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Lewis Couchman[1]* and Tony Edge[2]

[1]Viapath, King's College Hospital, London, UK

[2]ThermoFisher Scientific, Runcorn, UK

*Corresponding author: Tel 0203 299 5881 e-mail: lewis.couchman@nhs.net

A report on the meeting organised by The Chromatographic Society and the Separation Science Group, Analytical Division of the Royal Society of Chemistry, Guy's Hospital, London, 19th September 2014

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 The Roben's Suite, Guy's Hospital, London, on the 19th September 2014. There were over ninety registrants who attended the day, including attendees from the six sponsors and exhibitors who kindly supported this event.

The morning session was chaired by Lewis Couchman (Viapath, King's College Hospital, London), who introduced the first speaker of the day, Richard Kay from LGC (Fordham, UK). Richard gave an excellent and highly topical presentation on the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the quantitation of plasma proteins and peptides. After a quick introduction to the work carried out at LGC, Richard moved on to describe a comparison of results from MS-based techniques with immunoassays for clinical assays, considering the cost-per-test, applicability and ease of use of each approach. The top-down and bottom-up protein analysis workflows were described, and were clearly illustrated using examples of clinically relevant proteins, from high-abundance target analytes such as factor seven-activating protease (FSAP) and its mutant form, Marburg I, via medium abundance insulin-like growth factor I (IGF-I), through to low-abundance glucagon.

Clinical analysis using dried blood spots (DBS), as described by Robert Guthrie in the 1960s, revolutionised the screening of newborn babies for phenylketonuria. After just over 50 years of DBS analysis, it was timely that the second speaker, Neil Dalton from Evelina London Children's Hospital, gave us an overview of the use of DBS and dried urine spots (DUS) in clinical analysis. He started the discussion with a history lesson of blood spotting and how it became used as a routine method for the analysis of in the classical heel prick tests used on new borns. Neil then moved on to describe how DBS have been employed in his own laboratory for diagnosis of rare metabolic diseases, before moving on to explore some of the challenges associated with this technique, including the well-documented problems associated with variation in haematocrit between samples for quantitative analysis, and the difference between plasma concentrations and red blood cell concentrations for some analytes. In summary, Neil concluded that despite these known problems for quantitative analysis, DBS and DUS have been an invaluable tool in clinical diagnostics for many years, and these analyses have no doubt contributed to the successful treatment of many newborn children worldwide.

After the first two presentations, the symposium moved on to a series of short vendor presentations from the four gold sponsors of the event, which were all of a very high quality. The first presentation was given by Mike Oliver (ThermoFisher Scientific), who discussed some of the issues associated with bioanalysis, focussing on the matrix effects caused in particular by phospholipids. Mike described the issues of 'memory effects', and the impact this can have on analytical systems, in terms of maintenance schedules and also interferences with the detector. This was followed by an introduction to a novel, frit-less, solid-phase extraction (SPE) technology, which can overcome some of the issues of voiding associated with traditional SPE cartridges. Mike demonstrated application of this method by presenting data of a method developed for the analysis of a series of 21 naturally occurring and synthetic opiates in urine, including phase 2 metabolites.

Following Mike, Pete Christensen (Agilent Technologies) presented some excellent data illustrating the use of novel RapidFire™ SPE technology, a stand-alone technique that is easily integrated with a detector, typically mass spectrometry, which permits the analysis of samples every 15 seconds for ultra-high throughput applications. Pete presented data on the analysis of methadone and its primary metabolite, EDDP, in urine samples, highlighting that it was possible to have very high-speed analysis without compromise of the data integrity. The second example was that of therapeutic drug monitoring (TDM) of tacrolimus, everolimus, sirolimus, & cyclosporin A. Again, the data presented showed comparable results to more traditional LC/MS-MS approaches, but with results generated ten-times faster.

The third vendor presentation was delivered by Jason Wrigley (Sigma, UK), who discussed the analysis of 25-hydroxyvitamin D metabolites, an ever-topical subject amongst clinical scientists. Jason discussed the importance of choosing the correct analytical column when developing a method for clinical analysis, and gave an excellent demonstration of this by discussing the rapid chromatographic resolution of isobaric 25-hydroxyvitamin D and 3-epi-25-hydroxyvitamin D species using an Acsentis™ F5 column. Jason also discussed the use of specialised phospholipid-removal plates, describing how this technology worked using the selective nature of zirconia for the extraction of phosphates, and provided some excellent data showing the successful removal of commonly-occurring phospholipids from samples.

The final presentation of the morning session was given by Andrew Davison (Royal Liverpool & Broadgreen Hospitals) on behalf of Phenomenex Ltd. Andrew continued from the previous presentation by highlighting the importance of 25-hydroxyvitamin D analysis, and emphasised that high-throughput, robust analytical techniques are required to meet the ever-increasing clinical demand. Continuing the 'phospholipid-removal' theme of the vendor presentations, Andrew presented some compelling data demonstrating the use

of Phree™ phospholipid removal plates for successfully depleting clinical samples prior to LC-MS/MS analysis, and discussed how this may reduce the frequency of instrument maintenance and instrument robustness.

Lunch followed the vendor presentations, which was a welcome opportunity for delegates to have a break, some food, and a chat to old colleagues and new friends. This is the one aspect of this particular meeting that is quite unique. The delegates were made to feel at home and everybody was engaged in the discussions whether it was with vendors or with other delegates.

