Emerging Separations Technologies

A report on the meeting organised by The Chromatographic Society and the Separation Science Group, Analytical Division of the Royal Society of Chemistry, on Thursday, 26th March 2015 at RSC Burlington House, Piccadilly, London.

A one-day symposium, organised by The Chromatographic Society and the Separation Science Group, Analytical Division of the Royal Society of Chemistry, was held at RSC Burlington House, Piccadilly, London, on the 26th March 2015. There were almost ninety attendees who attended the event, including attendees from the six sponsors and exhibitors who kindly supported this event. This was the first ever meeting in this planned series and it was designed to provide an insight into the latest separation science technologies, with the timing being such that it was an ideal opportunity to engage customers who were not able to attend the PittCon event in the USA where new products are normally launched. The meeting covered new innovations in column technologies, instrument technologies (specifically on the theme of multi-dimensional chromatography) and novel applications and utilisation in life sciences. The impressive scientific programme gave attendees a range of highly interesting topics from highly regarded international speakers and also new emerging professionals making it a highly interesting event.

The morning session was chaired by the principal event organiser, Dr Adrian Clarke (The Chromatographic Society / Novartis Pharma AG, Switzerland), who introduced the first speaker of the day, Professor Peter Schoenmakers, University of Amsterdam (The Netherlands) who is a ChromSoc Martin medal recipient. Peter as usual gave an excellent and highly informative presentation on 'the current status and novel developments in multi-dimensional chromatography'. This was a comprehensive update on 2D LC x LC acknowledging the work of other pioneers in the field as well as highlighting his own group's work and often drawing comparisons with its GC equivalent and the work of Luigi Mondello. Initially he discussed the principal benefit of two-dimensional chromatography, which is the greatly enhanced separation power compared to one-dimensional chromatography, stating that this is particularly critical when sample complexity increases.

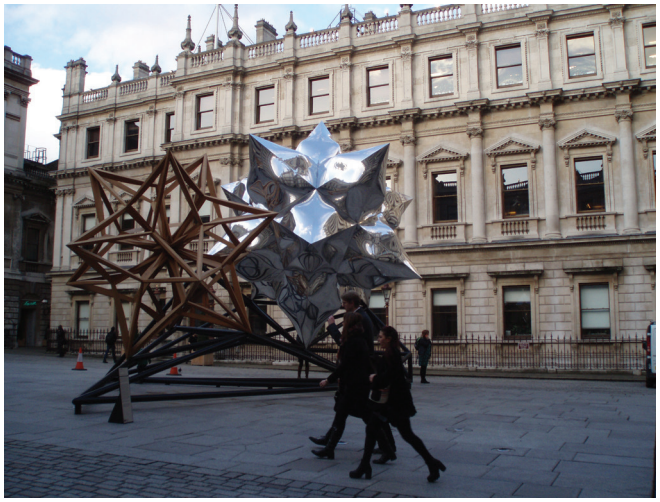
He then went onto comparing and discussing the relative merits of both two-dimensional GC (2D-GC) and two-dimensional LC (2D-LC) where he focused the talk on showing that that comprehensive 2D-LC (LC×LC) is now also gradually approaching maturity as good equipment and adequate data-analysis software are now commercially available. Thus, LC×LC is ready to find more applications stating there are two directions in which these will materialise: (1) separations that cannot be achieved without the combination of two different selectivities and (2) separations of very complex samples, which require the maximum possible separation power. He used synthetic polymers as a clear example of the first category, highlighting that when polymers feature more than one type of intermolecular

Two student attendees at the meeting. Joe Russell from the University of the West of England and Lucy Evans from the University of Sheffield.

distribution (e.g. molecular weight and chemical composition) they can be rigorously and elegantly characterised by LC×LC there are few alternatives because of the low compatibility of mass spectrometry and synthetic polymers. For the separation of very complex samples LC-MS (and LC-MSn) yields a lot of structural information on the sample and its constituents. But quantification is cumbersome, because standards (and frequent calibration) are required for each analyte and because matrix effects are often hard to negotiate. Although LC×LC yields little structural information it often allows better and easier quantitation and LC×LC-MS is the most attractive option as structural information can be obtained and matrix effects can be drastically reduced and quantitation is performed using a separate LC detector. Thus, there are plenty of incentives for the proliferation of LC×LC.

He set the scene for the remainder of the meeting and the future of 2D-LC by discussing the fact that there are also a few bottlenecks that are gradually being addressed. These include the required analysis time, the incompatibility of (first-dimension and second-dimension) eluents, the cumulative dilution in multi-dimensional separations and the time, effort and knowledge required to develop separations.

