains.

"We take great pride in having our Responsible Sourcing status confirmed and are particularly pleased that our customers appreciate our Ethical Trading Initiative," commented Hans-Christian Schumacher, Managing Director of LGC's Standards division.

With members in over 150 countries, Sedex is the largest collaborative platform for sharing ethical supply chain data. The online database allows companies to store, share, and report on information in the four key areas of labour standards, care of the environment, business ethics, and health and safety.

LGC employees across many departments underwent auditing by Sedex, with findings indicating LGC is an ethically run company with good workers rights and a commitment to reduce its burden upon the environment.

By informing customers on the ethical practices of companies, Sedex hopes to reduce risk and improve supply chain practices — simultaneously providing an efficient and cost-effective method of data sharing for companies.

For more information please visit **www.lgcstandards.com** and **www.sedexglobal.com.**

Novel Fatty Acid Analysis

Researchers from the USA have developed a simple and derivatization-free gas chromatography (GC) method for the quantitative analysis of oleic acid and related fatty acids.[1]

A common pharmaceutical excipient, oleic acid is widely used for long-term stabilization, solid formation bulking, and for the therapeutic enhancement of active ingredients including serving as an emulsion agent in topical pharmaceutical formulations and a solubility enhancer for gastrointestinal tract delivery.

The importance of oleic acids to the pharmaceutical industry has led to a variety of analytical methods being developed with high performance liquid chromatography (HPLC) the most common.[1]

However, HPLC separation of fatty acids is not perfect. The absence of chromophores or fluorescent functional groups[2] means the majority of HPLC methods in the literature require a derivatization process prior to analysis; those without a derivatization process suffer from poor sensitivity.[3]

Gas chromatography offers an alternative for the analysis of fatty acids and is commonly used. Unfortunately, GC methods suffer from a similar issue to HPLC, requiring a derivatization process because of the high boiling points of fatty acids.[4]

A laborious, tedious, and time-consuming process, derivatization, while effective, often results in lower accuracy and precision[5] — two undeniably unwanted side effects. The aim of this study was to develop a simple method for oleic acid and related fatty acid analysis, free from a derivatization process, but capable of accurate and robust analysis suitable for use in a quality controlled laboratory.

The method developed used a nitroterephthalic acid modified polyethylene glycol (PEG) capillary GC column as well as a flame ionization detector (FID). The sample preparation procedure was simple and straightforward requiring no derivatization. The method successfully separated 15 fatty acids in a total run time of 20 min. This was validated and proved to be specific, precise, and accurate for the analysis of oleic acid and related fatty acids. — L.B.

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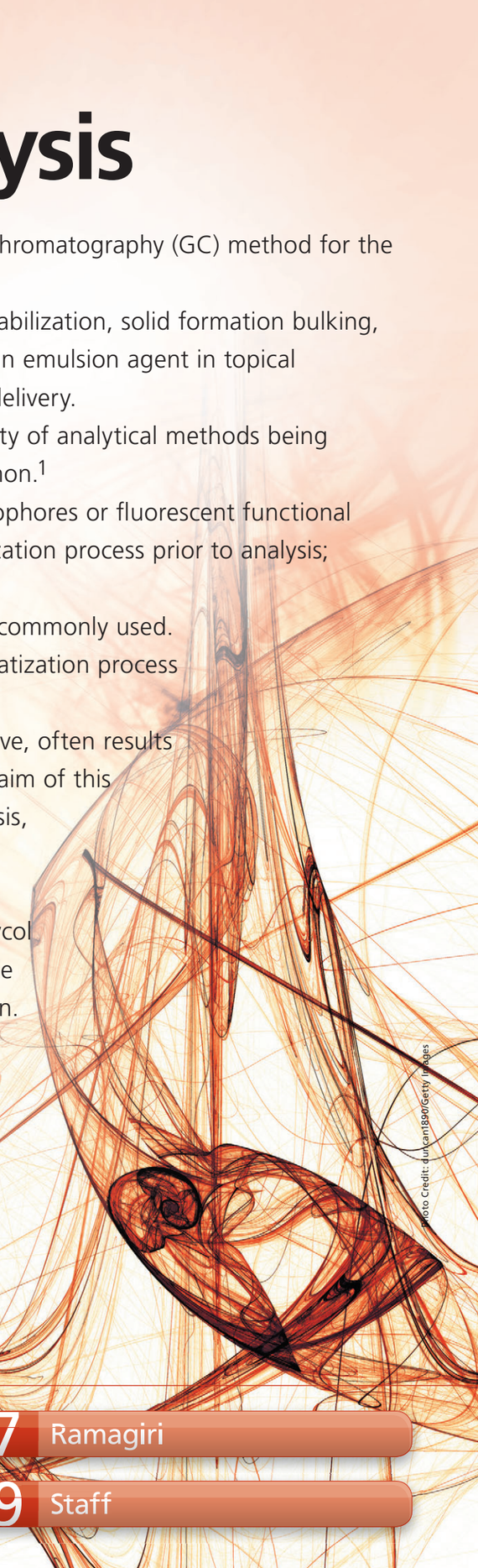

Photo Credit: duncan1890/Getty Images

Investigating Meat Authenticity

Researchers from Universidade Federal de Minas Gerais, Brazil, have used multiple analytical techniques including ion-exchange chromatography (IEC) and attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy to investigate bovine meat adulteration.[1] An increasing concern across many nations, food authenticity represents a considerable challenge for analytical chemists.

Numerous scandals have been reported in recent years highlighting the impact of food fraud on consumers. Recent examples include the horsemeat scandal within Europe[2] and the major scandal within Brazil with several slaughterhouses in the Belo Horizonte region found to be committing fraud through the addition of non-meat ingredients.[3]

Fraud with regards to meat can be categorized into four major areas: meat origin, meat substitution, meat processing, and non-meat ingredient addition.[4]

"Substitution of one species with another is the most reported type of meat fraud, such as in the recent horsemeat scandal [that] occurred in Europe in 2013," explained Marcelo M. Sena from the Universidade Federal de Minas Gerais, Brazil, and lead author of the study. These type of substitutions have been widely reported with a number of analytical methods for detecting this fraud discussed and developed, however, the addition of non-meat ingredients has received much less attention. This current research aims to address that gap in the literature.

"We studied this type of meat fraud due to a real case investigated and elucidated by the Brazilian Federal Police in 2012. Adulterant substances were found in loco in the denounced slaughterhouses, including salts, such as sodium chloride, phosphate, tripolyphosphate and acid pyrophosphate, and maltodextrin. Specific equipment used for these frauds were also found there, such as injection and tenderizing machines," said Sena.

Often the intention of adulterant substances is to increase the water holding capacity (WHC) of meat, thereby increasing the weight of the meat and its potential sale value. A standard method exists for detecting this based upon the water/protein ratio. However, it is much harder to detect water binding to the meat induced by the addition of salts and proteins. This water binding leaves the water/protein value ratio close to its natural value and to identify such fraud the detection of proteins and/or exogenous salts is required.

