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Advances in liquid chromatography coupled to mass spectrometry for metabolic phenotyping

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ABSTRACT

We review the separations currently employed in the field of global metabolic profiling and based on the use of liquid chromatography (LC) coupled to mass spectrometry. Currently, most LC separations are performed using reversed-phase (RP) methods, with hydrophilic interaction chromatography a popular choice for polar compounds that are not well served by the RP mode. The use of ultra-(high)-performance LC [U(H)PLC] is seen to be increasingly replacing conventional high-performance LC (HPLC) in metabonomic/metabolomic applications as its benefits are seen as essential for rapid, high-resolution sample analysis. We discuss alternative, and emerging, methods of sample profiling, based on the miniaturization of HPLC-type separations, or the application of supercritical high-performance and ultra-high-performance chromatographic separation.

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Contents

1. Introduction 181
2. To separate or not to separate? 182
3. Coupled approaches 183
   3.1. UPLC-MS 183
   3.2. High-temperature UPLC 183
   3.3. Polar metabolite analysis LC-MS 186
4. Future technologies 188
   4.1. Miniaturization 188
   4.2. Supercritical fluid chromatography (SFC) 188
5. Conclusions 189
   References 189

1. Introduction

The use of metabolites as biomarkers for diagnosis and understanding of disease, disease monitoring, evaluation of drug safety and basic studies in physiology (and then understanding the underlying biochemistry) is not new. The most recent advances in the use of metabolites in these roles employ broad profiling methods in order to generate metabolic phenotypes {or metabotypes [1]} and utilize high-content analytical chemistry to generate global metabolite profiles. This type of "omic" profiling, known variously as metabonomics or metabolomics by their adherents, was first described in the late twentieth century {discussed in [2]}. The general aim of metabotyping is to provide information on the changes in the relative concentrations of metabolites as a result of a certain biochemical state so as to detect biomarkers (individual metabolites, or more often "fingerprints") that are specific to that particular condition. Ideally, these biomarkers should also provide new insights into the biological impact on cells, organs and whole organisms of, e.g., physiological change, disease states, and response(s) to therapy or toxic insults. The process of metabolic phenotyping is, in the first instance, based on hypothesis-free, untargeted (and ideally unbiased) measurements. These often

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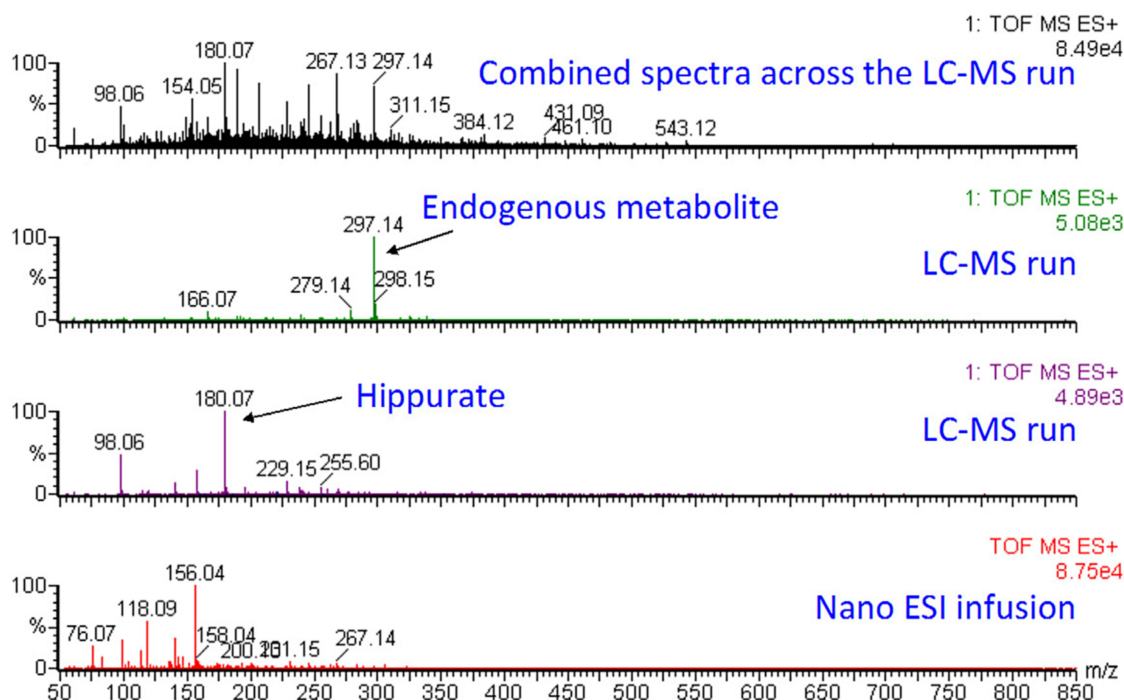