The second presentation entitled 'Comprehensive 2D GC combined with novel MS instrumentation: powerful unified tools for the analysis of complex samples' by Professsor Peter Quinto Tranchida (University of Messina, Italy, sponsored by Shimadzu), a late replacement for his colleague Professor Luigi Mondello who was unable to attend the event. Comprehensive 2D GC was first developed by Phillips et al. in 1991 [1], and in the authors' opinion can be classed alongside the invention of open tubular capillary columns as the two most



Venue for the Emerging Separation Technologies Meeting – Burlington House, London. The home of the Royal Society of Chemistry.

important innovations in the GC field. In this respect, the separation-power leaps from the packed to the capillary column, and from the latter to GC×GC, are comparable. The talk covered the principles of GC×GC and GC×GC-MS and then discussed the group's present research work which focuses on the combination of GC×GC systems with recent mass spectrometry (MS) instrumentation, such as rapid-scanning quadrupole (Q) and triple quadrupole (QQQ) MS. It was shown that such powerful separation-science tools are unified ones in as much that they can cover practically all the requirements of an untargeted, targeted or fingerprint-based application. The present state-of-the-art of advanced GC×GC-MS instrumentation was illustrated with several applications involving complex samples (Analysis of Allergens in Perfume, Headspace solid-phase microextraction GC×GC-qMS analysis of pesticides in drinking water and finally the analysis of sulphur compounds in coal tar using on-line LC-GC×GC combined with QQQ MS. He discussed various potential future scenarios such as new stationary phases (e.g. ionic liquids) and compatibility of MS for the analyte classes could further extend the application of comprehensive 2D GC.

He concluded by saying that the popularity of GC×GC-MS is continuously increasing (as demonstrated by the significant increased number of GC×GC-MS publications during the 1999-2014 period), as both hardware and software become more robust and easy to use. Cryogenically-modulated GC×GC-MS are unified tools suitable for both untargeted and targeted analyses and the use of QqQ MS boosts the selectivity and sensitivity in targeted experiments. Finally, he touched upon LC-GC×GC-MS as a potential separation-science 'magic box' covering a great number of the possible requirements for the analysis of mixtures of volatiles.

After the first two presentations, the symposium moved on to a series of vendor presentations from the sponsors, which were all of an extremely high scientific quality. The first presentation was given by the ChromSoc 2012 Jubilee medal winner Dr Monika Dittmann (Agilent Technologies, Waldbronn, Germany) on the topic of High Resolution Impurity Profiling using Multiple Heart-Cutting 2D-LC. Unlike full comprehensive 2D-LC where each fraction coming from the first dimension is analysed and very fast second dimension runs are required. Whereas in the heart-cutting mode usually only one cut per peak of interest is analysed in the 2nd dimension. This way the second dimension is decoupled from the first dimension and second dimension runs can be longer without compromising total separation speed. In full comprehensive mode each fraction coming from the first dimension is analysed and very fast second dimension runs are required. This presentation focused on the possibilities offered by a third mode of operation namely multiple heart cutting 2D-LC that allows for high resolution separation of regions of interest without sacrificing speed of separation. This mode of operation was shown to be particularly useful for quantitative impurity analysis as shown in specific application examples from Dow Chemicals and pharmaceutical impurity profiling. The remainder of the presentation discussed elegant software solutions and improvements showing that it is now more user friendly and practical.

Dr Ken Cook, (Thermo Fisher Scientific, UK) then presented on the topic of 'Innovations in UHPLC and multi-dimensional chromatography'. This showed that UHPLC is now common place in laboratories and commercially available from several instrument manufacturers. He discussed several design concepts and bottlenecks, which need to be overcome, including column chemistry for multiple application areas (e.g. MAbs, IEX, SEC, reversed phase). In addition he discussed the novel approaches to multidimensional chromatography in UHPLC and stated the 1st dimension need higher sample loading capacity and challenged the concept that the 1st dimension should be on a narrower, longer column and the 2nd dimension using a small wider-bore column, for fast analysis time. Employing high resolution in both dimensions and stated the optimisation of each separation dimension is essential. Many approaches to this require optimisation of each instrument module, including complex plumbing, additional pumps and specific timing of valve changes. He discussed the implementation of high resolution & speed fractionation and re-injection to simplify multidimensional chromatography and extend the level at which this can be achieved with ease.

