

Celebrating 25 years of Biomedical Chromatography

A report on the Royal Society of Chemistry, Separation Science Group Meeting, held at the Wellcome Centre, London, 25-26th November 2011

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A meeting to celebrate twenty-five years of the journal *Biomedical Chromatography* took place at the Wellcome Centre, London, 25–26 November 2011. The event was organised by the Separation Science Group of the Analytical Division, Royal Society of Chemistry, with the second day held in co-operation with the British Academy of Forensic Science. As well as a celebration of the journal, the occasion also marked the achievement of Chang Kee Lim, founding Editor and now Editor-in-Chief. The event across the two days attracted over 80, mainly UK-based, delegates.

The meeting was opened by Chang Kee Lim (University of London, UK), who reviewed the history of the journal. He discussed the inception of the journal in 1985 on the initiative of Heyden and Son in London, and how it has grown to the twelve issues that are now produced each year by Wiley. An aim that has been maintained is clear, detailed presentation of methodological detail. In the early days most of the papers submitted for publication concerned the use of GC (with or without MS), with HPLC being still in its relative infancy. Now, however, most submissions involve use of HPLC–MS or other hyphenated techniques.

Chang Kee was in turn thanked and presented with a token of appreciation for his efforts with *Biomedical Chromatography* by Paul Trevorrow, Executive Journals Editor at John Wiley and Sons, Ltd. The Chair of the Separation Science Group, John Langley (University of Southampton, UK), then gave an account of the contribution Chang Kee had made to the development of separation science in general, and to the field of porphyrin analysis in particular, work that had resulted in the elucidation of several previously unknown metabolic pathways. John was then pleased to present Chang Kee with the Separation Science Group's Knox Medal (Fig 1). The award was initiated in 2009 and named after Professor John Knox FRS, University of Edinburgh, and one of the original members of the Editorial

Board of Biomedical Chromatography, and recognises an outstanding contribution to separation science over a prolonged period.

The morning's scientific session was chaired by John Langley and featured five presentations on the theme of 'Analytical Developments'. John Halket (King's College London, UK) reviewed the development of GC and GC-MS techniques. He showed how the size and overall complexity of the instruments had decreased over the last 25 years. Added to this better mass spectral libraries, peak deconvolution software, and detectors that can measure at attogram sensitivity had been developed, although overall the range of biomedical applications of the technique had hardly changed. Next, Laura Owen (University Hospital of South Manchester, UK) spoke on the problems and pitfalls of using LC-MS/MS in clinical chemistry. The great growth in the use of the LC-MS/MS methods had been accompanied by greater awareness of traps that lay in wait for the unwary. These included matrix effects (ion suppression/ion enhancement), isobaric effects and interferences, the formation of adducts and clusters, and problems caused by impurities in reagents and plasticisers. Next, Paul Thomas (Loughborough University, UK) and Christopher Benton (University of Leicester and King's College Hospital London, UK) spoke in turn on aspects of ion mobility MS. Essentially this is a gas phase electrophoretic technique that separates ions according to their apparent shape, size, mass, and charge, commonly known as their collisional cross-section.

Paul gave an insightful talk on the recent developments in ion mobility hardware and showed how the technique is being miniaturised. The potential of the technique in finding people trapped following an earthquake or other disaster by the sensitive detection of natural metabolites in breath was discussed. Chris extended the theme to the measurement of 5-aminolaevulinic acid, porphobilinogen, and porphyrins, and showed how ion mobility MS could be used as a screening tool in the diagnosis of porphyrias. The method has the great advantage of being able to separate and detect fragment ions and doubly-charged ions, allowing additional identification and structural characterisation. In addition, travelling-wave ion mobility spectrometry has allowed the separation of interfering contaminants, previously misinterpreted as porphyrin precursors. Finally, James Heaton (King's College London, UK) and discussed the use of hydrophilic interaction liquid chromatography (HILIC, also known as normal phase chromatography using unmodified silica) with MS for the analysis of basic drugs.

The theme of the afternoon session, chaired by David Perrett (University of London, UK), was 'Clinical Applications'. Melissa Hanna-Brown (Pfizer, UK) first gave a perspective ('Pisse Pot to

Metabolomics') of 2000 years of biomedical analysis. Of particular note was elegant work undertaken by Charles Dent (University College Hospital, London, UK) using two-dimensional TLC that led to the discovery of a many diseases associated with amino acid metabolism. She also highlighted work from the mid-1960s at the Oakridge National Laboratories, USA that used a range of chromatographic and spectroscopic approaches for profiling biological fluids. Next, Lewis Couchman (King's College Hospital, London, UK) outlined the complexity the human vitamin D metabolism and discussed some of the analytical challenges caused by the C3-epimerisation of the compound, and the problems of finding suitable reference standards for LC-MS/MS vitamin D assay. Although many clinical laboratories undertake vitamin D analyses routinely, the analytical challenge is in fact complex since there are many possible metabolites of this vitamin. In time, there could even be 'vitaminomics'!