The Brazilian Federal Police provided 55 samples, with 12 controls taken directly from the bovine carcass. Physico-chemical parameters were determined for proteins, ash, sodium, chloride, and phosphate using the Kjeldahl method, gravimetry, and IEC. The research team also introduced the use of ATR-FTIR spectroscopy. A strategy of data fusion was implemented combining the outputs of multiple analytical techniques resulting in 91% of the adulterated samples being correctly identified. The analytical method used should preferably be "simple, rapid, of low cost, non-destructive, and involving no or a minimum of sample pre-treatment," clarified Sena.

Further work is currently being carried out by Karen M. Nunes as part of a PhD thesis to obtain more robust models and an in-depth characterization of the fraudulent bovine meat. "The aim of this project is to prepare adulterated meat samples, simulating various types of frauds. These samples will be analyzed by analytical techniques that generated bidimensional data, such as image vibrational spectroscopy, and more complex chemometric methods, such as multivariate curve resolution and unfolded partial least squares," explained Sena. — L.B.

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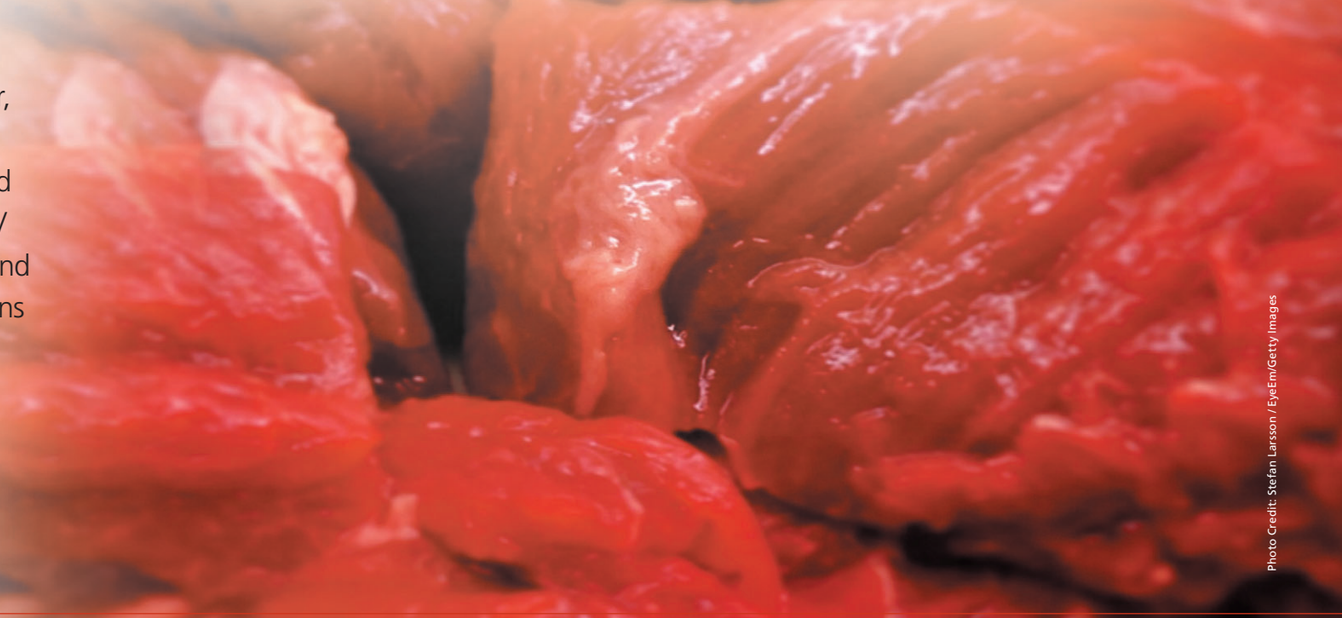

Photo Credit: Stefan Larsson / EyeEm/Getty Images

LCGC TV Highlights

***LCGC* TV: Choosing a Mass Spectrometer: What to Consider**
Different considerations will point you in different directions when selecting a mass spectrometer. Barbara Larsen from DuPont Central Research and Development talks through the various key aspects to consider when making this choice.
Watch Here>>

***LCGC* TV: The Future of 2D LC**
Comprehensive liquid chromatography (LC) is a multidimensional technique that has the potential to increase peak capacity resolution when separating complex mixtures, especially in food analysis. Luigi Mondello from the University of Messina, Italy, gives his opinion on the future of 2D LC in food analysis.
Watch Here>>

Peaks of the Week

The *LCGC* Blog: Practical HPLC Method Development Screening — Tony Taylor considers the other important aspects of "screening" in HPLC. Discussing the importance of the mobile phase composition, gradient parameters, and flow rate when developing new HPLC methods. Offering tips and advice to ensure good efficiency, peak shape, and practice. **Read Here>>**

Retention Time Drift — A Case Study — LC Troubleshooting editor John W. Dolan asks what should you do if your retention times drop from one injection to the next? A systematic approach to troubleshooting can help to quickly identify the problem source. **Read Here>>**

Photo Credit: David Malan/Getty Images

Chromatographers Get Into Cannabis — As the cannabis industry grows, demand for analytical robustness is increasing for analytes such as pesticides, residual solvents, and terpenes. GC and GC–MS are effective tools to address the demands of laboratories, growers, manufacturers, and consumers. This article provides an overview of the types of compounds that can be analyzed by GC, reviews the strengths and weaknesses of the analytical methods, and discusses areas of opportunity for chromatography.
Read Here>>

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News In Brief

By using GC-olfactometry researchers from Oregon State University, USA, have evaluated the aroma potential in early and late-maturity pinot noir grapes. Forty-nine main odour-active compounds were detected by aroma extract dilution analysis (AEDA) and quantified. Early harvest grapes released less β-damascenone, vanillin, 4-vinylguaiacol, and 4-vinylphenol suggesting they were important aroma compounds.
doi: 10.1021/acs.jafc.5b04774

Researchers from Bohai University, China, have developed a method to determine ractopamine (RAC) in pork using magnetic molecularly imprinted polymers (MMIPs) as the adsorbent followed by HPLC. RAC is a common food additive in livestock promoting leaner meat and less fat, however, serious side effects have resulted in restrictions and bans in countries such as Russia, China, and most of Europe.
doi: 10.1016/j.foodchem.2016.01.070

High-performance thin-layer chromatography (HPTLC) has been used to identify and quantify Oleanolic acid in the roots of *Achyranthes aspera linn*. Oleanolic acid is a secondary metabolite with potential medicinal uses as identified by the World Health Organization's drive to identify all medical plant species. Results indicate this method to be sensitive, specific, and reproducible.
Int. J. Green Pharm. 9(4), (2015)

The Importance of Specifications for Method Transfer

Incognito shares his thoughts on the importance of specifications for method transfer in gas chromatography (GC).

Photo Credit: PhotoAlto/Ale Ventura/Getty Images

I'm often unpleasantly surprised at the lack of essential detail, or just plain inaccuracy, in the way chromatographic methods are specified — often rendering a validation exercise pointless because of the large amount of subjectivity possible when implementing the method. My fear is that these "missing" instrument or method parameters may be simply invented or system defaults accepted without due consideration of the impact on the data produced — possibly resulting in unnecessary repeat analyses or, in the worst cases, results that are not fit for purpose. Certainly these "missing" values are often the cause of problems when transferring chromatographic methods between laboratories.

