Are Separation Scientists suited to Metabolomics?

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Metabolomics focuses on metabolite profile changes in diverse living systems caused by a perturbation. These metabolite signatures can be measured using numerous analytical instruments. Separation techniques used include gas chromatography, high-performance liquid chromatography, ultra-high-performance/pressure liquid chromatography (UPLC), capillary HPLC (cap-HPLC), capillary electrophoresis, and capillary electrophromatography, often linked to a mass spectrometer. These techniques are ideal for the identification and quantification of small molecules, so naturally they are a big asset for metabolomics studies. The above mentioned techniques have been applied extensively in the field, with applications covering phytochemistry, toxicology and clinical research.

Metabolic fingerprinting is not a new concept and fingerprints were analysed in laboratories as soon as the capabilities of an instrument were evaluated. In October 2010 at the Desty Memorial Lecture, Milos Novotny commented on how urine fingerprints were collected between coworkers and analysed by GC-MS using what were in the early 70’s newly developed columns, with dietary experiments being performed on a regular basis. The last two decades, thanks to data processing advancements, have seen increasing interest in the “omics” sciences, in particular genomics, proteomics, metabolomics and metabonomics.

Metabonomics and metabolomics focus on metabolite profiles found in very diverse systems, from living cultures to humans. Although these two terms have been used interchangeably in some papers, their differences have been described and they are considered two separate “omic” disciplines. In broad terms, metabolomics is a qualitative and quantitative study of all metabolites in tissues or biofluids (rather than a metabolite-targeted analysis) and metabonomics, having a time-dependent aspect, will follow changes in metabolites during time points in the experiment.

The unbiased and simultaneous determination of multiple metabolites is far from easy. Three principal issues arise. First of all, the quantity of metabolites is very large. For example, there are around 600 metabolites in the microbe Saccharomyces cerevisiae, while a plant can contain approximately 200000 metabolites. This is arguably the main challenge for metabolic fingerprinting. Not surprisingly, the number of metabolites in humans is far greater than those occurring in microbes and plants. Estimations of 1 million metabolites for humans are conservative and available databases cover at best 2% of the total number of metabolites. The second issue, from an analytical point of view, is that the metabolites present in tissues or biofluids vary in their physico-chemical properties and abundance. They range from small inorganic ions to hydrophobic lipids at picomolar to millimolar concentrations, not to mention the presence of many larger molecular species and potential interferences such as proteins. The third issue, which should not concern a separation scientist, is the tendency of this omic to oversimplify analytical methods.

For simultaneous analysis, metabolite fingerprinting, nuclear magnetic resonance (NMR) and mass spectrometry (MS) are the main techniques. NMR, an unbiased, non-destructive, robust and high-throughput technology, is the predominant tool for metabolite profiling, as all compounds with NMR-measurable nuclei can be detected. Sample preparation is minimal, but its sensitivity and sample volumes requirements can limit some applications.

MS is favored for its high sensitivity and selectivity. However, it is not as robust as NMR, with low reproducibility and the possibility of failing to discriminate between certain classes of compounds due to the ionisation methods employed. Direct infusion MS is not suitable for complex samples since this type of sample will have poor ionisation as a result of matrix effects.

By linking MS to a separation technique, the data obtained is three-dimensional: retention time of compounds, mass-charge ratio (m/z) and peak intensities/areas. This delivers higher resolution and facilitates identification of metabolites. There are a number of published reviews on techniques used in metabolomics. Most of them focus their attention on MS rather than on separation science.

Separation prior to detection mainly reduces ion suppression due to ionisation inefficiency when co-elution of compounds occurs. So what are the prospects for a separation scientist in the field of metabolomics? Are GC, HPLC, UPLC, capillary HPLC, CE and CEC experts necessary in the field? Should they embrace metabolomics?

In our opinion, although many may disagree, MS based metabolomics can only be attempted by separation scientists (here we are flexible enough to include novel methods of separation like ion mobility). There is one simple reason: who else can assess the quality of the raw data? Too many so-called biomarkers are being found by following a “one-night stand” protocol. How to separate a compound from its impurity is of no concern in a fingerprinting method, let alone the possibility of quantifying a group of biomarkers by an FDA approved method. We can understand when our colleagues view the science with suspicion and regard it as an approximation. However many people see the potential, if you get a clue by approximation, then you can be onto something. In the US metabolomic-based jobs are spreading like wildfire. New available instruments and a notion “it’s simple, just follow a protocol” can
certainly promote labs into investing serious amounts of money into a metabolomics programme. This is a bandwagon that cannot be stopped.

The -omic is going global and no clear guidelines are defined for people to use. We have read several manuscripts in high impact factor journals - higher than the highest analytical chemistry journal – with inconsistencies. These include markers being identified in the void of a chromatogram, standard errors of identified compounds which are too small, for example can the variation of a metabolite in a biofluid be less than the variation of an analytical method with LC-MS? We have seen molecule identifications which are plausible from a biological point of view but do not follow fundamental laws of chromatography. Not to mention manuscripts where the statistics and modeling have completely hidden the quality of the raw data.

Biomarkers are found in several correlation-causation studies, but translation to the real world will take time. LC-MS metabolomics is not that easy. It is not that difficult either; a comment we overheard by a pioneer of separation science while at a dinner at the Royal Society – “Metabolomics? You look at metabolite profiles after a drug has been given to someone?”…yes, that is one of the applications, in fact one that is relatively easy and gives excellent results.

There is no doubt in our mind that any experienced separation scientist would have an advantage on the acquisition of metabolomic data, understanding the data, its accuracy, its limitations and how sometimes, even if your MS accuracy is sub-ppm the molecule cannot be identified. Therefore, we foresee a time when separation scientists combine efforts and achieve the rapid analysis of hundreds of thousands of metabolites in a biofluid, tissue, etc- a new age in healthcare will unravel. Computers already handle this amount of information and statistics will certainly become better at facing uncertainty. Quite how we will achieve the separation of 1 million molecules in one go is something that remains to be seen.

References

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