

Meeting report

Symposia show strong synergy

A report on the ExTech-8 and HTC-9 Symposia at York, UK, 6–10 February 2006

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1. Introduction

These three-day meetings, being held in the UK for the first time, were organized by the Royal Flemish Chemical Society (KVCV, Chair Robert Smits) and the Chromatography and Electrophoresis Group (C&EG, Chair Tom Lynch) of the Royal Society of Chemistry (RSC). Also being held were a one-day conference on Endocrine Disrupting Chemicals on 8 February and parallel sessions for HTC, so the program (122 lectures) was very full. Add to this, short courses, tutorials, 230 posters, 25 exhibitors, manufacturers' presentations, and an evening social program, and it is clear that the 450 or so delegates from some 40 countries had no trouble filling their time.

Overall, the blend of formal lectures and informal presentations, often with time for discussion, proved very successful. The meetings benefited from characteristic HTC hospitality with a beer-tasting evening, a reception at the National Railway Museum, and a symposium dinner in the 14th century Merchant Adventurer's Hall. With so much on offer, what follows is inevitably a personal distillation.

2. ExTech-8

Although due attention was devoted to environmental applications, there was much of clinical relevance. Micro-extraction techniques were the rule – music to the ears of one of your reporters, as his first analytical task was to transform a GC method for plasma barbiturates (2–5 mL plasma, 2 × 15 mL chloroform, total sample-preparation time >60 min, became 0.05 mL of both sample and

solvent, total sample preparation time 10 min with vortex-mixing). Essentially the same procedure is still in daily use for hundreds of analytes 35 years later.

However, liquid-phase micro-extraction (LPME), as detailed by Knut Rasmussen and Stig Pedersen-Bjergaard (Oslo) using polypropylene tubing (cheap, disposable!) crimped at one end, is a much more recent development. For GC, the tubing is pre-washed with acetone, and extraction is into a water-immiscible solvent, such as octanol or dihexyl ether contained in the lumen of the tube. Conventional pH manipulation was used, if necessary, and extraction times were up to 45 min – not a problem for batch analysis as only mild shaking was needed.

For HPLC, pre-washing was not used, and extraction could be further manipulated by impregnating the tubing with immiscible solvent and then placing an appropriate buffer solution in the lumen – analyte trapping could be achieved by an analogous mechanism to that by which basic drugs appear in stomach contents after i.v. injection. Even ion pairing of polar analytes with trapping of ionized species in the buffer and electrokinetic migration has been demonstrated.

To reinforce the message, Hian Lee (Singapore) outlined LPME applications in environmental analysis, and the role of simple liquid-liquid extraction in handling plasma and brain microdialysate and tissue in conjunction with HPLC-ESI-MS-MS was discussed by Mareika Lutz (AstraZeneca).

But increased automation is an important goal, and bioanalytical applications of SPME were discussed at length by Heather Lord (Waterloo), Marcel Musteata (Waterloo) and Jochen Schubert (Rostock), amongst others.

Even *in vivo* sampling was mentioned, the small amount of analyte removed being a major consideration if sampling in young children, for example, ever became feasible.

Immersion SPME is an equilibrium technique and the problem of variation in extraction conditions has been

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addressed by pre-loading of the extraction phase with an internal standard (IS) and then measuring both analyte uptake and IS loss from the extraction phase.

The problem of protein binding seems more intractable (SPME measures “free” analyte), and there are vanishingly few indications for “free” drug measurement at present. Headspace SPME, in contrast, seems exciting, as it is not an equilibrium technique and offers the ability to concentrate vaporized or aerosolized analyte prior to transfer to GC, for example. As such, it has clear advantages over headspace GC *per se*.

With salting out, non-volatile analytes, such as amitriptyline, can be detected in human-hair digests using conventional HS-SPME. A newly-developed cooled HS-SPME probe could further advance this area and give good sensitivity with volatiles, such as amphetamine, at the same time.

Single-drop microextraction (SDME) using a non-volatile or immiscible solvent and thin-film microextraction using poly(dimethylsiloxane) (PDMS) sheet represent further variants on the theme with their own unique features. Notably, the use of thin PDMS membranes, as presented by Inge Bruheim (Hovdebygda, Norway), has the advantages of faster and more sensitive extraction when compared with conventional thick-film formats.

Presentations by Karl-Siegfried Boos and Rosa Morello (Munich) summarized some current ideas on fully-automated HPLC-MS of plasma for drug/poison analysis. Post-column analyte infusion provided a clear demonstration of MS signal suppression with various techniques, such as off-line protein precipitation. In the method adopted, two sample-extraction columns (restricted access media (RAM) to remove protein and other unwanted high relative molecular mass compounds) – an initial methanol concentration of 5% (v/v) being sufficient to release protein-bound analytes without causing protein precipitation – mixed-mode phase (MMP) to extract the analytes and any IS were used in series prior to HPLC. Production of a commercial system based on this work is under way.

It seems that on-line use of ideas developed from HPLC and SPE in bioanalytical sample preparation is the way to go, and the overall impression was that off-line use of SPE in such applications did not feature prominently in this meeting. By contrast, preparation and use of molecularly imprinted polymers (MIPs) and sol-gel and other immunoaffinity columns in SPE for targeted analytes in environmental samples was discussed by several speakers, including Valérie Pichon and Carine Maisonne (Paris), and Margit Cichna-Markl (Vienna). In addition, Valerie Pichon presented the rational design approach to preparing MIPs for particular analytes. The selection of monomer components to target particular interactions (electrostatic, H-bonding, non-polar) with the analyte of interest was very clearly outlined. Marie-Claire Hennion (Paris) summarized research on

pre-concentration in microfluidic devices, including liquid-liquid extraction and SPE approaches.