The afternoon session was chaired by Dave Perret (Queen Mary University of London, UK) who gave a warm introduction to the first speaker, Nicola Gray from the Phenome Centre at Imperial College London. She presented a brief overview of the very impressive activities at Imperial College, before proceeding to the main part of her presentation which described the analysis of amino acids using LC-MS/MS. Nicola guided the audience thorough the thorough development of the derivatisation-based sample preparation and LC-MS/MS methodology, which has allowed the isolation and identification of thirty-eight amino acids in less than eight minutes. Nicola also discussed future plans for scaling down the method to reduce solvent consumption and sample volumes used. The data presented were very impressive, and it is clear that this application will have significant benefits for patients.

Following Nicola, Andrew Davison gave his second presentation of the day, this time providing the audience with a more general, high-level overview of the application of LC-MS/MS technology in routine, high-throughput clinical laboratories. Andrew started the presentation by highlighting the problems of the current situation in the UK, where it may be argued that clinical laboratories have suffered due to an over-reliance on fully-automated analytical platforms to generate clinical results, resulting in a somewhat de-skilled workforce unfamiliar with the analytical processes and rigours required to develop novel methods based on chromatographic and mass-spectrometric technologies. Andrew then went on to provide an insightful overview of some of the various approaches to sample preparation for a range of clinical applications, including further discussions of SPE and specialised SPE including phospholipid depletion, but also other techniques such as turbulent flow chromatography, immuno-affinity extraction, liquid-liquid extraction and finally simple sample dilution.

Analytical considerations aside, an incredibly important concept during method development is that of assay calibration. With this in mind, Sarah Belsey (Viapath, King's College Hospital, London) presented some very encouraging data produced using a novel approach to assay calibration for LC-MS/MS methods. In this approach, multiple isotopically-labelled internal standards were added to each sample at different concentrations covering the calibration range, and these are used as 'surrogate' calibrators to produce individual, fully matrix-matched calibration curves for each injection. The presentation raised some valid discussion points regarding the regulatory aspects of such an approach, though it was noted by all the delegates who asked questions that this was a fascinating concept that would reduce the workload in the lab and also potentially improve the quality of the data being produced as it would remove the need to try and match the sample matrix when producing calibrators.

Following a short coffee break, Tony Edge was handed the chair for the final session of the day, and he introduced his colleague Norman Ramsey (ThermoFisher Scientific). Norman gave the audience a whistle-stop tour of the world of electrochemistry, demonstrating that it was not always necessary to use LC-MS/MS for clinical applications. Indeed, data were presented to suggest that there were occasions when the use of electrochemical detection was favoured over LC-MS/MS, in particular where data on the oxidation state of a molecule is sought. Norman also gave some examples of how the sensitivity of LC-MS/MS could be enhanced using an electrochemically-induced modification to a compound before entering the ion source. Norman also discussed how it was possible to generate 'metabolites' of some compounds electrochemically, and that by using this approach it was possible to generate sufficient amounts of these 'metabolites' in a single overnight analysis to satisfy the requirements for structural NMR characterisation.

The penultimate presentation was an enthralling one delivered by the head of MS at Waters Corporation, and Lecturer for the British Mass Spectrometry Society, Mike Morris. Mike gave an overview of clinical LC-MS/MS method development, highlighting some of the common mistakes and misconceptions associated with the technique, but also discussing a number of the ongoing issues regarding assay validation and assay standardisation. Mike started his presentation by listing some of the fundamental requirements for the development of a successful clinical assay using LC-MS/MS, including sample stability, column stability as well as assay calibration, providing exemplary data for some commonly prescribed therapeutic drugs. Mike also presented some thought-provoking data, showing the inter-laboratory variation that exists when using calibration standards prepared in-house. Whilst laboratories invariably had the ability to produce linear calibration curves, the slope of these curves differed, sometimes significantly so. With this considered, a number of questions followed Mike's presentation regarding the best use of reference materials, and in particular whether solid reference materials (which require weighing prior to use) of reference solutions (which may degrade or suffer stability problems) would be preferable.

Once the reference material debate had concluded, Tony introduced the final speaker of the day, Zoltan Takats (Imperial College London), who described some of his groups' excellent work carried out using ambient mass spectrometry and associated techniques. Zoltan gave an overview of the use of desorption electrospray ionisation (DESI) for MS imaging of tissues, both for providing spatial information regarding the distribution of drugs within tissues, but also using sophisticated principal component analysis of unique mass spectral 'fingerprints' for the identification of tumour cells. Zoltan proceeded to describe the use of rapid evaporative ionisation mass spectrometry (REIMS) for real-time, in vivo mass spectrometric identification of tumour tissue. In this approach, electrosurgical tools are used to generate ions which are transferred to a mass spectrometer, allowing data interrogation against a spectral database in real-time, providing almost instantaneous feedback to the surgeon regarding whether the tissue they are cutting is healthy or not. Zoltan certainly provided a truly fascinating finish to an excellent event.

The meeting was finally closed by Tony Edge. Thanks are due to the committee members of The Chromatographic Society and the Separation Science Group of the RSC, and also to the hospitality staff of Guy's Hospital for their efficient organisation of the event. Finally, we thank the sponsors and exhibitors (ThermoFisher Scientific, Agilent Technologies, Sigma Aldrich, Phenomenex, HiChrom, Gilson, and Chromsystems); without their generous financial support the event could not have been held.