Chromatographic Society Meetings Round-Up

Advances in High Resolution and High Speed Separations

Chromatographic Society 1-day meeting

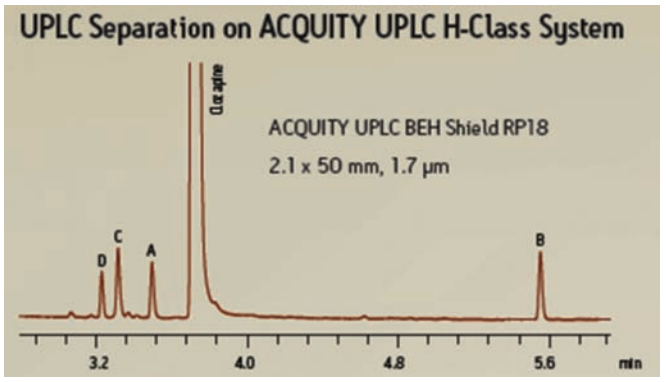
Thursday 18th March 2010, AstraZeneca R&D, Alderley Park, Cheshire, UK

by John Lough

Belying the general gloomy atmosphere prevailing over the developed world pharmaceutical industry, an encouraging number of approximately 116 delegates descended upon AstraZeneca's new Alderley Park conference centre to engage with the latest advances in high resolution and high speed separations. Appropriately there was also a good turn out of exhibitors (12) led by Gold Sponsors, Waters. In terms of quality of scientific meeting the bar has recently been raised by Spring Symposia drawing speakers not just from the UK but also from nearby mainland European countries. This meeting followed the same pattern, UK speakers in fact being comfortably outnumbered.

The scene was set for the meeting by Peter Schoenmakers who elegantly introduced the key issue of peak capacity, comparing the improved peak capacity of gradient LC over isocratic LC. Drawing from his own experience of GC (where a peak capacity of 1000 is fairly normal) and 2D GC, Schoenmakers went on to deal with 2D-LC. He described his own SEC x RPLC work and work from Peter Carr involving indole metabolite standards in which there was a peak capacity of 1800. Instrumentation for 2D LC is not available commercially and so it was very enlightening when Schoenmakers showed his own homemade system to underscore the fact that 2D LC is accessible and not far-off science fiction. His system was capable of generating 1000 peaks in 50 minutes when taking realistic cuts. While pointing to the advantages of 2D-LC and drawing on illustrations from his work on block copolymers, he also alluded to issues such as the need, in both dimensions, for the peaks to be evenly spaced out, the value of focussing fractions as they moved onto the second dimension, the problem of quantifying peaks spread over several chromatograms in the second dimension and for the second dimension to be different in its selectivity from the first dimension. He finished his presentation on an upbeat note, speculating about a future outlook involving 2D-LC with MS detection and even multi-dimensional LC.

Opening the batting for the home team was Patrick Pettersson (AstraZeneca) R&D Lund, Sweden) who addressed "Implementation of U(H)PLC within a global pharmaceutical company – A new way of working." This involved discussing the perceived advantages at the outset, the choice of Waters Acquity, the actual implementation and through to the lessons learned and the conclusions. Amongst the many interesting points that were raised were that method development and validation had become much faster, sample preparation had become a new bottleneck and there had been a 62% reduction in the number of LC systems with a concomitant increase in benchspace and reduction in solvent consumption. The adoption of U(H)PLC had brought about a new way of working. Walk-up systems with a 'buffer bar', a booking system, automatic column reordering, column selection maps and standardised screens were being enjoyed by increasing numbers of users. Along the way some lessons had been learned. The entire exercise had not been without its problems though. It had become apparent that buffers should not be kept too long because of the growth of microorganisms. The concept of "finger-tight" connections had been challenged during over-ambitious work at greater than 80% of maximal pressure. Poor baselines at low wavelength had been encountered when using trifluoroacetic acid as an additive. However, the entire exercise had been more successful than expected, thanks in part to help from Lean Six



Separation run on a new Acquity H class instrument which can accommodate HPLC and UPLC methods

Sigma management tools. During discussion it emerged that an additional U(H)PLC system which could not be booked was kept in reserve as back-up.

The next speaker was also from AstraZeneca. Chris Smith, a co-worker of Prof Ian Wilson in the Alderley Edge group working on metabolomics, gave a presentation on high-temperature LC. He started by proclaiming that he would be using chromatograms to rather than equations to illustrate his points. During the talk, practical issues were discussed and the application areas of ICP-MS and metabolomics were covered. With respect to the practical points it was pointed out that it was necessary to go to 150 ^{O}C before the van Deemter C term looked really flat and also that, as in GC, temperature gradients could be used highly effectively. There was a reminder of the need to cool the liquid post the GC oven and advice was given that more stable systems could be had by increasing the flow rate to increase the pressure in the system. Smith raised a few smiles when recounting of an incident when there was a very strange smell pervading in his laboratory and how, after a long time, it was eventually traced to PEEK tubing in the high temperature LC system burning at oven 180 ^{O}C. The focus of the ICP-MS work described was on drugs containing halogens, phosphorus or sulphur (for which ICP-MS allows quantitation which is independent of drug structure). In hot water LC using temperature gradients solvent issues are less of a problem. While this approach is not universally adopted in the Wilson/Smith group, all ICP-MS assays are run at at least 60 ^{O}C to minimise the use of organic solvents in the mobile phase with a consequent reduction in the amount of these solvents going into the MS source. For the metabolomics the attraction of high temperature LC was the higher efficiency leading to much greater peak capacity. Topics covered in discussion following the lecture included stability of the analytes (not so much a problem as might be imagined), effect on the system (pump can be a problem), column stability (~200 injections but better phases coming out) and whether there was much need for occasional organic column washes to clear compounds stuck on the column.

