The 18th International Reid Bioanalytical Forum

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Figure 1. University of Guildford – The venue for the 18th International Reid Bioanalytical Forum

The International Reid Bioanalytical Forum, initiated by Dr Eric Reid in 1975, is held every two years and offers an opportunity for industry, academia and analytical vendor organisations to come together and discuss the latest issues around bioanalysis. The Forum is run by the Forum Syndicate under the auspices of a sub-committee of the Chromatographic Society and draws on members from academia and industry. Its current members are Dr Howard Hill (chairman and co-organiser), Dr Derek Stevenson, Forum organiser, Dr John Lough, Treasurer, Dr Stephen Westwood, Dr Robin Whelpton, and Dr Ian Wilson.

The 18th International Reid Bioanalytical Forum was once again held on the Campus University of Surrey, from Monday 6th – Thursday 9th July 2009 with 120 registered delegates. Lectures were held in the Austin Pearce Building with the adjacent exhibition hall providing space for vendor stands and a poster exhibition.

The presentations, stretched over three days covering a number of topical subjects including the new Metabolites in Safety Testing (MIST) guidelines, dried blood spot analysis, large molecule analysis, incurred sample reproducibility (ISR) and an update on current thinking on the subject of bioanalytical method validation from the FDA. Interspersed amongst these were also a number of scientific vendor presentations. There were two social networking events organised on the Tuesday and Wednesday evenings. On Tuesday, the evening meal and welcome drinks were served at Denbies vineyard, England's largest vineyard situated in Dorking, Surrey. On Wednesday evening the conference banquet and pre-dinner drinks were served in the University Season restaurant on campus.

Mini-Exhibition

As an integral part of the symposium, Tuesday saw 12 scientific vendor companies with tabletop displays of their latest product offerings. On Wednesday, it was the turn of the bioanalytical CROs which included ABS Labs, Cyprotex Discovery Ltd, Quality Assistance, York Bioanalytical, Quotient Bioresearch, Covance, BASI and HLS.

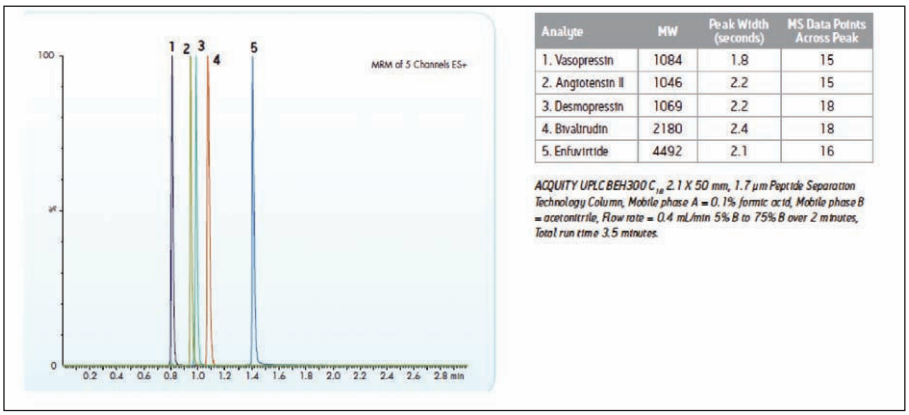
Company	Key Products On Display	Contact
Biotage	Sample preparation consumables including SPE and SLE extraction cartridges	www.biotage.com Tel: +46 18 56 59 00
Grace	VisionHT UPLC columns	www.discoverysciences.com Tel: +44 (0)1480 32 4430
Hichrom	Hypersil Gold, Nucleodur and Zorbax RRHT UPLC columns and chromatographic training services	www.hichrom.co.uk Tel: +44 (0)118 930 3660
IDBS	Advanced data management solutions	www.idbs.com Tel: +44(0)1483-595000
Laboratory	Electronic laboratory notebooks Data Solutions	www.labnotes.com Tel: +44 (0) 1904 686067
Presearch	Laboratory solutions including separation equipment, automation equipment and supplies	www.presearch.co.uk Tel: +44 (0) 1256 365492
Shimadzu	Recent Developments in Column and Instrument technologies	www.shimadzu.co.uk Tel: +44 (0)8708 375209
Spark Holland BV	Online SPE extraction systems for sample cleanup	www.sparkholland.com Tel : +31 591 631700
Supelco Analytical	Ascentis™ LC Columns	www.sigma-aldrich.com Tel: 0800 717181
Thermo Fisher Scientific	Hypersil Gold UPLC columns	www.thermo.com Tel: +44 (0)1442 233 555
Varian	HPLC systems and a wide range of column types and packing materials including Pursuit® and Polaris® LC Columns	www.varianinc.com Tel: +44 (0)1865 291 500
Waters	Acquity UPLC™ hardware and columns	www.waters.com Tel: +44 (0)208 238 6100