This was followed by lunch and a vendor exhibition, which was a welcome opportunity for delegates to have a break, enjoy some food, and take a look at the latest novel developments from the instrument and column vendors as well as doing some networking. The second half of the lunch break contained a vendor workshop from Dr David Bell, Sigma-Aldrich, Bellefonte, Pennsylvania, USA. The topic covered was 'Method development using alternative stationary phase and chromatographic modes'. This was a unique aspect of this particular meeting. The educational workshop focused on that many of the problems encountered executing HPLC methods are a result of decisions made during the early method development process. The objective of this workshop was to discuss the critical variable of stationary phase chemistry. In practice, many analysts will reach for their favourite C18 upon commencement of method development; however alkyl (Cn) phases are often not the most appropriate tool for a given separation. When retention or resolution is not readily achieved, analysts will often employ the addition of ion-pair reagents or resort to other complicated mobile phase preparations to force them to work. These common practices often result in the development of complex methods that suffer from lack of transferability, robustness and ruggedness.

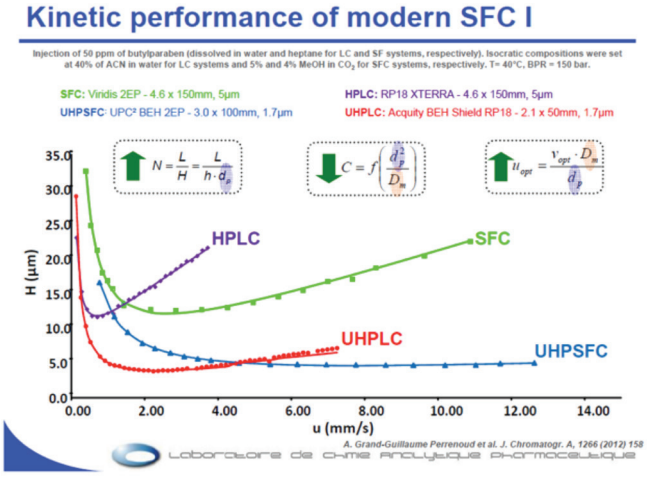
There are many choices for alternative stationary phase chemistries that render the phase decision difficult. In this workshop two stationary phase classes were discussed, namely embedded polar group (EPG) and aromatic that are highly complementary to alkyl phases from a fundamental molecular interaction point of view. To Bell's credit, this was not a vendor hard-sell sales pitch, He went into detail on the chemistry of these phases and constantly discussed

them in the context of the Euerby / Petterson approach to stationary phase characterisation. For the embedded amide phase at least, he backed up 'highly complementary' with data on poor log k v log k data compared to C18 (r = 0.44 for 30 – 40 compounds). The work on the PFP (pentafluorophenyl-) phase was illustrated by separations on diphenols, flavonoids and tamoxifen and metabolites.

Alternative modes of separation, such as HILIC were also discussed. An understanding of the contrasting interactions that these different classes of stationary phase chemistries and chromatographic modes provide leads to more effective decisions during the process. It was claimed that this critical information promises to facilitate method development and generate simpler and more reproducible separations. Bell was able to show quite clearly when a partition mechanism would take place and when ion-exchange would take place, relating this to the thickness of the water layer and noting that even PFP- phases exhibit some ion-exchange behaviour. The ion-exchange eluent of the retention can be suppressed by using a buffer.

The afternoon session was chaired by the chairman of The Chromatographic Society, Dr Paul Ferguson (AstraZeneca R&D, UK) who gave a warm introduction to the first speaker, Dr Stephanie Harden, from Waters, Switzerland. She presented on the ACQUITY QDa Mass Detector, specifically 'Applications in small molecule and biotherapeutic analysis'. The QDa detector was launched in 2014 and is proving very popular for a range of applications due to its versatility, ease of use and low maintenance burden (having only two user serviceable parts the disposable sample cone and inlet capillary). The mass detector has a mass range up to 1250 m/z and is available in a standard and high sensitivity configuration (through the addition of an additional rotary pump), and is compatible with all Waters LC instruments and purification systems. This latter point was demonstrated by the low level detection of a potential genotoxic impurity (PGI) 1-phenyl piperazine in SIM mode with an LOD of 0.1 ppm and an LOQ of 0.5 ppm (%RSD = 1.5%). Dr Harden also highlighted the precision of the instrument for the analysis of the non-chromophoric surfactant / drug excipient CTAB with RSDs of less than 1.0%. As well as small molecule analysis, the application of the QDa to peptide analysis was presented. For glycan analysis of mAbs, Waters have developed the RapiFluor MS derivitisation tag, which includes quaternary amine functionality for MS detection (the previous AccQ Fluor tag, provided only fluorescence/UV detection enhancements). Finally, Dr Harden highlighted useful new functionality in Waters Empower 3 CDS which allows for automated mass ion labelling of peaks.

