The Chromatographic Society Spring Symposium & Annual General Meeting

Banqueting Suite, Stadium of Light, Sunderland - May 30-31th, 2012

As trailed in the previous issue of Chromatography Today (5(2)), the 2012 Chromatographic Society Spring Symposium was held in Sunderland on Wednesday, 30th May and Thursday 31st May, a relatively focussed event to mark the quarter of a century existence of the University of Sunderland's BSc Chemical and Pharmaceutical Science course (provider of many graduate separation scientists for the UK pharmaceutical industry) having metamorphosed into a more wide ranging event. At a time of austerity and hardship for R&D in the UK pharmaceutical industry (itself one of the themes addressed at the meeting), around 90 delegates assembled at the Stadium of Light. With restrictions on travel prevailing, delegates from Big Pharma were thin on the ground but, gratifyingly, there was a good proportion of students in the audience thereby allowing the Chromatographic Society to fulfil one of its aims of fostering the separation scientists of tomorrow.

The meeting got off to a disappointing start - it was announced that it would not be possible to hold Stadium tours (the pitch and arena were being prepared for imminent concerts by Coldplay and luminaries such as Bruce Springsteen and the Red Hot Chilli Peppers)! However, such let-downs were soon behind the audience once first speaker Chris Riley (Riley and Rabel Consulting) got going on '60 Years of Liquid Chromatography for the Determination of Impurities in Pharmaceuticals'. While Chris's talk did in fact start with paper chromatography, in introducing the question of whether analytical developments precede pharmaceutical breakthroughs or vice versa, most of his time was spent on recent examples of impurity determinations. In particular, he focussed on genotoxic impurities. Until the late-1990s, the focus of the ipharmaceutical ndustry was on so-called 'ordinary' impurities, which were regulated by the ICH Guidelines Q3A and B. Of the three key parameters in pharmaceutical analysis (speed, selectivity and sensitivity), sensitivity had been less of issue than the other two, due to the ever increasing need for faster cycle times. However, he declared that the need to measure ppm levels of mutagens and neurotoxins in pharmaceuticals had now brought the need for greater sensitivity to the forefront, challenging the limits of conventional techniques such as UV-DAD and bringing more sensitive detectors, such the mass spectrometer into routine application in pharmaceutical analysis. Not unnaturally given his current position as a consultant on quality and regulatory matters for the pharmaceutical industry, Chris concluded with a brief discussion of risk-based approaches to the control of genotoxic impurities in pharmaceuticals.

The Spring Symposium was the fourth ChromSoc meeting to have been held at the Stadium of Light - so, unbelievably, one of the tasks set was to obtain different images of the venue from those used the last time. The Stadium is very close to the city centre but seen here from the other side of the River Wear it appears to have a positively rural setting

Another excellent overview lecture in the traditions of Spring Symposia of the 90's was given by Adrian Clarke (Novartis). Adrian, the first of eight University of Sunderland graduates on the programme, spoke on 'Chiral Separations in the Pharma Industry - Current Status and Future Possibilities'. He discussed conventional screening

approaches to chiral LC method development using short columns and isocratic mobile phases which showed an 85% success rate for a set of around 50 compounds. A polar organic screen using Chirobiotic® macrocyclic antibiotic phases was also used. This proved to be complementary to the normal phase derivatised polysaccharide screen and highest success rates (96%) were obtained by combining both screens. There was not too much difference in selectivity when using immobilised derivatised chiral stationary phases (CSP) rather than the original coated versions but of course the use of immobilised versions (IA, IB) allowed the use of non-standard mobile phases. It was noted on the Chirobiotic CSP that the most recent versions are better for preparative work and that they exhibit a degree of class selectivity with e.g. Chirobiotic V being best for β-blockers. Despite the high success rates of the screens Adrian reminded the audience that other CSP such as the immobilised-protein Chiral-AGP phase were still needed. Also mentioned were the ion-exchange QD-AX and QN-AX CSP, new chlorinated derivatised immobilised polysaccharides from Phenomenex and higher generation Pirkle CSP. The IC was said to be the best of the Daicel immobilised polysaccharides. In fact Adrian's coverage of recent developments was comprehensive. GC and CE screens even got a mention but most notably he discussed experiences with SFC at Novartis. It was used in conjunction with MS detection so that the trace enantiomer impurity in chiral drugs could be distinguished from other impurities. Also chiral SFC had proved to be a considerable success as a production technique.