The Scientific Program

Session 1 - Mixed theme including metabolites in safety testing (Chaired by Derek Stevenson)

Derek Stevenson opened the spring symposium with a welcome address and then passed to Howard Hill speaking on 'The Changing Face of the Pharmaceutical Industry: Impact on Bioanalysis'. He outlined the drivers influencing the industry including new analytical technologies and the rise in the number of large molecule therapeutics undergoing development. He commented that the regulatory bar is being raised, in part by pharma companies, to exclude new players. Also, the increasing importance of India & China to pharma companies in the market place both as cost effective areas of the world to carry out drug development activities but also, with the emerging wealth of both, potentially huge markets to sell drugs. The closing remarks were directed towards the Bioanalytical Forum itself. As part of the organising committee, Howard invited comments on where the future of the meeting lay

The following two presentations were on the subject of the recently published FDA guidelines on Metabolites in Safety Testing (MIST). Firstly Dennis Smith (Pfizer) outlined the development in thinking that has led to the guidelines and highlighted the danger of using plasma metabolite concentration alone in classifying major and minor metabolites as the volume of distribution may mask the true toxicological potential of a metabolite. He then went on to describe the four classifications of drug side effects, type A, B, C & D and illustrated each with examples. Mark Seymour (Xceleron) described the advantages of using of Accelerator Mass Spectrometry (AMS) in combination with C14 microtracer doses of drug for metabolite identification in Phase 1 studies. This negates the requirement for separate mass balance studies saving development time/money. The final presentation of the session was from Michelle Gradley (Novacta Biosystems) who described the synthesis of metabolite reference standards using microbial systems which have been shown to have the capacity to mimic mammalian metabolism of drugs, carrying out the same highly region and stereoselective modifications as seen in the liver and other organs.



Analyte	MW	Peak Width (seconds)	MS Data Points Across Peak
1. Vasopressin	1084	1.8	15
2. Angiotensin II	1046	2.2	15
3. Desmopressin	1069	2.2	18
4. Bivalirudin	2180	2.4	18
5. Enfuvirtide	4492	2.1	16

ACQUITY UPLC BEH300 C_{18} 2.1 X 50 mm, 1.7 µm Peptide Separation Technology Column, Mobile phase A = 0.1% formic acid, Mobile phase B = acetonitrile, Flow rate = 0.4 mL/min 5% B to 75% B over 2 minutes, Total run time 3.5 minutes.

Figure 3. Slide from Anne-Marie Orkild's presentation on 'Development of Selective and Sensitive Bioanalytical Methods for Peptide Therapeutics in Human Plasma'.

Session 2 - Mixed theme including peptide quantification (Chaired by Jaap Wieling)

Magnus Knutsson (Ferring) presented on 'Quantification of peptide drugs in biological fluids of low pg/mL levels using LC-MS/MS'. He outlined the generic approach to large molecule analysis employed at Ferring. Molecules <5000 amu are analysed by LC-MS/MS whilst >5000 amu remain the realm of immunoassay. With method sensitivity remaining the main challenge with LC-MS/MS analysis of large molecules, he described the use of large sample volume extraction using SPE (Oasis WCX), miniaturisation of coupled LC columns (use of 1.0 mm i.d.) and a Sciex API5000 instrument enabling LLOQs of 5 pg/mL to be achieved for some peptide molecules. However, the potential for non specific binding to some polypropylene plates and solubility of proteins and peptides remain challenges that must be overcome during method development. The final presentation of this first day was from Chris Harrington (University of Surrey) on 'High accuracy and high sensitivity methods for analysis of platinum cancer drug DNA adducts in cells'. The cytotoxic efficacy of platinum-DNA adducts and the relationship between adduct concentration in tumour cells and blood is not well understood. By measuring these biomarkers, information on a patient's clinical drug response could be obtained. By coupling a highly specific enzyme based adduction isolation method with a sensitive detection based on HPLC coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) it was possible to measure platinum-DNA adduct formation from a number of different sample types. The methodology opens up the possibility to tailor cancer treatment to an individual's potential to form and repair DNA-adducts, making for a targeted treatment regime.