Fig. 1. Comparison of the positive ion MS signal obtained from LC-MS and direct infusion (DI). Top signal shows the combined spectra from an LC-MS run and the bottom trace shows the MS signal acquired from the same urine sample acquired by DI. The middle two traces show the extracted ion spectra for the endogenous metabolite $m/z = 271.14$ and hippurate (Castro-Perez et al., unpublished data).

provide only relative quantification and are aimed at detection and measurement of as many components in the sample as possible. Currently, global metabolite profiling is achieved using high-content analytical platforms, such as ^{1}H nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) [3–6]. The latter can be used for profiling via direct infusion (DI) of samples or extracts, or coupled to a chromatographic or electrophoretic separation {i.e., gas chromatography (GC)-MS [7], liquid chromatography (LC)-MS [8], supercritical fluid chromatography (SFC)-MS [8], or capillary electrophoresis (CE)-MS [9]}. As global metabolic profiling has matured and become a mainstream technique for systems biology and biomarker detection, new challenges have emerged. These include the desire for affordable, rapid, high-throughput, high-capacity methods that nevertheless deliver comprehensive metabolome analysis for epidemiological and clinical metabolic phenotyping. Here, we survey the current state of metabolic phenotyping by LC-MS with emphasis on the developments in liquid-phase separations.

2. To separate or not to separate?

In the case of MS-based analysis, one obvious way of reducing the overall complexity of the analytical system is, of course, to eliminate the separation step. The ability of MS to separate molecules on the basis of their mass-to-charge (m/z) ratios has led many researchers to conclude that results may be obtained faster using direct introduction [i.e., direct infusion/direct insertion (DI)] methods, and that the separation of molecules on the basis of their m/z is sufficient, especially when very high-resolution mass spectrometers are employed. And, indeed, there is a case for DIMS in terms of both speed and ease of implementation {for some interesting applications, see, e.g. [10–12],}. However, in untargeted analysis of unknown samples, problems associated with ion-suppression/enhancement effects, and the inability to distinguish between regioisomers and stereoisomers and isobaric compounds, etc., limit the value of the approach. The benefits of a separation *versus* direct MS infusion for more comprehensive untargeted metabolic phenotyping are illustrated in Fig. 1, where comparison of the spectra data obtained from the DI of rat urine, via a nano-spray device, with those derived from the summation of all of the data across the LC-MS chromatogram is shown (Castro-Perez et al., unpublished observations). The same quadrupole time-of-flight (QToF) mass spectrometer operating in positive ion electrospray mode was employed for both experiments; wherever possible, the operating conditions were kept constant. From the data displayed in Fig. 1, it is clear that there are significantly more ions detected by LC-MS analysis than in the nano-spray infusion. The selective detection of the DI approach is highlighted by detection of both hippurate and a peak of $m/z = 297.1$, as two of the most intense ions in the HPLC-MS analysis, which are barely detected by DI.

Whilst LC-MS clearly provides a more comprehensive profile, enhancing its potential for biomarker discovery, it is clearly possible to use DI approaches for screening once the biomarkers are known. This is illustrated by the analysis of rat and dog bile, previously characterized by UPLC-MS [13] using an atmospheric solids analysis probe (ASAP) [14] {first described by McEwan et al. [15]}. With appropriate sample preparation to disrupt the micelles present in the bile, this simple approach allowed both the rapid detection of many bile acids and the discrimination of bile obtained from rats and dog. Had this been, e.g., a disease-specific profile, then such an approach, with comprehensive profiling performed first to identify phenotypes and a simple DI method for subsequent targeted analysis could provide a route to robust, high-throughput screening (HTS) in, e.g., a clinical laboratory.

The increasing availability of ion-mobility separations allied to mass spectrometers may also improve the metabolic profiles that DI approaches offer by including a very rapid separation step, but they are still unlikely to remove all of the limitations of the DI approach {e.g., see [16], where preliminary results on rat urine still demonstrated the superiority of an approach employing an LC

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