Mass spectrometry and metabolomics: past, present and future

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Published online: 17 February 2013
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From humble beginnings, the scientific discipline of metabolomics has developed with a multitude of small but significant steps in the last 15 years. Today, we are interrogating the metabolite complement of biological systems in significantly greater depth and with higher accuracy and quality than we could ever predict 15 years ago. Many developments that have emerged during this journey have been related to new tools, from analytical instruments to computational software and including novel methodologies associated with instruments and software. With these innovative tools we are deriving new knowledge related to metabolism, biochemical synthesis and the regulation of complex biological networks in diverse organisms from microbes and alga, through plants and model organisms including *Arabidopsis*, *Daphnia* and *Drosophila* to mammals.

The application of analytical platforms is one hugely important area where development in the past has, and in the future will shape our capabilities. The technological advances which allowed us to detect hundreds or thousands of metabolites in an untargeted approach, applying mass spectrometry (MS) and NMR spectroscopy, was one of the significant driving forces in the expansion of metabolomics at the end of the 20th century and in the early years of the

21st century. The two techniques of MS and NMR are highly complementary and when applied synergistically in studies they can increase our capabilities to answer biological questions. Today, MS and NMR are applied in greater than 80 % of all published metabolomics studies.

Mass spectrometry, including its integration with separation techniques including liquid chromatography and associated variants, gas chromatography and capillary electrophoresis, is a powerful bio-analytical tool to apply in metabolomics. In studies where the metabolites of interest are known and chemical standards are available absolute quantification can be applied with high precision, accuracy and sensitivity; although the metabolites still have to be extracted from the samples in a robust and quantitative (or at least known) extent, which is an important aspect in these studies. These techniques have been applied in many areas including pharmaceutical drug development, environmental testing laboratories and anti-doping laboratories such as were applied in the 2012 Olympic Games. Mass spectrometry can also be applied to study thousands of metabolites in a single sample in untargeted studies where the metabolites of biological importance are not known *a priori*; a true discovery platform providing an unparalleled phenotypic readout comprising thousands of metabolites.

It is with great pleasure that as Guest Editors we present a special issue of the journal *Metabolomics* focused on the development and application of mass spectrometry. Here we will focus on past achievements, current tools, developments for the future and novel applications. We have planned this special issue to provide an overview of the current role and potential of MS for metabolomics which have not been previously observed. We wish to thank our contributing authors and provide special thanks to our sponsors for this special issue; Agilent Technologies, LECO and Thermo Fisher Scientific.

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Many MS applications are coupled with a prior separation techniques (for example, chromatography) that has advantages related to sensitivity, selectivity and metabolite annotation/identification but is limited with respect to sample throughput, typical analysis times range from 10 to 60 min. Flow infusion electrospray-mass spectrometry (FIE-MS), also known as direct infusion or injection mass spectrometry (DIMS), removes the separation technique and directly introduces complex samples to the mass spectrometer. Sample analysis requires a few minutes only and increases sample throughput; thousands of samples can be analysed each week. Draper et al. expertly review the development of FIE-MS as a high-throughput metabolite fingerprinting tool applied to screen sample sets and where feasible to derive subsequent targeted studies. The authors discuss the workflow applied to acquire and analyse data and the important steps in relation to data quality, the lack of importance of ionization suppression and the many applications of FIE-MS observed. However and even with high mass resolution instruments, isobaric ionic species cannot be separated. Ion mobility spectrometry (IMS) has the potential to separate isobaric species. Martínez-Lozano et al. present their work focused on the exploration and characterisation of a Differential Mobility Analyser coupled to a Q-TOF mass spectrometry. The results are highly encouraging and show significant advantages to applying the technique, including resolution of isobaric species, with a reasonable analysis time of less than 5 min.

A significant bottleneck in metabolomics currently is the annotation and identification of metabolites, this process is critical to derive biological knowledge from mass spectral signals. Dunn et al. review the traditional and emerging tools being applied to annotate or identify metabolites detected in untargeted studies and the current limitations where significant further developments are required. Three complementary papers are included to highlight emerging tools being applied to annotate and identify metabolites. Bird et al. describe all-ion HCD-based fragmentation to acquire MS/MS data applied to characterise qualitatively mitochondria isolated from rat livers. Neumann et al. describe a novel nearline method to acquire, in a data dependent manner, MS/MS data applying knowledge from previous analytical runs to minimize the acquisition of irrelevant mass spectra. This is a large problem in on-line data dependent MS/MS experiments. One of the major problems in metabolite annotation is the selective identification of isomeric species. Li et al. discuss a methodology for the targeted identification of methylated flavonoid regioisomeric metabolites applying integrated enzymatic semi-synthesis and LC-MS/MS.

Many metabolomics studies eliminate spatial resolution of metabolic activity during sample collection and preparation; cells and tissues are typically homogenized prior to

analysis. The spatial distribution of metabolites in subcellular compartments can provide complementary information and enhance our biological knowledge. Armitage et al. review one of the techniques applied for 2D and 3D metabolic imaging of single cells and tissues, Secondary ion mass spectrometry (SIMS). The re-emergence of traditional tools is also an important aspect to expand our analytical capabilities and Warren investigates the application of chemical ionization (CI) with gas chromatography-mass spectrometry (GC-MS), with particular emphasis on how the complementary data from CI and electron impact studies can be applied for metabolite identification. The high intensity trimethylsilyl-derived fragment ions dominate the structural specific and higher mass ions in electron impact GC-MS; this issue is resolved when collecting CI data. The complementary nature of different liquid chromatography separation techniques is well known. Okazaki et al. describe a method of applying HILIC to class separate lipids in plants including glycerophospholipids and metabolites derived from secondary metabolism, to some an unexpected alternative to reversed phase chromatographic separations. Soltow et al. clearly highlight how two different liquid chromatography separations, ion exchange and reversed phases, coupled with FTMS can significantly expand the detectable coverage of the metabolome.

The impact of MS in metabolomics has and will continue to be derived from high quality biological studies. Five papers have been included to highlight the diversity of sample types and application areas where MS is applied. Viant and Sommer have comprehensively highlighted the use and capabilities of MS in environmental metabolomics along with current challenges which remain to be solved. The taxonomic relationships between different fungal species has traditionally been characterized applying hyphal anastomosis testing and molecular methods. Aliferis et al. describe the potential of GC-MS metabolic fingerprinting and footprinting to chemotaxonomy of *Rhizoctonia solani* species. The unique characteristics of wine are derived from the 'terroir', an interaction of the region's soil, local vineyard topography along with macroclimate and microclimate. Dreyer et al. have applied UHPLC-MS to understand the contribution of 'terroir' during the cultivation of *Vitis vinifera* L. in California. The production of biofuels from oil-rich algae is an environmentally important and interesting research field. Ito et al. have applied CE-MS and LC-MS to understand the metabolic changes of the oil accumulating trebouxioophycean alga in nitrogen-rich and nitrogen-deficient conditions. Obesity and diabetes are becoming global epidemics. Despite the introduction of hypoglycemic drugs, diabetes and related complications continue to be a major medical problem. Godzien et al. have applied the streptozotocin

(STZ) diabetic rat model with capillary electrophoresis-MS to study the effect of a nutraceutical treatment.

The special issue introduced here highlights the importance of MS as one of the analytical tools to be applied in metabolomics studies, the types of studies where MS can be applied but also the current limitations to be overcome.

Of course, MS is only one of many tools applied, metabolomics is a true multi-disciplinary research field. We hope to stimulate researchers to develop the tools and methods currently still required, or to further refine methods that are not performing at their optimum.