

Mass spectrometry for the 21st century

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The recent IBC conference, *Mass Spectrometry 2001: Technological Advancements for Drug Discovery and Drug Target Identification* (9–10 April, London) was attended by approximately 60 delegates. It blended together presentations from MS instrument vendors, academia and industrial researchers.

The majority of innovation in the MS field is driven by the major vendors, with one eye on academic research and the other on industrial applications to meet commercial need. The conference addressed the latest developments in triple quadrupole MS from Applied Biosystems (IBS)/MDS Sciex (Foster City, CA, USA). Tom Covey described the API 4000, which seeks to address some of the fundamental limitations of sensitivity encountered when doing atmospheric-pressure ionization MS. It has long been thought by those using LC–MS that to achieve ultimate sensitivity one has to exploit low flow-rate chromatography (capillary or nano-LC at flow rates of 10 μL –50 nL min^{-1}). Sciex are seeking to turn this paradigm on its head. Data presented indicate that the API 4000 can be ninefold more sensitive than the API 3000 predecessor in multiple reaction monitoring (MRM) mode (as widely used in target drug clinical and drug metabolism MS), however, this performance advantage can increase to a factor of 50 at flow rates of 0.5–1.0 mL min^{-1} . Using conventional flow-rate LC has some advantages in terms of throughput, injection-volume capacity and system reliability, and it also presents less of a training burden for analytical managers. These improvements are a result of adopting a truly orthogonal spray-injection system, new orifice design and, most interestingly, by using 800°C gas

inlets to assist spray drying while preventing surrounding gas molecules from being pulled into the ionization environment of the instrument. This is claimed to give rise to a large amount of chemical noise, which ultimately limits signal:noise ratios in LC–MS and LC–MS/MS.

Applications

Matrix-assisted laser desorption ionization-time of flight MS (MALDI-TOF MS), and related techniques, continue to expand in application base. Gary Siuzdak (Scripps Research Institute, La Jolla, CA, USA) has been involved in research that seeks to avoid the need for matrix by employing porous N-type silicon surfaces, hence making low-MW TOF-MS possible when this would normally be masked by signal contributions from the matrix. The technique still has low sensitivity and poor upper-mass range limits compared with MALDI, but the nature of the surface lends itself to chemical modification which might lead to potential applications in affinity and/or ligand fishing exercises. Such applications have been the driving force behind research by Ciphergen (Fremont, CA, USA), as described by Scot Weinberger, who presented the latest applications of their ProteinChip™ Array technology. An example of this is the ability of the system to visualize, by differential display, the progression of prostate carcinomas following on-chip collagenase digestion. In certain cases the resultant mass spectrum is viewed as a pattern to reveal clinical information without the mass/charge (m/z) values being investigated further. A similar message emerges from those working in the field of SNP genotyping. Charles Cantor (Sequenom, San Diego, CA, USA) and

Ivo Gut [Centre Nationale de Genotypage (CNG), Evry, France] are using MALDI to rapidly locate SNPs in human and non-human species by employing very high-throughput Bruker systems and have described acquisition rates of a spectrum in one and two seconds, respectively. CNG is using the new Bruker Autoflex™, which has plate-twister robotics and a rather monolithic appearance; highly appropriate for a conference with the date 2001 in the title! With this technology, CNG is using tissue samples, which are removed when animals are ear-tagged in the course of standard registration procedures, to generate SNP genotype profiles for prion-mediated diseases, such as bovine spongiform encephalitis (BSE), with a view to eventual progress in the field of variant Creutzfeldt–Jacob Disease (CJD) in the human population. Sequenom is using its MassARRAY™ and MassEXTEND™ technology to probe the genome for SNPs and has modified existing software to make visualization less challenging. CNG uses the GOOD assay, which employs a chemical modification stage and software adaptation of Bruker's own core product to provide an easily interpreted result. In a recent iteration of the GOOD assay, several clean-up stages have been circumvented to minimize chemical intervention steps.

The pressures of ever-increasing efficiency targets in the pharmaceutical industry is driving analysts who support discovery and early-phase development to achieve more assays in unit time or on a given instrument. Multiplexing LC–MS and LC–MS/MS was exemplified by workers at GlaxoSmithKline (GSK, Stevenage, UK) and Bristol-Myers Squibb (BMS, Stamford, CT, USA), respectively. This approach allows four or eight wells of a

96-well microtitre plate to be analyzed simultaneously. At GSK, Andrew Organ has been applying a novel multipump solution from Jasco (PAR-1500) to enhance the chromatographic performance of an eight-way Micromass MUX™ system based on the LCT™ (liquid chromatography-TOF mass spectrometer, Micromass, Manchester, UK). This provides metered, high-pressure liquid streams to eight lines fed into a Gilson 215/889 liquid handler (Gilson, Middleton, WI, USA). Greater reliability is also claimed for the overall system. David Wang-Iversen at BMS is using a four-way MUX, employing MRM analysis and typically achieving better sensitivity on a multiplexed Micromass Quattro Ultima™ than with a previous generation of instrument (Micromass Quattro LC™) running in serial mode. Both GSK and BMS are using short columns (20 or 30 mm in length) with rapid, ballistic gradients. BMS claims that interchannel crosstalk is ~0.07%. GSK supports its bead-based combinatorial synthetic efforts by using MS constructs, which make use of tags to aid detection (fluorescence) and ionization in an electrospray MS source. Steve Lane (GSK) is also

using mass-difference specificity afforded by incorporating mixtures of non deuterated and deuterated tags to deconvolute cleavage products by use of the cluster analysis functionality within MassLynx™ software (Micromass).

Proteomics

MS plays a pivotal role in the science of proteomics, which has stimulated the development of many spin-off organizations as well as a significant level of investment in large pharmaceutical companies. Among the contributions from academia, Peter Roepstorff (University of Southern Denmark, Odense, Denmark) remains convinced that two-dimensional (2D) SDS-PAGE gels still have a valuable role to play. Roepstorff's group is using high-resolution gels with techniques such as enzymatic digestion in mixed ¹⁸O/¹⁶O water and ³²P-labelled protocols to reveal semi-quantitative and phosphorylation data, respectively. Roepstorff's view about recent MS developments is that MALDI-Quadrupole-TOF combinations look promising but do not yet offer sufficient sensitivity, and that TOF-TOF technology (M. Vestal, IBS) could offer a better way forward. By contrast,

David Goodlett [Institute for Systems Biology (ISB), Seattle, WA, USA] argued that complex protein studies could be attempted without gels. To avoid gels the ISB group makes extensive use of Sequest, in conjunction with tandem MS, to generate large numbers of potential MS/MS spectra. They then match these spectra to those observed after segmentation of the tryptic-fragment mass range of 400–2000 Da into either three, or at one extreme 16, m/z windows for data-dependent MS. This approach is not for the computationally faint-hearted – parallel processor arrays of 10–100 computers are used to handle the data in real time. In one example, the use of isotope coded affinity tag (ICAT) reagents, developed by Abersold (ISB) led to 30 separate ion exchange and avidin cleaned-up fractions analyzed by 30 slow capillary LC-MS/MS runs, giving rise to 10,000 Sequest scores.

Overall, the conference was a useful summary of developments in MS at the beginning of a new millennium, with particular relevance to the pharmaceutical sector, which provides most of the financial impetus to this dynamic field of analytical instrumentation.

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