As we move towards the situation where there is less and less need to "get under the hood" of our instruments, it's vital that through the method itself, we properly specify the instrument acquisition and data collection parameters.

I've recently been transferring several gas chromatography (GC) methods and thought it might be useful to show how we might properly specify a GC method, to serve both as a template for the level of detail required in method specifications but also to allow readers an insight into all of the parameters that are required for GC method development and validation.

Our "ideal" GC method is shown below, with my commentary on each section following:

Carrier Gas:

- Carrier gas: Helium (99.999% purity or greater)
- Flow-rate: 1.00 mL/min (35 cm/s)
- Mode: Constant flow

Sample Introduction:

- Injection volume: 1 µL
- Injection solvent: Dichloromoethane (cite also grade and purity)
- Syringe size: 10 µL, cone tipped
- Autosampler routine: Wash needle: Solvent A (3 × 10 µL) pre-injection
- Sample priming: Aspirate sample (3 × 10 µL) and dispense to waste
- Sample pumps: (3 × 10 µL) with 5 s viscosity delay
- Wash needle: Solvent B (3 × 10 µL) post-injection

Sample Inlet:

- Mode: Splitless
- Temperature: 250 °C (no oven tracking)
- Pressure: Track column pressure (no pressure pulse)
- Splitless time: 35 s
- Split flow: 100 mL/min
- Split ratio: 101:1
- Split (gas) saver: 15 mL/min after 2 min
- Liner: Single lower gooseneck 4 mm i.d. splitless liner, deactivated, containing 1 cm deep plug of deactivated quartz wool packing, positioned to wipe the needle tip

Column:

- Phase: 14% Cyanpropylphenyl methylpolysiloxane
- Length: 30 m
- Internal diameter: 0.32 mm
- Film thickness: 0.25 µm
- Phase ratio: 320
- Upper temp. limits: 280 °C (isothermal)/ 300 °C (gradient)

Oven:

- Initial temp.: 40 °C
- Initial time: 1 min
- Ramp 1: 20 °C/min; temp. 1: 250 °C, hold 1: 5 min
- Ramp 2: 50 °C/min; temp. 2: 300 °C, hold 2: 2 min
- Equilibration time: 1 min

Detector:

- Type: Flame ionization detector
- Temperature: 300 °C
- Fuel gas: Hydrogen @ 30 mL/min
- Oxidizer gas: Air @ 400 mL/min
- Make-up gas: Nitrogen @ 35 mL/min
- Make-up mode: Constant flow
- Attenuation: Specify if required

Ideally the required carrier gas purity should be specified; I have also seen statements relating to the necessity to filter out moisture, hydrocarbons, and oxygen from the carrier for optimum performance. Flow rate or carrier linear velocity should be specified for the carrier rather than the column head pressure (or pressure drop across the column). The pressure drop across the column is a function of the carrier flow, oven temperature, and column dimension and any slight deviation in these parameters (poorly calibrated oven thermocouple, trimmed column length) will require an alteration in the column pressure drop to achieve a given flow rate (or linear velocity). Further, the column dimensions and carrier gas type must be correctly entered into the instrument for flow rate to be correctly calculated when using systems with computerized pneumatics — this is especially

important after column maintenance where the column inlet has been trimmed to improve chromatographic performance. Specifying the mode of carrier operation is also important with modern computerized pneumatics. In this instance we have specified constant flow, which raises the carrier gas pressure as the column temperature increases to achieve a constant carrier gas flow rate into the detector. This mode has several advantages such as increasing the signal-to noise ratio of late eluting peaks, decreasing overall analysis time, and producing flatter baseline profiles when using mass-flow sensitive detectors.

It's vital to specify both the sample volume and the solvent used. Apart from the obvious reasons for this, it's important to be able to calculate the sample vapour volume produced within the liner to asses if "back flash" — a liner overfill problem, which can lead to carryover and insidious baseline artifacts — will occur. Many autosamplers use a fixed volume syringe with a stepper motor to measure the sample volume (with a 10 µL syringe installed, one step will represent a 1 µL injection). It's therefore important to specify the syringe size to avoid injecting the wrong volume. One step of the plunger driver motor will result in a 0.5 µL injection when a 5 µL syringe is installed, so check the installed syringe on the instrument as well! Specifying the cleaning regime of the needle is also useful to ensure minimized carryover — usually two separate solvent bottles are used, one pre-injection, the other post injection. In addition, the requirements for sample priming into the syringe and the speed of the plunger used to avoid cavitation with more viscous samples should be specified. These needle washing parameters are very often omitted from methods — however, without them, the performance of the analysis may be irreproducible between instruments or operators.

Sample inlet conditions are often incorrectly specified, which is problematic because they are arguably most important in terms of analytical reproducibility and performance! The major variables are usually well specified and the mode of injection, inlet temperature, and split ratio (for split injections) appear in most documents that I review. It's important to remember that both the split flow and split ratio should be specified for clarity — remember that the split ratio is calculated as (split flow + column flow)/column flow):1. However, I often see splitless injection conditions specified without a "splitless" time (also called "split on" time or "purge" time), which represents the time after injection when the split valve is opened to allow lingering components to escape the inlet, leading to better solvent and early eluting analyte peak shapes as well as reducing the amount of baseline rise during the analysis. It is vital that this parameter has been established for the method and is clearly specified. Further, it's rare to see "gas saver" times and flows specified, but these are important because some instruments are capable of automatically reducing the split gas flow after the inlet has been flushed to save gas — why waste 100 mL of helium every minute for the length of your analysis when a much more moderate flow is possible? Further variables may also be important such as inlet temperature, pressure tracking, or the use of pulsed pressure when injecting larger sample volumes and should also be carefully specified. Remember that the inlet pressure is likely to increase during programmed temperature analysis if "constant flow" mode is chosen. There are over 300 different liner designs currently available and the liner volume, construction, deactivation, and packing can have a drastic effect on both the quantitative and qualitative nature of the resulting chromatogram. These parameters should be fully specified and in some instances I have even seen manufacturer part numbers quoted to maintain absolute reproducibility of the method.

The minimum requirement for specifying a GC column is the information required to purchase it. That is, the nature of the stationary phase, the column length, internal diameter, and film thickness. I have seen a multitude of methods in which the film thickness is omitted. It's also good practice to specify the phase ratio in case the method needs to be adapted or changed, in which case the use of an equivalent column (say of lower internal diameter) can be easily specified by adjusting the film thickness to maintain the required resolution. Showing the correct upper temperature limits of the column is also useful for column conditioning purposes. Of course, when installing a column into a GC system, the exact column length is often unknown (even your new 30 m column is unlikely to be exactly 30 m long!), and in order for the instrument to correctly set a pressure to generate the required flow-rate, the column should be calibrated using the retention time of an unretained component. Most data systems will calculate the column length if the retention time of methane or air is known (take care that your column does not retain these species!).