Dr Davy Guillarme, School of Pharmaceutical Sciences, University of Geneva, Switzerland gave an excellent reminder of the potential of SFC in life-sciences 'The use of modern supercritical fluid chromatography in pharmaceutical analysis'. Dr Guillarme started his presentation with a potted history of SFC stretching back to 1960 and a comparison of the chromatographic properties of gas, liquids and supercritical fluids. He also discussed the latest generation of SFC instruments, namely the Agilent Aurora and Waters UPC2 systems noting that the extra column dispersion of the systems (85 µl2) was still too large to maximise system chromatographic performance by this approach. However, UHPSFC provides significantly better chromatographic performance than UPLC at much lower back-pressures providing higher efficiency and higher speed separations. Dr Guillarme continued his presentation focusing on the analysis of basic pharmaceutical compounds offering alternative approaches via mobile phase additives or appropriate stationary phase selection. In all presented cases, a Waters BEH silica column with a 0.1% NH4OH mobile phase additive performed better than the 2-EP stationary phase without modifiers. He then went on to discuss the potential for superficially porous particles in SFC illustrating that they could provide faster and more efficient separations than sub-2 micron fully porous materials. He also demonstrated the versatility of SFC for analysing structural isomers, and the broad range of molecule hydrophobicities that can be measured via this approach ranging from lipids (log D ~10) to peptides (log D ~-5) - with more hydrophilic analytes such as polysaccharides under investigation. Dr Guillaume concluded his talk with research on doping control for drugs of abuse in sport. The use of UHPSFC-MS/MS provided similar performance to UHPLC but with complementary orthogonality and relatively straight-forward sample preparation for complex matrices such as urine.



The third presentation in the session was provided by Dr Matthew Lewis (MRC-NIHR Phenome Centre at Imperial College London) highlighting the technical rigour required when analysing huge sample sets required for genome analysis and the work involved in processing the data and extracting information from them in his presentation 'Application of Hyphenated Techniques in Population Phenotyping'. He started by setting the scene on how microbes in gut flora can change human genetics and these flora populations are in turn influenced by diet. The centre's work studies 'molecular epidemiology' probing for subtle effects within populations or sub-populations of individuals with unique and dynamic metabolomes analysed through identification of biomarkers in body fluids. The use of high efficiency separation techniques coupled with mass spectrometry (MS) is mandatory for these activities. Time-of-flight MS instruments are used for biomarker discovery and triple-quadrapole MS is required for quantitative measurements. However, the need to identify biomarkers requires the study of massive sample sets in order to identify biomarkers. A large component of this work is therefore the industrialisation of sample batching and continuous sample analysis and Dr Lewis gave an eloquent insight into the maximum analysis time allowable for analysing sample sets>1000 based on, available working patterns, instrument constraints and sample stability which was 15 minutes running at high flow rates on long columns to maximise peak capacity. Analysis times greater than this may lead to aging and metabolome change. This can be corrected to some degree by data smoothing, but it is imperative that true variance within the sample sets is maintained and analytical variance is minimised. Reliability

and reproducibility of the analytical method is therefore key. In terms of MS performance, for quantitative phenotyping signal sensitivity is much more important than MS spectral resolution but a continuous decrease in MS signal intensity is observed with continual analysis from sample matrix contamination and great attention is paid to this. He also noted the issue of analysing large sample sets in terms of the volume of data produced which can be in the order of 9.6 Tb per day! Some of this is achieved by deoptimising the MS signal, data feature extraction and removing data on the fly from analytical systems for on-line processing, but work continues in this area.

The final session was chaired by Dr John Langley (Separation Science Group of the RSC/The University of Southampton). The first speaker was Dr Andreas Gargano, University of Amsterdam) who presented on the novel topic of 'Active modulation in comprehensive LC×LC'.

In order to achieve high separation efficiency, LC×LC setups are typically based on a small internal-diameter first-dimension column coupled to a second-dimension column with larger diameter. Shallow gradients making use of low volumes of mobile phases can be applied to the first separation dimension (1D), while fast analysis at high flow rates can be run in the second dimension (2D). Limitations of this approach include the low loadability of the first-dimension and the dilution caused by the increase in column diameter in going from 1D to 2D. Moreover, the relatively high flow rates used for the second dimension make this combination difficult to directly hyphenate to mass spectrometry.