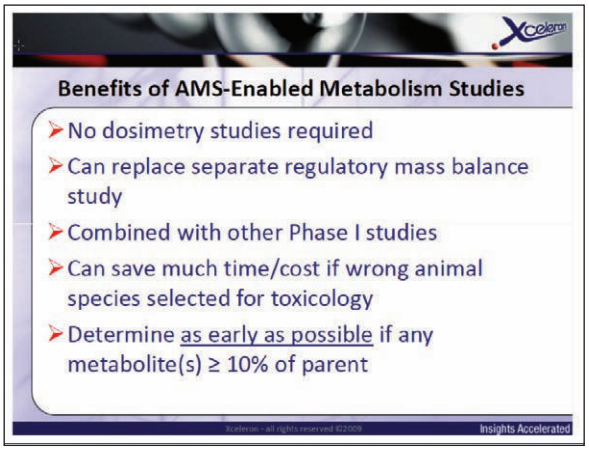


Figure 2. Slide for Mark Seymour's presentation on 'HPLC-AMS: seeing through the MIST'.

Session 3 - Mixed theme including a number of vendor presentations (Chaired by Robin Whelpton)

This session, covering the Tuesday morning of the conference, included a number of vendor presentations. These included Anne-Marie Orkild (Waters) on 'A Comprehensive Approach to Developing Selective and Sensitive Bioanalytical Methods for Peptide Therapeutics in Humana Plasma, Klaus Buckendahl (Sigma-Aldrich) on 'Hybrid SPE in the removal of Phospholipids and Proteins in Biological fluids', Jonathan Coffey (Shimadzu) on 'Analysis of impurities in streptomycin and dihydrostreptomycin by hydrophilic interaction chromatography/electrospray ionisation quadrupole ion trap/time-of-flight mass spectrometry' and Pär Davidsson (DiLab) on Automated Blood Sampling.

Mark Bayliss (Pfizer) presented on 'Halo Columns; what are they, what are their advantages' describing the pelicular particles as "inside-out maltesers" i.e. the honeycomb bit on the outside. His practical tips were that a pre-column filter must always be used, they can be used with ballistic gradients, are only 20% less efficient than sub 2 µm columns, can be used back-to-front, up to temperatures of 80oC and in his experience,

will last for up to 6000 injections. He illustrated the use of the columns with applications including the analysis of cholesterol and fluticasone as well as peptide analysis. His conclusion was that they are a convenient and cheap alternative to uHPLC.

Richard Houghton presented on 'Generic approach to validation of small molecule LC-MS/MS biomarker assays'. He described the successful use of synthetic surrogate matrix calibration procedures to overcome the presence of endogenous analyte. This was illustrated with data from a number of steroid and amino acid assays that had been validated using this approach. Martyn Hlhorst (PRA International) presented on 'Quantitative Determination of steroids using LC-MS/MS. He describes the theory behind Atmospheric Pressure Photoionisation (APPI) and illustrated the potential for sensitivity gains over more conventional APCI of ESI ionization techniques for the analysis of steroid molecules.

Session 4 - Dried Blood Spot Analysis (Chaired by Ian Wilson)

This 90 minute session featured a series of talks from Matthew Barfield (GSK), Tony Edge (AZ), Graeme Clark (Pfizer), Lee Goodwin (Covance) and Shirish Yakkundi (Queens University, Belfast) discussing the experiences of each of these organisations with the technique of dried blood spot analysis (DBA). GSK have pioneered the technique and have the most experience in this area. They have validated over 60 bioanalytical assays using this technique of which two compounds are now in Phase 1 clinical development. The advantage of using DBA is that it only requires very small volumes of blood allowing serial bleeds from preclinical species such as mice. This leads to much better preclinical pharmaco and toxicokinetic data. The logistics of collecting DBA samples is much simpler in the animal facility or clinic with blood being spotted onto special filter paper card and then allowed to dry for 2 hour. Samples are then stored at room temperature in plastic bags containing a desiccant. Under these conditions, samples have been shown to be stable for some considerable time. Samples can be posted in the mail, avoiding the conventional difficulties of shipping frozen plasma packed on dry ice.

With demonstration of good stability of labile metabolites, good reproducibility of sampling across blood spot (avoiding the "halo"), simple solvent extraction methodologies and comparative (and in some cases superior data) to plasma, the technique is fast becoming established in discovery and preclinical arenas of large pharma companies. With some challenges still to overcome, such as method sensitivity, this is a technique that looks like it is here to stay.