After a short interval, the afternoon resumed with Tim Liddicoat (ThermoFisher) discussing 'SPE-LC-MS/MS Analysis of Biological Extracts' with the focus very much on ion suppression and how to eliminate it. As an illustration he pointed out that with gradient elution phospholipids in plasma, if only protein precipitation is used as sample pre-treatment, take eight column volumes to clear the column. The problem is of course much exacerbated when repeat injections are made. The use of sample preparation techniques, it was pointed out, can significantly reduce matrix effects. Tim compared the degree of extract cleanliness from solid phase extraction and protein precipitation protocols carried out on human plasma and gave particular attention to the use of full scan Q1 MS data (contour plots) of multiple extract injections under different extraction conditions to investigate the effectiveness of matrix removal.

Young separation scientists at the Spring Symposium; (left to right -Jaspreet Sihra (University of Surrey), Beng Tan (University of Sunderland), Mina Kalantarzadeh (University of Surrey), Jo Gan (BP Laboratory), Dorota Nawrot (University of Surrey)

A major development in the pharmaceutical industry is the increasing move towards using biopharmaceuticals as medicines. However, given that tis very issue had been the theme for the previous ChromSoc event in March at GSK, Stevenage, this trend was represented only by the late afternoon talk by Jim Thorn (Beckman-Coulter) on 'Use of Capillary Electrophoresis in a Quality Control Environment for Therapeutic Proteins'. Given that CE is not as commonly used as it once was, Jim gave an overview of the means available to separate proteins (by RMM, charge, isoelectric focussing and by carbohydrates attached to the protein) and explained why a commercial approach of providing "supported kits for biopharma" to obtain the same results worldwide was appropriate. One of the highlights of Jim's talk was on glycoprotein microheterogeneity. This may be measured by glycan profiling using CE (Figure 1). Functional variants such as the fucosylation of monoclonal antibodies