Gargano discussed a study aimed at demonstrating that solid-phase-assisted modulation (active modulation) can be implemented in an on-line comprehensive two-dimensional LC (LC×LC) setup to overcome this paradigm. Active modulation makes possible to choose virtually any combination of first- and second-dimension column diameters without loss in system performance. In this setup a 0.30-mm internal diameter first-dimension column with a relatively high loadability is coupled to a 0.075-mm internal diameter second-dimension column. This actively modulated system is coupled to a nano-electrospray high-resolution mass spectrometer and applied to the separation of the tryptic peptides of biologically relevant samples. This was another impressive LC x LC discourse, but like Schoenmakers before him, Gargano did not really spend much time on the difficulties associated with the required orthogonality of the two phases used or the possibility of using the second phase for modulation as well as separation.

Following Andrea, David Bell (Sigma-Aldrich, USA) gave his second presentation of the day, this time providing the audience with an update on the column technology area, entitled 'Column technology: current status – looking forward, blue sky, novel developments'. Although liquid chromatography is often considered a mature technique, there have been numerous advances over the past decade. In this presentation some of the more important developments in particle technology, device designs and instrumentation were discussed. Particular attention was paid to how small sub-2 µm and superficially porous particles have changed the HPLC and hyphenated HPLC landscape. Newer developments in monodispersed particle technology and how it is expected to fit into the future of HPLC were also covered. Bell did well to avoid duplicating material from his lunchtime session and concentrated on particle geometry rather than stationary phase chemistry. The presentation was concluded with Insights into a few interesting up-and-coming integrated devices.

Chair, Langley introduced the final speaker of the day, Professor Peter Myers (Department of Chemistry, University of Liverpool), who as always provided a challenging and thought provoking lecture which provided the ideal finish to an excellent event. His talk on 'Separations without Silica' started by reviewing developments in chromatography instrumentation much less favourably with developments in NMR, MS and even UV and IR instrumentation over the same time span.

"Now look at the developments in the chromatography instrumentation over the past 20 years. What has changed?," he stated. In many chromatographic instruments not a lot has changed. In liquid chromatography we have pumps that can pump at higher pressures. However, that is only to accommodate the availability of sub 2-micron particles. Maybe the advantages of the sub 2-micron particles is all that has changed in that manufactures say they provide faster analysis times, higher resolution, higher sensitivity and lower costs

But 95% of all HPLC and UPLC columns are manufactured from silica. Reviewing the specifications of the silica, he pointed out that.it still has the same surface area, it still has the same pore volume and for the majority of materials it still has the same pore size.

Similarly he went on, the hydrophobic bonding applied to the silica. 20 years ago was generally C18 bonded onto the silica via a silane bridge. Today the most common bonding is a C18 bonded onto the silica via a silane bridge.

Professor Myers proposed that it is time we moved away from silica particles to progress chromatography. His view was that it was time to look at new alternatives to using the old amorphous silica that has caused so many problems over the years and has held back the development of separation science. His alternatives to simply continuing to use silica-based materials included chromatography on a CD, addressing issues of detection, and use of a self-indicating liquid crystal-based stationary phase which gives qualitative information on the nature of stationary phase – analyte interaction were some way from the market. However these ideas were thought provoking and demonstrated Myers' out-of-the-box imagination and very good breadth of scientific knowledge. This was a lively, fitting final talk of the day.

The meeting was finally closed by Dr John Langley on behalf of the Separation Science Group of the RSC, offering gratitude to the speakers for their excellent scientific contributions in this impressive scientific programme. Thanks were also given to the committee members of The Chromatographic Society and the Separation Science Group of the RSC, and also to the hospitality staff of RSC Burlington House for their efficient organisation of the event. As ever, The Chromatographic Society is greatly indebted to the sponsors and exhibitors (Agilent Technologies, Sigma Aldrich, Shimadzu, ThermoFisher Scientific, Waters, and HiChrom); without whose generous financial support such events could not be held. Profuse thanks were proffered.

Chromatographic Society bursaries to attend the meeting were awarded to Joe Russell (University of West of England) and Lucy Evans. Joe and Lucy (pictured) respectively will be writing about the benefits of ChromSoc bursaries (for the ChromSoc website) and the role of chromatography in her PhD studies (for ChromCom, a new members only Chromatographic Society newssheet).

References:

1. Lui ZY and Phillips JB. J. Chromatogr. Sci. 1991, 29, 227-231