Oven temperature programmes are usually well specified and the parameters are often shown in the form of a table for ease of understanding. One parameter that I often see omitted, however, is the "oven equilibration time". This parameter is used

to account for the time delay in the carrier gas achieving the same temperature as the air inside the GC oven, which is created by the thermal lag of the GC column walls. Not including an equilibration of at least 30 s (in my experience) can lead to significant retention time variability, especially with earlier eluting peaks. Obviously the wider the column bore and the thicker the stationary phase film thickness, the longer the equilibration time that is necessary.

The main detector operating parameters such as temperature and flow rates of the fuel and oxidizer gases are generally given — note for most flame ionization detectors (FID) the optimum flame stoichiometry is approximately 10:1 oxidizer:fuel. However, I have seen a number of cases in which the make-up gas type or flow rate have not been specified. This is a major omission because both of these variables can affect the flame stoichiometry and hence the response or sensitivity of the FID detector. I also specify (usually as a caveat to the conditions) that the gases should be filtered and the nature of the filter material — just to remind operators in the laboratory of the need to check the viability of the gas traps prior to analysis. Some modern instruments offer the option to ramp the makeup gas flow to "mimic" a constant carrier flow into the detector even though the instrument is operating in constant pressure mode. If this option is available, then the required operating mode should be clearly specified. Most modern detector and data system combinations are self-attenuating and as such the specification of absolute attenuation is not required, however, if your detector type requires an absolute attenuation, it should also be clearly specified.

This imaginary specification is written entirely from a personal perspective and in the form that I personally like to see methods set out. Your own preferences or those of your employer may differ; however, unless we properly specify methods both on paper and within the data acquisition settings of our instruments, we will continue to have unnecessary difficulties with instrument and operator variability, especially during method transfer exercises. Next time you use a GC (or high performance liquid chromatography [HPLC]) method, have a look at the specification and ask yourself how many parameters are left unspecified and whether these "assumed defaults" may be the cause of any issues you have with your analysis? If you can influence the quality of your method specifications lobby for an improvement — it can be your good deed for the day!

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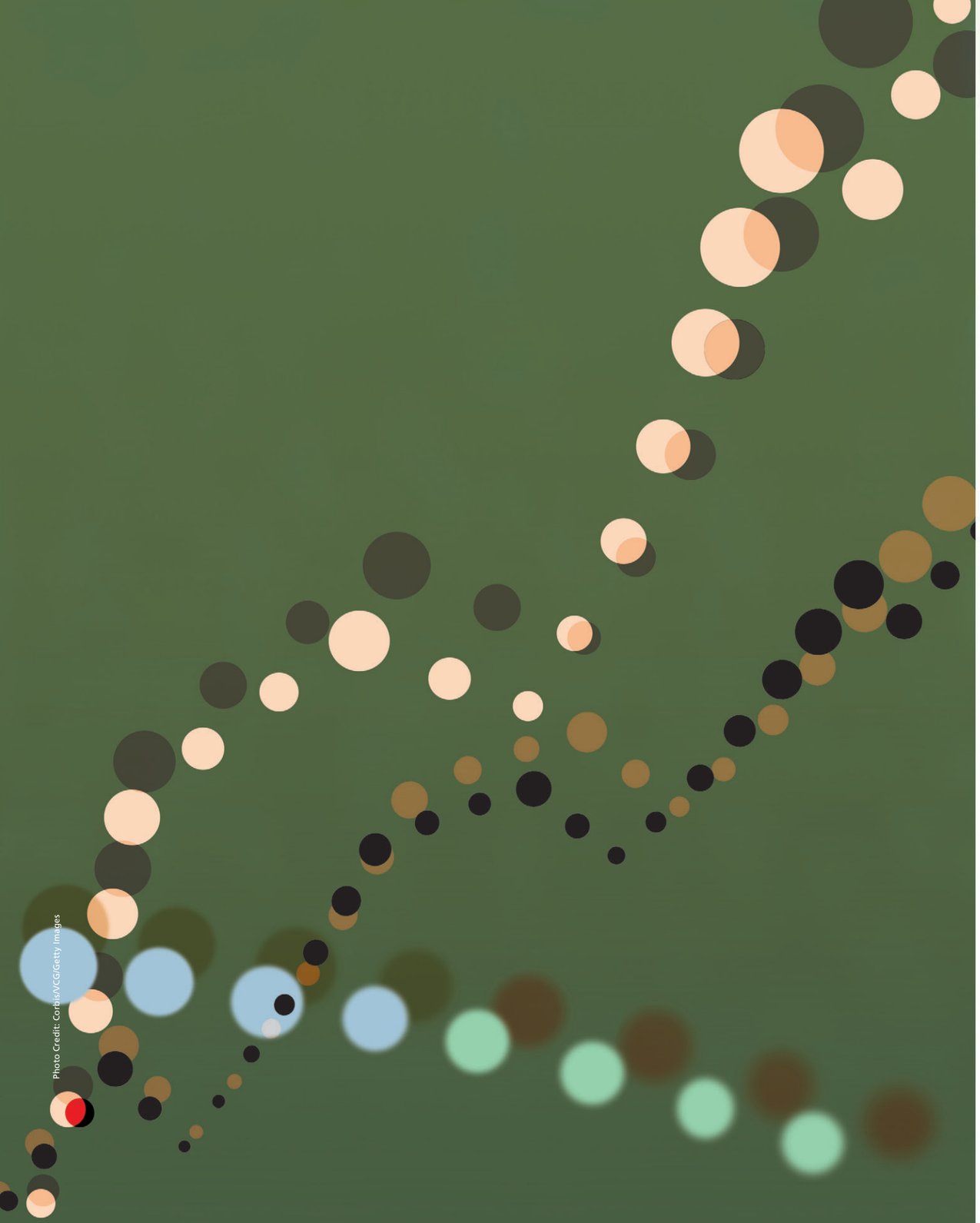

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Trends and Challenges for Bioanalysis and Characterization of Small and Large Molecule Drugs

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Structural, bioanalytical, characterization, and quality control studies are critical for successful drug development. These studies must be as accurate, sensitive, and selective as possible, and liquid chromatography coupled to tandem mass spectrometry (LC–MS–MS) has been the technique of choice for many areas of small molecule analysis for the past 30 years. During that time, rapid improvements in analytical technologies have supported the development of more sensitive and robust methods. However, the pharma and biopharma industry continues to need more powerful instruments and more diverse methods, particularly because therapeutics have expanded to include large molecules. This work follows on from an earlier article that explored the limitations of LC–MS–MS for bioanalysis of biologics.[1] This article considers some of the current issues for analysis of small and large molecules, and emerging trends in method development.

Liquid chromatography coupled to mass spectrometry (LC–MS)-based techniques have been widely used in drug discovery and development laboratories since the 1990s because of their powerful abilities to identify and quantify low levels of compounds very quickly, within samples containing hundreds or thousands of other substances.[2] LC coupled to tandem MS (LC–MS–MS) has become particularly important for structural elucidation, ADME, and bioanalysis studies of small molecule drugs. These challenging applications require extremely accurate and reproducible compound detection at ever-decreasing concentrations. In recent years, there has been rapid growth in the development of biotherapeutics, and analysis of these larger molecule drugs introduces a number of challenges that are also driving new developments in technology and methods.