In the final talk of the morning session given by Dr Philip Grace (Quotient, UK) on "Sorting Needles in Haystacks: Resolving Problems in Nutrition and Fitness Testing" the application of UPLC to the study of nutritional supplements and nutritional biomarkers was addressed. Philip's talk was all the more interesting as he was able to touch on one or two well known cases that had reached the popular press. The technical content included discsussion of matrix effects, the difficulty in distinguishing between ephedrine and pseudoephedrine, phytoestrogens, catechins in green tea and the analysis of Vitamin D in dried blood spots. While the intention was to highlight the effectiveness of UPLC for these applications, it was clear that in some cases the success could be attributed to differences in stationary phase selectivity rather than UPLC *per se*.

As befitting a meeting of this nature, the lunchbreak was very busy with even some excellent food still left over for those heavily wrapped up in networking. As can be seen from the photograph above, it was all too much for this group of speakers and organisers – they were literally jumping up and down with excitement!

Following lunch was a vendor's session. However, this was no graveyard slot and gone are the days when vendors turned up to deliver a straight-on sales pitch. Barry Upton spoke on behalf of the Gold Sponsors, Waters UK.. Barry talked about the historical evolution of UPLC and how Waters, with different column chemistries (to allow more ready adaptation of legacy HPLC methods), 7-port, 6-position switching valves (stackable for up to 6 columns) and quaternary solvent managers, were responding to the reactions of customers. This all felt a little different from the early days of UPLC when Waters boldly led the customers. However, genuinely new chemistries were promised for HPLC 2010 and indeed this subsequently proved to be the case. Speaking for Agilent, Monika Dittman described how the kinetic plot model (Poppe Plot) could be used to investigate peak capacity in gradient LC and to study the effect (very small) of diameter of the connection capillary diameter on overall peak variance in gradient LC. As with Peter Schoenmakers, there was an acknowledgement that, because of the uneven nature of the distribution of the physicochemical properties of analytes the actual peak capacity for individual separations of complex mixtures was often much less than the theoretical. Ken Cook of Dionex passionately advocated the use of ion exchange stationary phases, particularly for the analysis of ionic and polar compounds such as organic acids, carbohydrates, nucleotides and amino acids when carrying out metabolomics and described how salt buffer problems could be dealt with using online de-salting when using ion exchange LC-MS.

There was a change in direction after the afternoon tea break with two talks on GC. It was demonstrated to those LC-orientated individuals in the audience that there were still interesting new developments to be explored in GC. Jaap de Zeeuw of Restek described how fast GC-MS separations using short 0.53 mm columns and a simple vacuum GC setup could be improved by employing a restriction positioned inside the injection port. Since the restriction is at constant high temperature and very short, there should be no negative impact on the chromatography. Wim Traag's (RIKILT, a "crisis organisation" dealing with e.g. terrorist attacks) talk involved the coupling of GC x GC to TOF-MS. This involved a range of interesting applications including the study of nicotine in mushrooms and pesticides in drinking water. Wim discussed the complexity of the data handling involved and how e.g. ut was useful to focus on chlorine clusters in the MS data.

Final speaker, Frederic Lynen's (University of Ghent) title was "Enhancing fluidity and maximizing stationary phase selectivity for faster high resolution pharmaceutical and bio-molecular HPLC analysis." This amounted to a microcosm of the entire symposium. Frederic covered a wide range of topics related to "high and fast resolution chromatography" including radial heating from frictional flow effects, coupling columns at high temperature, HILIC LC at 90 OC, use of zirconia-based RPLC materials, van Deemter shifts in SFC and improvements in RPLC (enhanced fluidity) and non-aqueous LC (modified selectivity) brought about by the addition of sub-critical carbon dioxide to the mobile phase. Intriguingly he concluded with the subject of gradient POPLC suggesting its use when a mixture is not quite complex enough to warrant using 2D-LC. This might seem counter-intuitive (if phases being coupled were orthogonal then early eluting peaks on one phase would not be early eluting peaks on the orthogonal phase and similarly for late eluting peaks so that there would be no need for a gradient mobile phase in the coupled system). However it seems that there is still value to be had in coupling columns which are little more than slightly dissimilar in their selectivity. The gradient separations shown were characterised by a very even distribution of peaks. This nicely turned things full circle serving as a reminder of one of the main problems in 2D-LC. Perhaps if each dimension was a POPLC gradient then the entire separation space could be occupied evenly by peaks!

["Meetings Round-Up" (ChromSoc Spring Symposium and HPLC 2010 including the Pete Carr Martin Medal award) will be continued in the next issue of Chromatography Today]