Can Spectroscopy assist Separations Scientists – Yes it can!

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.

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.

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VENDOR PRESENTATIONS

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 2µm 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

Opening the first session entitled 'Current state-of-the-art and the future' Keynote speaker Dr Frank Pullen from Pfizer UK talked about the different forms of ionisation developed during the 1960's through to the 1980's which was a period dominated by people who could not envisage Mass Spectroscopy ever being for the masses let alone used as a 'back end' to any other instrument. The break through came in 1987 when Atmospheric Ionisation was commercialised in the form of API and ESPCI interfaces and these led to greater use of the LC-MS technique. Not only did Instruments become more of a bench top size but one Instrument and one ionisation technique became the norm with increased reliability and quadruples replacing permanent magnets and control; of the LC and MS merging into one process. Some 'crystal ball' gazing finished an enlightening 45 minutes.

The second Keynote speaker was Professor Mario Thevis from the University of Cologne, Germany who spoke on the 'Current role of LC-MS (/MS) in Sports Drug Testing'. He told the tale of how the Chromatographic- mass spectroscopy world had gone to war with the sports cheats and illustrated the progress made with some illuminating case studies from as recent as The Beijing Olympic Games from 2008. The majority of analytical assays currently employed in drug testing rely on the identification power of retention time and (product) ion mass spectra derived from hundreds of target analytes. The nature of cheating athletes and their backers has necessitated comprehensive as well as sensitive and specific detection methods in both high and low molecular weight analyte ranges. Complex mixtures need to be analysed with a range of spectroscopic techniques allied to Liquid Chromatograms at the front end. It is on this front that the war is being waged.

Professor 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

Dr Florence Reynaud from the Institute of Cancer Research, Surrey, UK spoke on 'Optimisation of a method for metabolomic analysis using a UHPLC/QTOF system' with the new Agilent 1290 instrument was compared to the model 1200 series LC from the same manufacturer. LC was performed on a column containing 1.8µm diameter particles and a 7.5 min. water/acetonitrile (containing 0.1% formic acid) linear gradient. In either case the rear end was a Q-TOF mass spectrometer with an electrospray source in positive ionisation mode. Replicates of extracted plasma were analysed in full scan mode (70 -1000 m/z). Data was processed using molecular feature extraction software then analysed with GeneSpring MS. For each feature the peak height/area was imported into MS Excel and then compared and their reproducibility examined.

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 Professor 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 Professor 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. An example is shown in *Figure 1*.

Although the theme of the day centred on the LC side of hyphenated techniques, two interesting talks were given using GC as the front end instrument. Professor Frank David (RIC and University of Gent, Belgium) with his talk on 'Method Selection for the Trace Analysis of Potentially Genotoxic Impurities in Active Pharmaceutical Ingredients' discussed the options

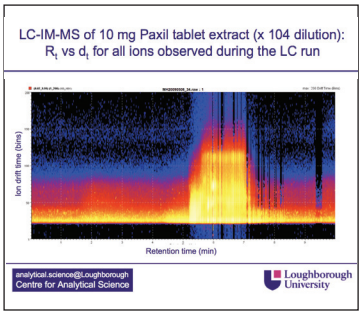

Figure 1. Example of LC-IM-MS.

available to the analyst with particular emphasis to the use of a single quadrupole MS.

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.