Bioanalysis and Small Molecule Drugs

For decades, drug developers have depended on bioanalysis studies to provide accurate measurements of drug concentrations in a given sample, at the time of sample collection.[3] The accuracy of these studies depends on the method and on the reliability of the laboratory's analytical instruments, which must be able to quantify the compound of interest with adequate selectivity and specificity. This has always been particularly challenging as a result of the typically high presence of structurally related and non-related compounds in bioanalysis samples, such as plasma, blood, and other complicated matrices. These can cause cross-reactivity with affinity reagents or co-elution of unrelated compounds, impacting the accuracy and reproducibility of the assay.[4]

Over the years, LC–MS–MS-based methods have been developed to overcome these challenges, offering increasing sensitivity, throughput, accuracy, and reproducibility for drug quantification. A common approach has been to use multiple reaction monitoring (MRM) on triple quadrupole mass spectrometers to reduce noise while improving selectivity and accuracy of quantification. More recently this has been extended to MRM^3, which increases the number of fragmentation steps for further improvements in selectivity.[5] Today's triple quadrupole MS systems with MRM have been used to develop validated methods for detecting small molecule drugs at low pg/mL concentrations, with good reproducibility, linearity, and signal-to-noise ratios.[6]

Some compounds are particularly difficult to separate out in biological samples because of matrix interference, which can cause unresolved peaks or high baselines, impacting the data reproducibility, accuracy, and dynamic range.[5] These effects have traditionally been overcome through incorporating additional sample clean-up steps or through using slower chromatography. However, these add significant time, cost, and labour that most drug development laboratories cannot afford, as they come under pressure to increase their sample throughput. Alternative technologies have become available over the past few years that combine ion mobility separation with LC–MS to offer increased selectivity. These are either in the form of ion mobility devices that can be attached to the front-end of time-of-flight (TOF) and triple quadrupole MS systems, or built in to a TOF-MS system, but the majority of these are unable to meet the balance of speed, selectivity, and robustness required for bioanalysis.[7] More recently, differential ion mobility separation devices have been developed for orthogonal separation of ions in between the LC and MS stages of the analysis. The ions are separated

in trajectory, based on the difference in their mobility, as opposed to separated in time. This removes background components, providing a system that can be used with short MRM cycle times for rapid and accurate detection of compounds at low levels in complex matrices.[5]

Bioanalysis laboratories are increasingly adopting microflow LC-based methods for analyzing compounds at very low levels of detection. This technique uses smaller diameter columns (under 1 mm) and electrodes, resulting in faster separations with improved sensitivity and resolution, with minimal post-column dispersion. The lower flow rate also increases the ionization efficiency to reduce ion suppression, while using significantly lower amounts of sample and solvent, offering economic and environmental advantages to pharmaceutical development. The lower sample volumes needed for microflow LC are well suited for the industry's growing interest in using microsampling techniques for toxicology and bioanalysis studies.[8,9] Moreover, microflow LC can be combined with differential ion mobility MS for extremely sensitive and selective analysis of compounds in biological samples.[10,11]

Bioanalysis and Large Molecule Drugs

The accuracy, robustness, and reproducibility of bioanalysis studies remain key concerns for drug developers and regulatory authorities. However, traditionally used LC–MS–MS methods for small molecule bioanalysis are usually inappropriate for studying large molecule drugs such as antibodies, growth factors, oliognucleotides, and recombinant peptides. The greater size and complexity of these molecules mean they typically require extensive sample preparation prior to analysis; their adsorptive properties and interference from background proteins can further affect quantification accuracy.

LC–MS–MS methods have been optimized for the direct analysis of small peptides (under 10 kDa); these typically require immunaffinity-mediated sample extraction and/or sample enrichment steps to enhance selectivity, prior to quantifying the intact analyte. For larger proteins, more extensive workflows are usually required that include proteolytic digestion of the protein prior to LC–MS–MS analysis of a surrogate peptide.[1,4,12] This indirect analytical method is widely accepted but can be highly laborious, and is associated with complications such as variable peptide release.[1] Furthermore, guidance for validation of these methods has yet to be issued by the regulatory authorities.[4]

Ligand binding assays (LBA) such as ELISAs provide a well-established technique for quantifying proteins, and for bioanalysis they have the advantage of being able to detect circulating "free" drugs as well as physiologically active forms. However, LBAs have a number of limitations that preclude their use in high throughput drug development.[12,13] In a recent development, researchers have begun combining LBAs with LC–MS–MS.[14,15] This approach brings together the selectivity and sensitivity advantages of LC–MS–MS with the specificity and broader immunocapture capabilities offered by LBAs.

These method developments have been supported by several technology improvements for triple quadrupole and QTRAP MS systems, including improvements in sensitivity that allow detection of large molecules at low nanogram to picogram levels.[16] These new technologies offer improved ionization and sampling efficiencies, increased dynamic range, and switchable (dual) mass ranges that allow ions of different mass to pass through the detector. As a result, validated methods are now available for quantifying a variety of challenging drugs such as the cytokine-inhibitor, adalimumab;[17] the glucose-raising hormone, glucagon;[18] the insulin analogue, glargine;[19] and antibody-based treatments such as infliximab, for autoimmune diseases, and trastuzumab for breast cancer.

Characterization of Large Molecules

Most large molecule drugs are prone to inconsistencies in the form of naturally occurring sequence modifications and biotransformations, as a result of their production processes. These changes can affect the efficacy of the drug, as well as its bioavailability and safety. As a result, pharmaceutical analysis laboratories regularly perform protein characterization studies to monitor sequence degradation and post-translational modifications, such as amino acid changes and glycosylations. These studies have previously been performed using LBAs or capillary electrophoresis (CE) technologies.[21] CE is powerful and robust but typically labour intensive for full characterizations, particularly when dealing with complex therapeutics such as antibody-drug conjugates that may require repeated runs for different analytics and incur complicated data processing.