3. HTC-9

The development of ever-more simple and selective sample-preparation procedures for environmental and bioanalytical purposes that featured so strongly in ExTech provided a marked contrast to HTC-9, where a dominant theme was the use of various forms of one-, two-, and even three-dimensional chromatography, often linked with MS, to the analysis of complex mixtures, often with no particular analyte in mind. The matrices used included harbor sediments, soil from beneath oil-storage facilities, river water, stored food, human urine, exhaled air, protein digests, and herbal remedies. Clearly, analyte or at least group identification/quantification is a primary aim, but overall the work is sometimes like looking for the proverbial needle in a haystack without knowing what the needle looks like. In part, this reflects the search for insights into perceived problems, such as endocrine disruption, but more speculative aims include pre-clinical diagnosis of disease.

Whilst environmental monitoring responds to, and in some cases perhaps leads, regulation of specific chemicals in this complex area, the role of some other applications seems difficult to define – is ever more emphasis on separation and identification a means to an end or a goal in its own right? The recent book by Professor John Timbrell (*The Poison Paradox: Chemicals as Friends and Foes*, OUP, Oxford, UK, 2005) presents a balanced account of topics such as endocrine disruption and other aspects of environmental toxicology. Moreover, we know of many factors associated with disease etiology from epidemiological studies, smoking, for example, being amongst the most obvious (a downside to international meeting these days to many non-smokers is the number of smokers attracted!). And we know of the interplay of factors such as diet, exercise, and genetics in preventing or at least minimizing the incidence of smoking-related and other diseases. It is the expression and regulation of enzymes in different tissues at different times that controls gene expression, not simply whether a given protein, for example, is present.

This being said, the use of multi-dimensional techniques may have a role in one seemingly intractable area, that of studying alternative (e.g., herbal, Chinese and ethnic) remedies, as indicated by Guowang Xu (Dalian). Although in some cases (banned) Western medicines and toxic metal salts may masquerade as “natural” remedies, in others the sheer multiplicity of possibly active ingredients seemingly defies logical analysis. Whilst issues of efficacy may become less important in time as new chemical entities (NCEs) are developed to treat specific ailments that at present elude

effective conventional treatment, in the meantime looking for either effects or components/metabolites may help in assessing efficacy/toxicity and any specific components associated therewith, when viewed in the light of modern pharmacology and toxicology.

Other interesting areas were those espoused by Hans-Gerd Janssen (Unilever) – hydrolysis of protein, separation and identification of the resulting peptides, and testing for biological activity, which has led to newly-identified peptides being patented – and by Andy Booth (Plymouth) – studying the toxicity of unresolved complex mixtures (UCMs) of hydrocarbons in mussels.

Other presentations in HTC-9 featured targeted analytes or groups of analytes, or specific problem areas. The question of how to cope with quantification in HPLC in the absence of reference material during pharmaceutical development, for example, was tackled by Ian Mutton (GSK). The evaporative light-scattering detector (ELSD), the chemiluminescent nitrogen detector (CLND), and the charged aerosol detector (CAD) had been compared with a proton NMR reference method – only the ELSD gave inconsistent results. The value of simple methodology is clear, since hundreds of thousands of NCEs requiring purity assessment are synthesized for testing every year, a fact that reinforced comments from the environmental analysts as to the range of ex-NCEs continually being marketed and about which they are given no advance information.

The problems posed by identification and quantification in the absence of reference material are not limited to pharmaceutical development, and Ian Sanders (Bruker) outlined work on accurate mass measurement ($\pm 0.002 m/z$) in HPLC-MS using ESI-TOF instruments with illustrations from forensic toxicology [1].

4. Round-up

There were a number of presentations and awards. The winner of the best poster competition sponsored by the RSC C& EG was Lucia Sanchez-Prado (Santiago de

Compostela) for her poster *Study of the Sunlight Photo-Decomposition of Five PBDEs by Photo-SPME*, the prize being a grant to cover registration and expenses to attend ExTech 9 to be held in Ålesund, Norway, 3–6 June 2007.

The Life Time Achievement Award (sponsor, LC.GC) was presented to Jim Jorgensen (North Carolina), well-known recipient of many previous honors.

The HTC award (sponsor, Elsevier) was presented to Luigi Mondello (Messina) for his paper on *Comprehensive Chromatography Approaches to the Analysis of Real Samples*.

At the symposium dinner, Stuart Laing (ex-GSK) was presented with an RSC Analytical Division Distinguished Service Award, and Ian Wilson (AstraZeneca) was awarded the Society for Analytical Chemistry Gold Medal for his services to chromatography.

There was an unusual highlight too in the form of a light-hearted debate between Phillip Marriott and Peter Schoenmakers as to whether 2-D, etc. chromatography was a (comprehensive) waste of time, with Peter Myers (X-tec) complete in full-bottomed wig and scarlet robe acting as Judge (and some thought jury too!). But, in the end, the audience was pretty evenly divided when it came to a vote.

This was an extremely friendly meeting, high-tech science was given due prominence, and the ancient City of York played its part in providing an elegant back-drop to everything.

For the longer term, an IUPAC committee to look at terminology involved in sample preparation was mooted, and a number of the papers from the meeting are to be published as a special issue of the *Journal of Chromatography*. Furthermore, as a result of this successful meeting, ExTech-10 and HTC-10 will again combine in February 2008 in Bruges, Belgium.

Reference

- [1] Ojanperä, A. Pelander, S. Laks, M. Gergov, E. Vuori, M. Witt, J. Anal. Toxicol. 29 (2005) 34.