Lewis was followed by Neil Dalton (King's College London, UK), who showed how modern LC-MS/MS instruments with multiple reaction monitoring are capable of detecting a wide range of inborn errors of metabolism using a single plasma sample and/or a dried blood spot. The technique includes a suite of isotopically-labeled internal standards to aid in accurate quantification of target metabolites, the forerunner perhaps of a metabolomic approach to the diagnosis of genetic disorders. In contrast to this 'bottom-up' approach, largely theoretical aspects of metabolomics were the subject of the talk by Lee Williams (University of Sunderland, UK). Although the different ways in which the large data sets generated in some such studies could be statistically analysed and displayed were discussed, as yet a clinical link was missing in the approach outlined. Clinical considerations were, however, the theme in the final presentations. Firstly, operation of HPLC-based assays for the diagnosis of haemoglobinopathies were reviewed by Martin Jarvis (North Middlesex University Hospital, London, UK). He showed that all haemoglobin A2 methods in common use gave different mean results on the UK National External Quality Assessment Scheme and that many laboratories were incapable of reliably distinguishing between normal and β -thalassaemia carriers. Marked differences in results for some rare haemoglobin variants between 'in-house' and proprietary HPLC methods were also reported. Finally, Phillip Morgan (King's College Hospital, London, UK) gave a brief history of the development of point-of-care devices and showed the range of systems currently being used within different health care settings. He highlighted some of the developments that are taking place with devices such as lab-on-a-chip systems, micro-pillar array chromatographic columns, ion-mobility spectrometry (e.g. for emergency analysis of ethanol and other volatiles), single cell assays, and the potential for personal devices that can offer a range of biochemical tests.

The second day was chaired Robert Flanagan (King's College Hospital, London, UK), the theme being toxicology/forensic science. David Holt (St George's, University of London, London, UK) opened proceedings by discussing aspects of hair analysis especially as regards use of ethyl glucuronide as a marker for ethanol consumption. Although many drugs and metabolites can be detected and measured in hair at high sensitivity, there are a range of issues that often confound interpretation of results in individual cases such as the possibility of external contamination, or loss of analyte following cosmetic treatment. Moreover, there is a great lack of control data for naturally occurring analytes including ethyl glucuronide and γ -hydroxybutyrate (GHB). This was followed by a talk delivered by David Perrett on behalf of Hannah Brown (Queen Mary, University of London, UK) on the analysis of steroids (testosterone, stanozolol) in fingernail clippings from body-builders and control subjects. After hydrolysis in strong alkali, analytes were extracted into ethyl acetate and analysed by LC-MS. Preliminary results suggest that fingernails could provide an alternative biological matrix for detecting illicit steroid use.

Paul Russell (Unilever, Northampton, UK) gave an overview of the use of dried blood spots in clinical chemistry and in pharmacokinetic studies. Using dried blood spots (typical spotted volumes are 10-20 μ L) can allow for serial bleeding of, for example, laboratory animals such as rats and thus can not only reduce the number of animals that are needed for a study, but also costs. There remain issues related to comparability to conventional blood sampling in human pharmacokinetic studies, but direct analysis of spots using DESI or DART techniques, or the special interface LC-MS developed for this purpose by CAMAG, are interesting developments. Finally, Andrew Taylor (Royal Surrey County Hospital, UK) reviewed trace elements analysis in clinical samples using ICP-MS. Although the technique has advantages including high sensitivity and multi-analyte capability, as with LC-MS/MS there are traps for the unwary. These include polyatomic (for example $^{40}\text{Ar}^{2+}$ on $^{80}\text{Se}^+$, $^{156}\text{Gd}^{2+}$ on $^{78}\text{Se}^+$) and isobaric, such as $^{48}\text{Ca}^+$ on $^{48}\text{Ti}^+$, interferences. An area with huge potential was 'metalomics' – the ICP-MS does lend itself to interfacing with LC, and studies of Se metabolism have identified six Se-containing species in human cerebrospinal fluid, for example.

The final session was opened by Susie Davies (Analytical Services International Ltd, London, UK), who described methods used to monitor the rapidly-changing 'designer drug' scene in the UK. New compounds are continually synthesised and sold, often over the internet, mainly with the intent of being outwith controlled drug legislation. She described the methods employed to detect and identify these compounds and the difficulties often associated with identifying them in urine. Next, Simona Francese

(Sheffield Hallam University, UK) reviewed the forensic applications of MALDI-MS imaging and showed how the method can not only resolve overlapping fingerprints, but also detect cocaine in a fingerprint. Clearly some of these developments have major implications in criminal investigations, although there is of course an added cost.

Simona was followed by Atholl Johnston (Bart's and The London, Queen Mary, University of London, UK) who discussed the problems associated with counterfeit and sub-standard drugs entering the market place via a number of sources. The World Health Organization estimates that 25 % of drugs traded in developing countries are counterfeit, but the problem is increasingly recognized in Western countries as well. Sub-standard drugs are also a major problem, with little control over manufacturing processes in many parts of the world, lack of control of potentially or actually toxic impurities being just one issue. The role of analytical chemistry is important on monitoring quality, but many developing countries do not possess adequate analytical facilities to support a laboratory inspection system even if one was introduced.

The concluding lecture was delivered by Rudi Fortson QC (Queen Mary, University London, UK), who highlighted some of the many problems associated with controlled drug legislation, both nationally and internationally. It was becoming impossible for legislators to keep up with the plethora of new drugs that are becoming available using definitions that rely on chemical structures, whilst use of other statutory measures such as the Medicines Act in an attempt to control sales is problematic because many such compounds are ostensibly marketed as 'plant food' or some such similar product. On the other hand, simply abandoning attempts at control had much wider implications than many realised. The underlying problem was the willingness of young and sometimes not-so-young people to put at risk their health or even their lives by taking such compounds in the first place.

Overall, the conference overall was a great success and was a fitting tribute both to the journal and to Chang Kee. We thank the staff of the Wellcome Centre for their efficient organisation of the event, and two the main sponsors (John Wiley and Sons Ltd. and ThermoFisher Scientific) and the other exhibitors (Agilent Technologies, Anatune, Crawford Scientific, Presearch, Shimadzu UK Ltd. and Sigma-Aldrich). Without their generous financial support the event could not have been held.