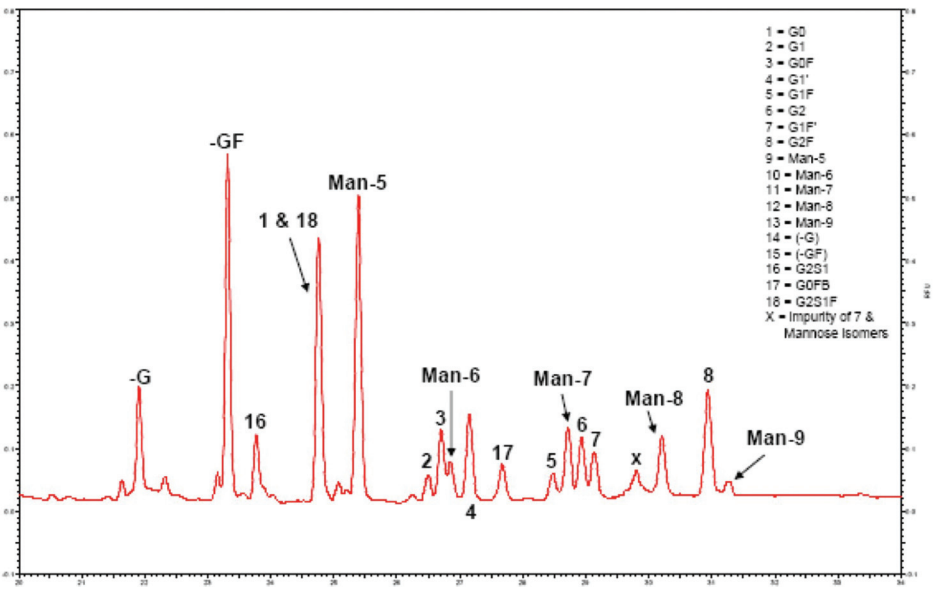
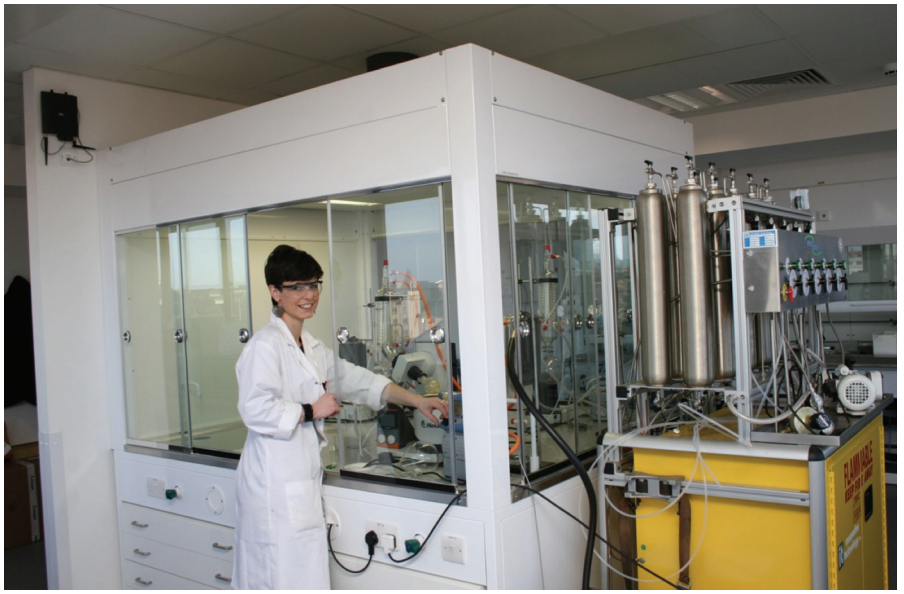


Figure 1: High resolution N-glycan profiling by CE



Multi-solvent drier and easy-access fume cupboard, organic chemistry research laboratory University of Sunderland

may be quantified by CE. The high resolution of this technique coupled with simple, cost effective workflow is leading to its preferential adoption. Finally, Jim touched on how novel approaches to coupling CE to mass spectrometry are driving characterisation of therapeutic proteins into new realms of sensitivity and resolution.

Norman Ramsey (ThermoFisher (unlike Tim Liddicoat – with Dionex heritage)) completed the afternoon session by addressing 'Novel Tools for Pharma – mass responsive detection and electrochemistry'. The main emphasis was on charged-aerosol detection (CAD) with its picogram sensitivity. The role that could be played by this detector in studying mass balance, analysing lipids, looking at active pharmaceutical ingredient (API) and counter-ion in one run and in studying ionic impurities was clearly established.

The Wednesday evening social function was held at the Sciences Complex of the University of Sunderland's City Campus. Sandwiched between a tour of the complex, recently refurbished at a cost of £8.5 million (the illustrative photograph shown here is of the new 25-hood organic research laboratory given that the analytical laboratories are equipped wall-to-wall with instrumentation from a single vendor), and a most delightful hot buffet with drinks was a discussion session on "The Evolving Pharmaceutical Industry: Regional and Global Perspectives". Because of time constraints, the anticipated lively debate on recent changes in Big Pharma R&D in the UK did not materialise. However, John Lough gave an account of the North East pharmaceutical industry's relatively buoyant state in troubled economic times. Also, Chris Riley gave a global perspective from a WHO viewpoint and in his discussion on drug counterfeiting highlighted that the pharmaceutical industry has more than just the high costs of R&D to worry about. A full account of this session will be given on the website of the North East Pharma cluster's First for Pharma website (http://firstforpharma.co.uk/).