In recent years, technology developments have led to several improved methods for protein characterization. These include combining differential ion mobility separation with LC–MS for improved and simplified analyses.[20] Elsewhere, the integration of CE technologies with electrospray ionization (CESI) has led to the development of CESI–MS techniques, which promise rapid and greatly simplified protein analysis. The development combines the high separation efficiency of CE with nanoflow LC, which maximizes the ionization efficiency and minimizes ion

suppression.[21] CESI–MS systems are designed with open-tube capillaries, thereby eliminating the occurrence of dead volumes, and resulting in improved sensitivity and peak efficiency. There is also no stationary phase, which is important for ensuring peptides are not lost or excessively retained. In a recent example, the breast cancer drug, trastuzumab, was fully characterized from a single protease digestion and a single run using CESI–MS. The method obtained 100% sequence coverage and identified several key amino acid modifications; comprehensive glycopeptide analysis was also performed from the same separation.[21]

Biotransformations such as deamidations, oxidations, and structural changes can be challenging to detect with conventional methods, such as LBAs. Trastuzumab is known to undergo *in vivo* deamidation at a crucial position in its structure, and antibodies used in validated ELISAs are unable to recognize the deamidation. Scientists recently developed an LC–MS–MS-based method for quantitative monitoring of this biotransformation, using tryptic digestion followed by quantification of a signature peptide with selected reaction monitoring. The method was shown to successfully quantify the deamidation-sensitive signature peptide and its deamidated products simultaneously.[22]

Conclusions

Successful drug development and drug safety depend on numerous analytical testing processes at several steps during the development pipeline, and during ongoing characterization of commercial large-molecule drugs. It is essential that the industry and patients can rely on the accuracy and reproducibility of this analytical testing. Over the years we have seen continual improvements in the capabilities of analytical technologies, driven by the highly competitive and strictly regulated nature of the pharmaceuticals industry. During recent years in particular there have been a number of innovative developments in instrumentation engineering, as well as in method development, that are allowing scientists to explore novel therapeutic molecules and increasingly complex compounds. In the future these developments are likely to require even more diverse analytical methods, with continual improvements in speed, selectivity, and accuracy.

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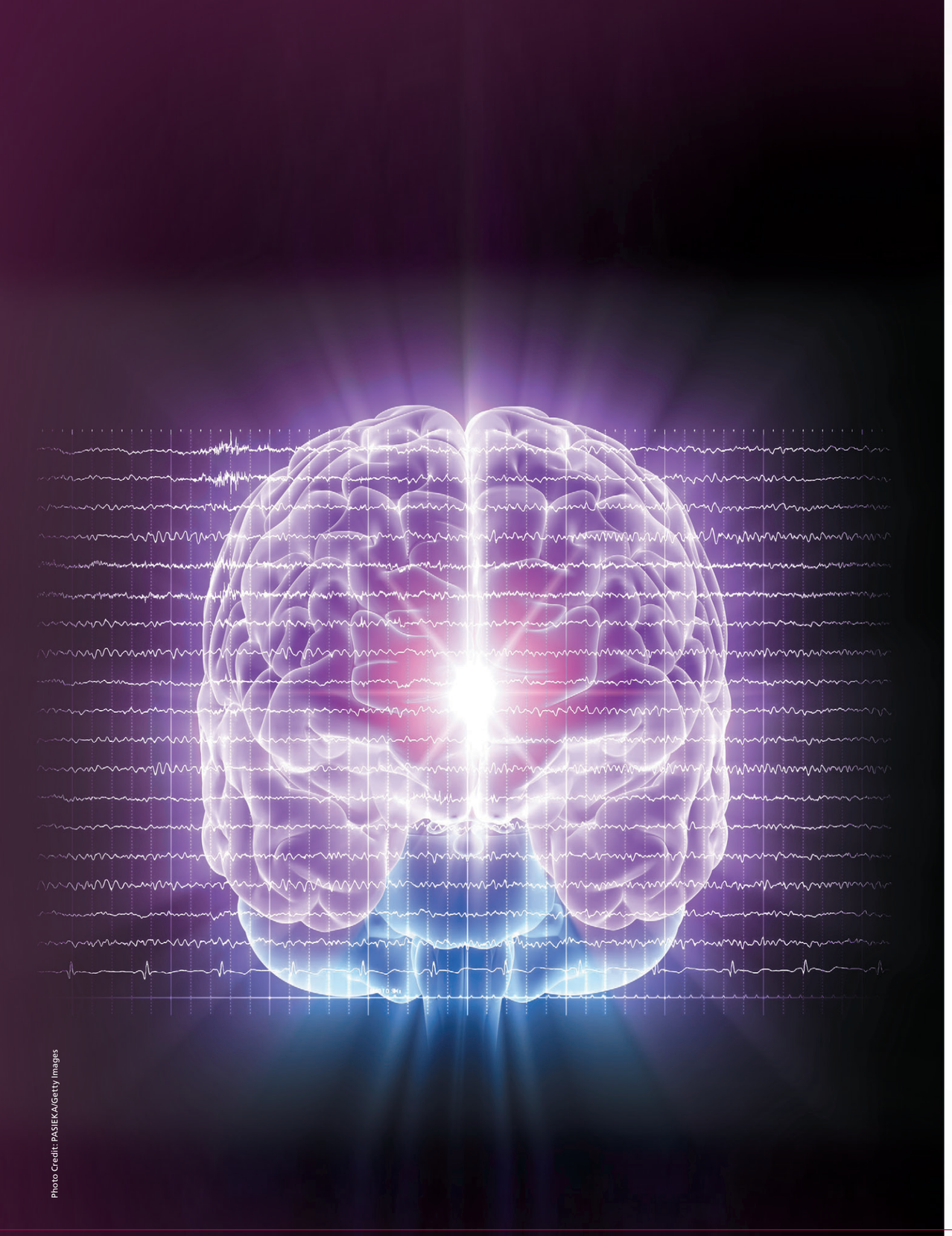

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Preview of Topics at HPLC 2016, Part 3: Reconsidering HIC for Top-Down Proteomics

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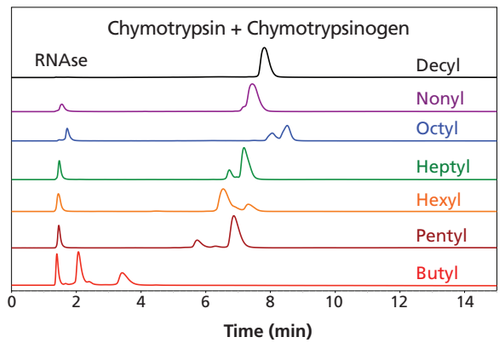
This is the third in a series of articles exploring topics that will be addressed at the HPLC 2016 conference in San Francisco, USA, from 19 to 24 June.

Until recently, mass spectrometry (MS) was limited in the information it could supply regarding proteins larger than 40 kDa. The most recent instruments have broken through that limit, but proteins smaller than 40 kDa are still more easily detected in MS and can suppress the collection of data from larger proteins. This situation has created a demand for better separation of proteins upstream from the MS orifice to facilitate top-down proteomics. At present, though, this separation of proteins is something of a bottleneck. Methods such as reversed-phase liquid chromatography (LC) that involve mobile phases compatible with MS are not compatible with many proteins. With those mobile- and stationary-phase combinations, the proteins may be eluted in peaks 15-min wide or not at all.[1,2] The conditions also tend to cause the loss of protein three-dimensional structure, precluding analysis of protein complexes.