Session 5 – Incurred Sample Reproducibility (Chaired by Derek Stevenson)

This final short session of the day featured a single presentation from Frank Mullins (Icon Development Solutions) on 'Case Studies in Incurred Sample Reanalysis – Problems Encountered'. How to measure Incurred Sample Reanalysis has been a hot topic of discussion around the industry over the last couple of years. However, this is only part of the challenge of ISR facing the bioanalyst. As the industry comes to some consensus about measurement, the bigger issue now is how to investigate cases of failed ISR. The presentation centred around two case studies describing the investigation process. The conclusions were that validation of assays in blank control matrix don't highlight issues involving labile metabolites. Pooled incurred samples are required to support freeze/thaw and long-term stability data. Constant vigilance is required by bioanalytical laboratories and speedy communication of sample instability must be communicated effectively to sample collection facilities.

Session 6 – Comparing Immunoassay Technologies (Chaired by Ray Briggs)

Wednesday's first session was delivered by John Allinson (Veeda) (FIBMS) who gave a comprehensive overview of emerging immunoassay technology platforms. John compared open immunoassay systems including Luminex, Mesoscale Discovery (MSD), Grifols Triturus and the Gyros Gyrolab, with closed system clinical analysers for immunoassays used in diagnostic labs such as the DPC Immulite. John asked the question 'Why Multiplex?' and presented the various advantages of using a multiplex approach including reduced cost, time and sample volume. However validation of a multiplex assay can be complex and failure of one analyte to meet acceptance criteria can lead to many repeats.

A more elegant approach which has all the advantages of multiplexing without the pitfalls of multiplexing is the single assay platform the Gyros Gyrolab. John presented interesting work which has been carried out on this platform on three biomarkers of Alzheimer's disease Abeta amyloid 1-40, Abeta amyloid 1-42 and total Abeta in CSF and plasma illustrating the benefits of the Gyrolab compared with DELFIA or Luminex.

Figure 4. The MesoScale Discovery and the Gyros Gyrolab Immunoassay platforms

Session 7 – Unwanted immunogenicity (Chaired by Ian Wilson)

Geoff Hale (BioAnaLab) kicked off the session presenting some of the challenges of anti-drug antibody (ADA) testing for the bioanalyst. With the rise in the number of biopharmaceuticals such as monoclonal antibodies in the drug development pipeline, ADA testing is becoming an increasingly important part of the regulatory package. An immune response to the drug is part of the body's natural defence's, gaining an understanding of this response is key to the success of the drug. Some biopharmaceuticals may be non immunogenic, an ideal situation, however where antibodies are evoked they can fall into a number of categories. Some ADA will have no effect, again a good result for the pharma company, however others may affect PK/PD by neutralising the biological effects decreasing efficacy, while others may lead to serious adverse events such as a reaction with a native protein. Recent white papers and EMEA guidelines were covered in this talk are leading to a common understanding of the validation parameters for these quasi-quantitative assays. From Geoff's presentation it is clear challenges still remain in the availability of appropriate positive controls, establishment of assay cut points, requirement for sensitivity and establishing the clinical relevance of the assay data, with many of these factors having to be assessed case-by case.

Christopher Kirton (Huntington Life Sciences) followed with a talk entitled In vitro cytokine release' posing the key question is this novel antibody therapeutic going to induce cytokine release syndrome (CRS)? This has been seen with antibodies such as Rituximab and more recently TGN1412, leading to release of TNF- , IFN- and IL-6, which can lead to serious adverse events. A bead-based muliplex immunoassay using flow cytometry detection has been set up and early results were presented at this meeting showing its potential to as a tool to predict CRS risk of a novel drug in vivo.

Small molecule re-analysis was picked up in the session too continuing the theme of the importance of this topic throughout the meeting with Jaap Wieling (Xendo) talk entitled 'Accuracy of incurred samples'. To complete the session before lunch David Perrett (Queen Mary, University of London) gave a rather gruesome demonstration of the inadequacy of sterilisation/decomtamination procedures in use in hospitals and dentists. Current procedures do not lead to the complete removal of Prion Proteins (PrP), the cause of new variant Creutzfeldt-Jakob disease (CJD). Even at very low levels PrP present a risk of cross-infection. Prion proteins are highly hydrophobic and have a high affinity for stainless steel, the material used for most surgical instruments. However new reagents are being developed and patented at Queen Marys to reveal contaminant PrPs in surgical instruments which will significantly reduce risk of cross-infection.