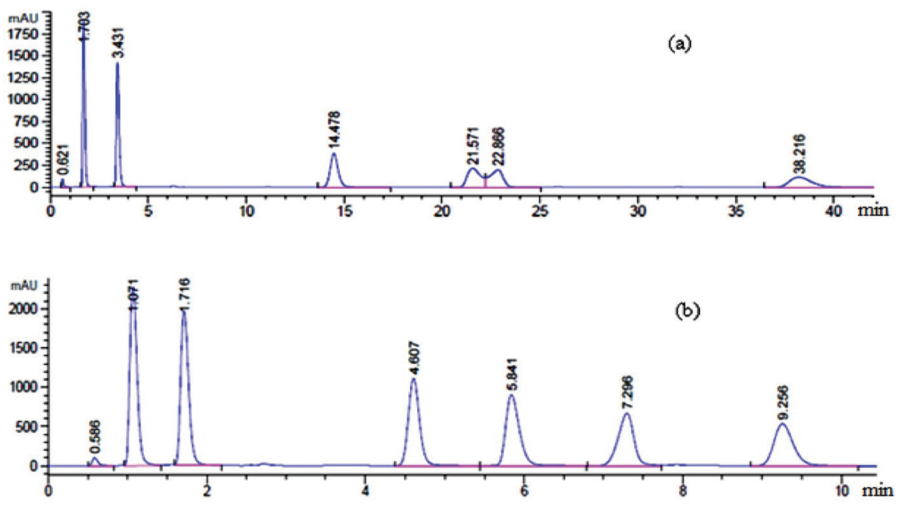


Figure 2: U-HPLC of steroids mixture (uracil as a marker, then prednisone, corticosterone, clobetasol-17-propionate, clobetesone-17-butyrate, betamethasone-17-valerate and beclomethasone dipropionate) with mobile phase methanol – water (60:40, v/v) and an Eclipse Plus C18, 50 × 2.1 I.D. column, at two different temperatures (a) ambient temperature (24°C), and (b) at elevated temperature (70°C) [from a study of temperature and pressure on k].

The opening talk of the second day was given by the principal meeting organiser, John Lough. Lough made it clear that he was speaking not on behalf of himself but on behalf of current students at the University of Sunderland. He noted that this included MSc and undergraduate students as well as PhD research students since teaching of pharmaceutical science at Sunderland is very much research-led. Research at Sunderland is a microcosm of research in the pharmaceutical industry, including elements of Discovery, Development and even Production and, as in industry, everything is underpinned by analysis. Areas of research mentioned were the search for drugs to treat cystynosis (Prof R J Anderson), metabolomics studies involving sulphur-containing compounds (Dr L Williams), total synthesis of antibiotics isolated from marine plants (Dr J Harburn) and isolation of active constituents from Indian medicinal plants (Lough himself). In each case LC had a key role to play. Lough went on to give illustrative examples where LC had been applied to good effect. Examples of chiral LC screening method development akin to systems used in industry were given. In the context of Development, systems had been developed using the Whelk-O1 chiral stationary phase which allowed the simultaneous determination of trace enantiomeric impurity and other related substances. Improved methods for cleaning validation carried out in Production had been developed directly on the Waters Acquity UPLC system and work carried out on 'legal highs' had mirrored work carried out to assess the quality of drugs available in the market place. Finally, it was demonstrated how the very modern LC systems in teaching laboratories readily allowed recent developments in LC column technology to

be assessed (e.g. Figure 2 effect of temperature on selectivity in U-HPLC), especially in terms of how these developments might be applied to typical pharmaceutical applications. Special mention was reserved for Hypercarb. It had been possible to deploy the unusual selectivity of this phase with volatile mobile phases like chloroform to carry out micro-preparative work on plant extracts and coal tar.