Alternative modes of chromatography for protein analysis include the following:

- **Size-exclusion chromatography:** Size-exclusion chromatography is helpful in separating large proteins from small ones but is otherwise a low-resolution method.
- **Ion-exchange chromatography:** Ion-exchange chromatography is a high-resolution mode but generally requires more salt than a mass spectrometer can cope with.
- **Hydrophilic interaction chromatography (HILIC):** HILIC has been used successfully for proteins that do not normally occur free in aqueous solution, such as histones,[3] membrane proteins,[4] and apolipoproteins.[5]

Figure 1: Retention of ribonuclease A (RNAse) and chymotrypsinogen (CHYGEN) on various columns. Columns: 100 mm × 4.6 mm, 3-µm d_p, 1500-Å (coating as shown); mobile-phase A: 1 M ammonium acetate; mobile-phase B: 20 mM ammonium acetate with 50% acetonitrile; gradient: 15-min linear, 0–100% B; flow rate: 1 mL/min; detection: 280 nm. (Adapted with permission from reference 6.)

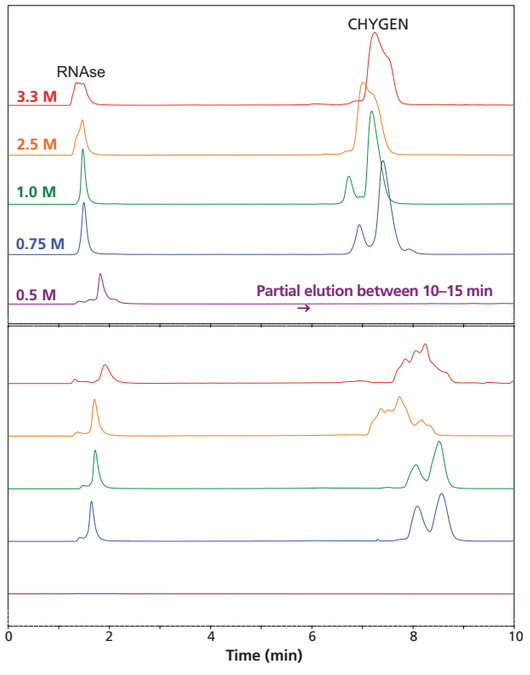


However, the high concentration of organic solvent required probably precludes its more general use.

- **Hydrophobic interaction chromatography (HIC):** HIC involves a gradient from a high to low concentration of salt, and the best-performing salts are nonvolatile. This condition would seem to eliminate it from consideration for protein separations on-line with MS. However, HIC separates proteins with high resolution, based on differences in the hydrophobicity of the surface of their tertiary structures. HIC is nondenaturing and extremely sensitive to differences in protein composition. As a result, we decided to take another look at HIC.[6]

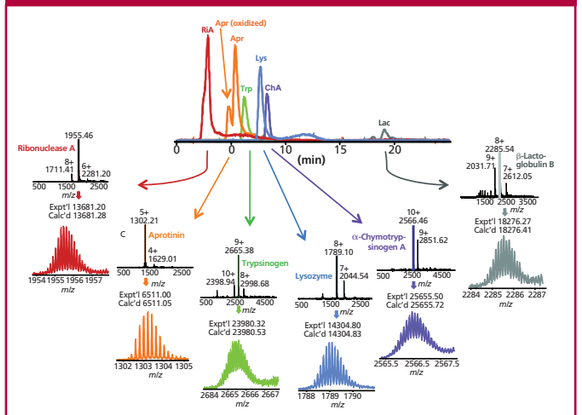
The use of HIC would be practical if a volatile salt could be used. Suitable salts such as ammonium acetate are poor at promoting retention in the HIC mode. Literature on the subject has involved concentrations in the 3–4 M range, which is too high for a mass spectrometer. Now, the more hydrophobic a material is, the less salt is needed for retention in HIC. We decided to increase the hydrophobicity of the stationary phase systematically in an effort to produce materials that could retain proteins at concentrations of ammonium acetate that were practical for MS analysis. Increasing the length of the functional ligand in the coating from C_3 to C_4 to C_5 resulted in dramatic increases in protein retention, in keeping with past observations about HIC.[7] However, lengthening the ligand from C_5 through C_{10} did not result in much change in protein retention times (Figure 1). Furthermore, in this range the retention of some of the protein standards was not directly proportional to the concentration of ammonium acetate (Figure 2). This

Figure 2: Retention of RNAse and CHYGEN as a function of initial salt concentration: (a) PolyHEPTYL A column, (b) PolyOCTYL A column. Mobile-phase A: ammonium acetate concentration as noted; mobile-phase B: 20 mM ammonium acetate with 50% acetonitrile; gradient: 15 min, 0–100% B, then 5 min at 100% B. Other running conditions were the same as in Figure 1. (Adapted with permission from reference 6.)



behaviour is normally associated with reversed-phase LC rather than with HIC. A concentration of 0.75–1 M ammonium acetate is still necessary in the starting mobile phase, but its function seems to be

Figure 3: HIC separation of standard proteins and QTOF-MS analysis. Column: 100 mm × 0.2 mm, 3-µm d_p, 1500-Å PolyHEPTYL A capillary; mobile-phase A: 1 M ammonium acetate; mobile-phase B: 20 mM ammonium acetate with 50% acetonitrile; gradient: 15-min linear, 0–100% B, then 5 min at 100% B; flow rate: 2.4 µL/min; detection: QTOF-MS. (Adapted with permission from reference 6.)



the maintenance of the tertiary structure of the proteins rather than promotion of binding. Mass spectrometers can handle such concentrations. The presence of some organic solvent in the final mobile phase was essential for elution of most proteins within the gradient. All in all, the behaviour of these new HIC materials resembles that of reversed-phase LC as much as HIC. The distinction between the two modes seems to have been blurred if not erased.

This project seems to have been successful in adapting HIC to the requirements of MS for top-down proteomics. It should be possible to adapt ion-exchange chromatography for this purpose as well. Figure 3 demonstrates that standard proteins are eluted from the new materials with good peak shape. Direct elution into a mass spectrometer produced mass spectra characteristic of proteins with their native structures intact. Notwithstanding the high concentration of acetonitrile in the final mobile phase, the kinetics of the chromatography was evidently faster than the kinetics of denaturation. Figure 4 is a demonstration of direct HIC–MS of an extract of *E. coli* proteins. A protein as large as 206 kDa was identified.

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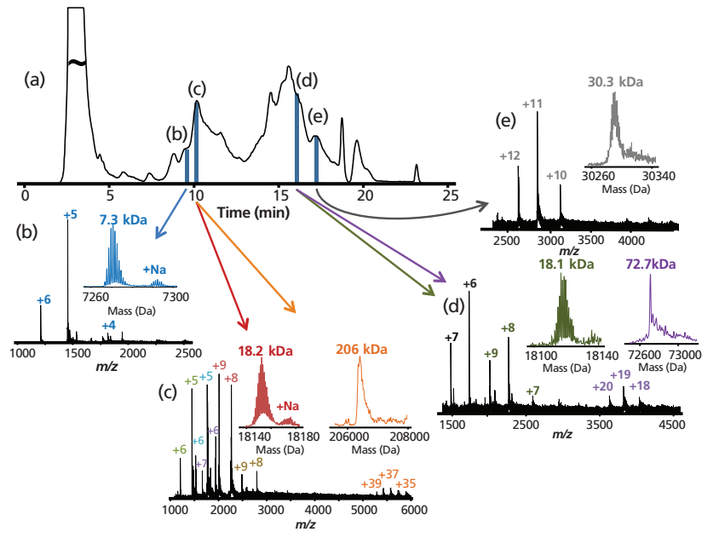
Figure 4: HIC–MS analysis of an *E. coli* lysate. Column: 100 mm × 0.2 mm, 3-µm d_p, 1500-Å PolyHEXYL A capillary. Other conditions were as in Figure 3. (a) TIC (10-fold zoom-in) of *E. coli* proteins; (b–e) average spectra and deconvoluted spectra from colour-coded regions as shown. (Adapted with permission from reference 6.)