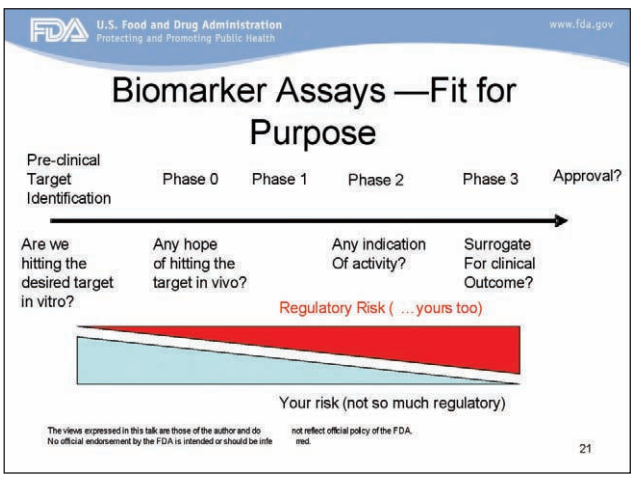


Figure 5. Slide from Brian Boothe's presentation on 'Bioanalytical Method Validation: (some of) What the FDA is thinking'.

Session 8 – Regulatory approaches to evolving technologies (Chaired by Steve Westwood)

Brian Booth (FDA) joined the meeting by teleconference and covered (some of) what the FDA is thinking offering a personal view on current bioanalytical guidelines. Amongst the take home messages from Brian were that some issues of method validation are clear cut, while others depend on the situation. How much validation is required really does depend on the particular goal and everyone has a responsibility to ensure that the method is fit for its ultimate purpose. Brian concluded with the comment that the FDA can't (won't) figure everything out.

An impromptu discussion was held covering the 2008 EMEA Concept Paper on Bioanalytical method validation including Graeme Smith and Howard Hill (HLS) and Ray Briggs. Overall there were mixed views about the wisdom of 'another' set of guidelines which may lead to lack of clarity about validation requirements, however some delegates felt that having EMEA guidance would push the field forward, and challenge the FDA, which would be welcomed. The final session of the day chaired by John Lough (University of Sunderland) stayed on a large molecule theme with Jaap Weiling (Xendo) and Matt Ewles (Covance) presenting on immunoassays with elemental tagged antibodies and an introduction to the Quantification of proteins using proteolytic digestion by LC-MS/MS.

Session 9 – MS of Biological analytes (Chaired by John Smeraglia)

John Smeraglia (Pfizer) chaired this session with three talks given by Eric Ezan (CEA Institute of Biology and Technology), Chris Barton (Quotient Bioresearch) and Hendrick Neubert (Pfizer). The theme of this session was the application of LC-MS/MS to proteins, and its potential to supersede traditional immunoassays. Eric lead with a side-by side comparison of ELISA vs LC-MS. The initial challenge laid down by Eric was that immunoassays are fundamentally flawed due to problems with lack of concordance of standards across platforms and questions the specificity of antibodies (what is measured? Free, bound or total analyte?). Therefore it is essential we look for alternatives. Could mass spectrometry be the answer?

Eric made a strong case for mass spectrometry as an alternative in the future particularly for biotherapeutics and in immunogenicity testing, linking in well with some of the challenges presented by Geoff Hale in earlier session. Further evidence was presented by Chris where the application of BioMS to biomarker testing has crossed species from equine testing looking at markers of drug abuse in horses, driven by the absence of suitable immunoassay methods) to the clinic where human samples are now being tested.

Hendrik brought the whole story together presenting his work on the development of an assay for low abundance targets of antibody therapeutics using immunoaffinity enrichment and LCMS-MS. This work demonstrates the power of combined approaches to answer complex sophisticated questions about percentages of free and bound drugs.

Session 10 – Concluding session (Chaired by Robin Whelpton)

In this final session John Stobaugh (University of Kansas) presented work on the evolution of chemical reactions for determination of protein 3-nitro-tyrosine, followed by Gavin O' Connor (LGC) talking about protein quantification of mass spectrometry.

Conference closing comments

John Lough (President of the Chromatographic Society) closed the conference commenting that it had been a good conference from a scientific standpoint with a wide variety of topics covered. Attendance was very good taking the present economic climate into consideration. (although down by around 20-30% on previous years). John thanked the organising committee and major sponsors of the event (Quotient Bioresearch Ltd, Waters and York Bioanalytical solutions).