Report on 2009 Spring Symposium of the Chromatographic Society

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It is the scientific argument equivalent of 'Which came first, the chicken or the egg?' only in this case it is 'Liquid Chromatography or Spectroscopy, primarily Mass Spectroscopy?' One could debate the criteria as to which form of each we are voting on but the premise remains the same as is the inescapable fact that the two do actually have a symbiosis and exist to each others mutual benefits on an increasing level especially at the life sciences end in the pharmaceutical industry.

Figure 2: Success Rates of Lux™ Phases in RP for over 200 Racemates (Rs>1.5) – Various Conditions

Presenter and Title
Gold sponsor seminar: **Dr Stefan Schuette** (Agilent, UK) – Infinitely Better LC for LC/MS
Vendor 2: **Dr Luisa Pereira** (Thermo Fisher Scientific, UK) – Evaluating the sensitivity of sub 2 m particle packed columns for the LC/MS analysis of complex biological samples
Vendor 3: **Dr Khalil Divan** (Dionex, UK) - Ion Exchange-MS Application for Ionic and Polar Compounds
Vendor 4: **Dr Jackie Mosely** (Durham University on behalf of Waters, UK) – Practical applications of an Atmospheric Pressure GC (APGC) ion source and an Atmospheric Solids Analysis Probe (ASAP)
Vendor 5: **Dr Jonathon Nielson** (ACDLabs, UK) - Data Overload! High- Throughput Data Reduction of Complex Datasets.
Vendor 6: **Pauline Leary** (Smiths Detection, USA) - Ion Mobility Spectrometry for the Identification of Microbiological Samples
Vendor 7: **Peter Ridgway** (TharSFC, UK) – Interfacing SFC with MS

Table 1. Vendor Presentations

The spring symposium of the Chromatographic Society debated this inter dependency in a fascinating manner with well practised speakers in the tandem field of LC- (almost any spectroscopic technique but primarily Mass) in a two day meeting entitled 'Advances in LC/MS and Related Hyphenated Techniques' held in Sunderland, UK during May 13/14th. Alongside vendors, some of whom made interesting presentations, almost 80 interested participants attended from the UK and Europe.

LC and MS are now inseparable and integral to the pharma industry, according to the first keynote speaker, Dr Frank Pullen, who gave a personal view of the historical background to techniques hyphenated to MS. After struggling for many years to link GC to MS, scientists can now link MS to virtually any, including SFC. The first MS, based on moving belt technology, could only be used in normal phase, but the invention of APCI using a simple ionisation source in 1987 was the real turning point. Advances in instrumentation and automation mean that non-MS experts can utilise the technique in bench-top, reliable systems. SFC-MS is an interesting development in that greater ionisation is achieved without using a voltage, giving greater sensitivity. In Dr Pullen's words, 'it has shunted NMR sideways'. Latest applications for LC-MS include the detection of anti-sense strands after including a drug into RNA duplexes – a novel drug delivery process. Further development in this area now lies more in the chromatography than the MS detection. GC-MS and Metabolomics are being used in the search for biomarkers. The future for MS will include reaction monitoring at a miniature level to enable chemists to

instantly monitor, control and stop a reaction at the correct place. Lead diversification could be helped by using a protein to change the reactions of a small molecule that are possible and flow chemistry may lead to a logical flow from synthesis to screening. 'Pharma needs to do more with less'.

The use of MS in Sports Science has moved on a long way since the white mouse 'Straub' test for alkaloids in the 1920's and 30's. A recent ruling, WADA 09, lists 12 clauses on prohibited substances and now 34 laboratories in the EU and USA specialise in this complex area using a 50:50 mix of GC- and LC-MS to reach the required selectivity. Typically, as Prof. Mario Thevis (University of Cologne) reported, 15,000 samples per year are analysed. Recent attempts at avoiding detection in sports include the use of rapid acting synthetic insulins and cross-linked haemoglobins that have a much higher uptake of oxygen. Ingenious ways to avoid detection include the use of proteases to degrade proteins prior to analysis – but LC-MS will now detect these via peptide mapping. New drugs of abuse include non-steroidal quinoline, hydantoin types that are highly selective for certain muscle groups.

Prof. Hubertus Irth from the Free University of Amsterdam opened the session relating to Bioanalytical applications with a talk entitled 'Integration of High-Resolution LC-MS, NMR and on-line screening for the Rapid Discovery and Characterisation of Bioactive Metabolites' where he talked about the use of Cytochrome P450 BM3 in this field. This molecule has been reported as having the capacity to convert drug, and drug-like molecules into metabolites with interesting drug-like properties. Using this approach allows medicinal chemists working in areas such as early drug discovery with information on so called 'soft spots' in scaffold molecules.