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The 6th International Network of Environmental Forensics (INEF) 2016

The 6th International Network of Environmental Forensics (INEF) 2016 conference will be held from 27–30 June at Örebro Castle in Örebro, Sweden.

The International Network of Environmental Forensics (INEF) was created in 2008 to provide a forum for scientists, environmental consultants, regulators, and attorneys to share information regarding the use of environmental forensics. The INEF conference is designed to provide a formal and informal setting to engage scientists working in this field, and to allow opportunities for in-depth discussion and questioning. Environmental forensics is the use of scientific techniques to identify the source, age and timing of a contaminant into the environment. The conference allows multidisciplinary expertise in natural sciences, such as chemical fate and transport; environmental, analytical, and isotope chemistry; environmental sampling, toxicity, and statistics; and also knowledge of law and communication to be bought together.

The conference will include a series of hands-on short courses followed by three days of plenary talks by invited leading scientists and oral and poster presentations from selected abstracts. The technical sessions of the conference will cover a wide variety of environmental topics including: contaminant and microbial source apportionment and tracking; use of isotope ratios in forensics applications; biomonitoring and human exposure; identifying emerging organic and inorganic environmental contaminants; multidimensional gas and liquid chromatography (GC×GC, LC×LC); applications of new technologies (atmospheric pressure gas chromatography [APGC]; development of signature techniques to source and age contaminant release; teaching in environmental forensics — challenges and strategies; and advanced forensic techniques — visualization, modelling, statistical analysis.

A number of keynote speakers have been invited to the conference. Kim Esbensen will present a short course on Monday 27 June focusing on "The Theory of Sampling". This will be followed by environmental case studies from both the inorganic and organic field. The programme will also have

a number of plenary lectures including "Robotics Olfaction and Environmental Forensics" by Amy Loutfi of Örebro University (Örebro, Sweden); "POP Measurements: Tracking Down the Part in the Quintillion" by Jef Focant, University of Liège (Liège, Belgium); "Detective Work in Food and Feed Incidents with Dioxins and PCBs" by Rainer Malisch from the Dioxin Laboratory, State Institute for Chemical and Veterinary Analysis of Food (Freiburg, Germany); "Environmental Forensics, Its elementary!" by Gwen O´Sullivan, Mount Royal University (Calgary, Alberta, Canada); and "Future of Environmental Forensics" by Court Sandau, Chemistry Matters (North America). Other leading scientists who will be presenting during the conference include Glenn Johnson, Jack Cochran, Paul Geladi, and Henk Bowman.

As well as the series of high-quality lectures there will also be breakout periods with poster presentation sessions and exhibitor booths for delegates to browse with complimentary refreshments provided. There will also be talks from students currently studying environmental forensics. To boost student participation INEF are offering several travel grants to prospective students to help them attend the conference. Students are also encouraged to enter the student competition for the best presentation when submitting their abstracts. The top three students will all receive a generous monetary prize.

The conference venue is medieval Örebro castle situated in the centre of Örebro city in the heart of Sweden. Örebro offers many interesting tourist attractions, the most famous being the castle, which dates back to the 13th century. Örebro has a rich cultural life with the County Theatre, the Swedish Chamber Orchestra, which performs at the Concert Hall, and various museums and galleries. The city also offers a wide range of sporting and leisure activities in the town, in the mountains, and by Lake Hjälmaren — the fourth largest lake in Sweden. Örebro is the capital of cycling in Sweden. Cycling in Örebro is easy; there are many cycle lanes and it is possible to hire a low-cost municipal bike easily.

The conference follows the major Midsummer holiday in Sweden. To continue these national festivities all delegates will experience the traditions of a Midsummer dinner on the Tuesday. During the Wednesday the conference dinner will be held at medieval Örebro Castle where dinner will be accompanied with entertainment by a historian. Daring delegates will also be invited to a ghost tour after dark!

E-mail: **inef2016@oru.se**
Website: **www.inef2016.com**

Training Courses

GC

The Theory of GC
Website: http://www.chromacademy.com/gc-training.html

Complete GC and GC–MS
13–17 June 2016
The Open University,
Milton Keynes, UK
Website: http://www.anthias.co.uk/training-courses/completeGC

The Technique of GC in Three Parts — Fundamentals/ Troubleshooting/Method Development
14 July 2016
Reading, UK
Website: www.hichrom.co.uk

HPLC/LC–MS

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

Practical HPLC Method Development
7–8 June 2016
Caledonian University,
Glasgow, UK
Website: http://www.crawfordscientific.com/training-online-calendar.asp

Basics of HPLC, Column Care, Troubleshooting, & Method Translations (4 Short Seminars)
5 July 2016
Manchester, UK
Website: www.hichrom.co.uk

HPLC Masterclass
2–24 August 2016
Laserchrom HPLC Laboratories Ltd,
Rochester, Kent, UK
Website: http://www.hplccourses.com/index.htm *(please mention* The Column *when you make an enquiry)*

SAMPLE PREPARATION

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

Sampling Techniques for GC & GC–MS
12 October 2016
The Open University, Milton Keynes, UK
Website: http://www.anthias.co.uk/training-courses/sampling

MISCELLANEOUS

Basic Lab Skill Training
Website: http://www.chromacademy.com/basic-lab-skills-training.html

Introduction to IR Spectroscopy
Website: http://www.chromacademy.com/infrared-training.html

GPC/SEC

Light Scattering and Viscometry Hands-On Training
23–24 June 2016
Mainz, Germany
Website: www.pss-polymer.com

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

Event News

28 August–1 September 2016

31st International Symposium on Chromatography (ISC 2016)
University College Cork, Cork, Ireland
Co-Chairs: Apryll Stalcup and Jeremy D. Glennon
Tel: +353 1 280 2641
E-mail: ISC2016@mci-group.com
Website: http://www.isc2016.ie/

26–29 September 2016

International Symposium on GPC/SEC and Related Techniques
Novotel, Amsterdam, Netherlands
Tel: +1 508 482 3129
E-mail: joy_longa@waters.com
Website: www.gpcevent.com

13–16 November 2016

27th International Symposium on Pharmaceutical and Biomedical Analysis
Guangzhou, P.R. China
E-mail: pba2016@hotmail.com
Website: www.PBA2016.org

30 November–2 December 2016

2nd ACROSS International Symposium on Advances in Separation Science (ASASS 2)
Hobart, Tasmania, Australia
Chairman: Brett Paull
E-mail: brett.paull@utas.edu.au
Website: http://www.utas.edu.au/across

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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.