The second speaker of the morning, Dr Lee Williams, was also from the University of Sunderland. Lee discussed the study design, analytical instrumentation and statistical methods needed for the practice of metabolomics. The main themes he developed were on soft ionisation techniques, databases for identification of analyte, pathway analysis techniques, statistical software packages and the availability of new, accessible instruments. He concluded that while metabolomics in clinical analysis is becoming more widely accessible, there is very much a need for expertise in all of statistics, instrumentation and biological pathways. He pleaded for patients and patience!

The first of a series of three shorter talks was by Paul Russell. His employer, Unilever, is not a pharmaceutical company but in 'The Use of Chromatography in Complex Toxicological Risk Assessment' in his company there was very much in common with the Pharma world. Kristin Downey's company, Newchem, is a 'spin-out' of the University of Newcastle that specialises, amongst other things, the development of pro-drugs. Kristin took her audience through case studies of how LC on both the analytical and preparative scale had been vital in delivering objectives in stability testing on drugs and prodrugs, She explained how these aim to mimic biological environments in terms of pH, with or without hydrolytic enzymes being present. These differing conditions led to challenges in analysis, in which resolution between prodrug, promoiety and drug must be achieved. Sarah Chow's talk on cleaning validation drew on her many years of experience with Merck Sharp and Dohme and part-time research at the University of Sunderland. While she gave examples of her use of on-column sample focussing on Hypercarb, CE, DryLab and UPLC the main impact was from her master-class on regulatory aspects.

Keynote speakers for the final afternoon session were Andy Teasdale (AstraZeneca) and Melissa Hanna-Brown (Pfizer), both Sunderland graduates. Andy managed the not inconsiderable feat of keeping his audience captivated at the end of a telephone line! Speaking from Macclesfield (!) on "Genotoxic Impurities: strategies for identification and control" he drew from his recently published book on the same subject (from www.wiley.com). Genotoxic impurities are a significant concern often requiring control to very low levels (ppm or below) in order to assure patient safety . Andy explained how, by nature, many GIs are highly reactive electrophilic agents; this reactivity along with the low levels involved makes their analysis a significant challenge. He described how a systematic approach can be taken for the analysis of GIs, examining how issues such as matrix interference / analyte instability and poor detection / retention can be overcome in a predictable way to quickly develop robust methods. Examples of analyses described included sulphonate esters, arylamines and halides. From a distance of over a hundred miles away he felt safe enough to finish off with a slide featuring Newcastle United football club!

Melissa Hanna-Brown addressed 'Recent advances in in-silico chromatographic method development for pharmaceutical analysis'. A detailed justification of the need for the *in-silico* approach was given. She also showed how increased resolution using state of the art techniques and rational selection of stationary phase selectivity can increase the probability of complete resolution of even the most complex samples without a corresponding need for method development or optimisation. Finally, she also demonstrated how the latest computational tools can further improve the effectiveness of the chromatographic method development process, reducing waste, and speeding up the process as well reducing the negative impact on the environment. No doubt there were sceptics in the audience but those asking questions were enthusiastic in their interest.

Melissa also played a leading role in the concluding debate on the future directions of separation science. However, session leaders and audience alike seemed satisfied with their long days work and we never really got to the bottom of whether the drivers will come from large molecule drugs or still from small molecule drugs or not from the Pharma world at all. Surely this will be a recurring theme in Spring Symposia for years to come.

As ever, the Society is indebted to vendor companies for sponsorship and exhibiting (and to Paul Ferguson (AstraZeneca) for urging them to come along!). On this occasion, many thanks go to ThermoFisher Scientific (who sponsored lunches), Agilent Technologies, ACD Labs, Beckman Coulter (UK) Ltd, Chiral Techologies Europe, Crawford Scientific, Hichrom Ltd, PerkinElmer, Presearch Ltd, Sigma-Aldrich and TTP Labtech.

For forthcoming meetings check out the Events page at www.chromsoc.com Following 'Clinical chemistry' in late October there will be another full programme of events in 2013.