Two case studies were presented where Cytochrome P450 BM3 acting as a biocatalyst was applied to screening of focussed library samples. In both cases the direct correlation of accurate molecular mass and affinity data of biotransformation products generated by the Cytochrome P450 BM3 mutants resulted in an efficient workflow to expand focused libraries with interesting novel chemical structures

Metabolomics is being studied by Dr Florence Raynaud at the Institute of Cancer Research, providing successful drug development targeted against specific cancers. A typical profile of biomarkers, peptides and metabolites with QTOF displayed around 100 – 1200 'features', of which around 30-40% were identified. Separation success relies on HPLC column dimensions and the use of JetStream technology for MS.

Results indicated that on the conventional system, at 0.4 ml/min, 1324 features were detected, 60% of which showed less than 25% CV. The number of features with CV<25% increased by 16% on the 1290 system and with the same LC conditions and by 45% at 0.6 ml/min. This was the optimal flow rate as the number of features detected decreased and variability increased at higher flow rates with the same gradient. Decreasing the length of the gradient did not improve the number of features or the variability. Peak capacity was also found to be optimal at 0.6 ml/min.

Naturally an over reliance on academic perspectives limits the ability of the research to make areal impact and so the third session was devoted to Industrial perspectives of the topic in hand. Firstly Prof. Ian Wilson (Astra Zeneca, UK) in his presentation entitled 'Hyphenation, hyphenation, hyphenation….' debated the endless, seemingly, opportunities to use hyphenated techniques to obtain better quality information faster. He claimed a world record for linking together an LC system to UV, IR, MS and NMR spectrometers. Experience of the analysis of complex (often biological) using various combinations of hyphenated techniques were discussed alongside the practical problems and limitations that arise out of the need to analyse complexity.

His final comment was that LC-MS was an answer to many analytical problems but was not THE universal answer and really LC-Anything should never be dismissed.

Dr Mark Taylor from Pfizer approached Ian Wilson's strategy when he talked on 'Improvements to the universality of response of Evaporative Light Scattering Detection using gas-flow and temperature programming for high-speed LC-MS-UV-ELSD of pharmaceutical compound libraries.' In promoting the use of Evaporative Light Scattering Detection (ELSD) as a "universal" quantitative detection system to augment LC-MS, he spoke on how this has now become a routine method of analysis to obtain quantitative quality assurance testing of large numbers of liquid file solutions in support of early plate based pharmacology screens. Non-linearity of the ELSD due to changes in mobile phase composition is known but recent advances in detector design could overcome this. Real-time control over detector variables is not too far away.

Finally closing the session on Industrial Perspectives was Prof. Colin Creaser (Loughborough University, UK) who discussed 'Combining chromatographic separations with ion mobility spectrometry and mass spectrometry'. Ion mobility spectrometry (IMS) is an electrophoretic technique in which ionised analytes are separated on the basis of ion mobility in the gas phase in the presence of a buffer gas and under the influence of an electric field. Ion mobility is determined by the charge, reduced mass and collision cross section (i.e. size and shape) of the ion. Two types of IMS were discussed and illustrated using applications of the hyphenated techniques in pharmaceutical and bioanalysis.

Prof Frank David (RIC and University of Gent, Belgium) reported the use of SPME, always with PDMS fibres for porfilling potentially genotoxic impurties. He noted that these are more sensitive for later eluting compounds (> heptanes) and that the technique is better than static headspace. There is a list of FDA 'alerts' that include boron containing compounds, azides, haloalkanes – both volatile and non-volatile and he showed the use of hexachloroformate derivatisation for amine-containing compounds to give retention on C18 and a greater mass for MS detection.

Dr Tony Bristow (AstraZeneca, UK) – moved onto 'Evaluation of a new interface to couple gas chromatography to time of flight mass spectrometry - GC-MS and LC-MS on one mass spectrometer'. His work centred upon the use of accurate mass GC-TOF-MS analysis of a series of AZ compounds used in the development of new drug molecules.

One type of hyphenation that could certainly be regarded as specialist owing to the cost of the spectrometer end would be LC-NMR. Dr Nicolas Haroune (ChemiSPEC, University of Sunderland) gave an entertaining and informative presentation entitled 'LC-NMR: Why would anyone want to do that?' He talked on the practical operational details of the technique along with advantages and limitations. Examples were shown illustrating its use to chemical structure problems and how best to use the information alongside that supplied by LC-MS.

Dr Karine Ndjoko (University of Geneva, Switzerland) closed with some interesting work entitled 'Application of LC-NMR-MS Techniques to the Identification of Bioactive Natural Products'. The advantage of LC-NMR resides not only in the fact that full structural and stereochemical information can be obtained (by the use of 2D NMR) but also in the fact that it is also a highly nonselective detection technique. 1H NMR spectroscopy will detect any hydrogen-containing compound present in the HPLC eluent in a sufficient amount regardless of its structure.

The possibility of using hyphenated techniques does really appear endless and so many possibilities exist to push the information boundary back that the future is extremely positive.