Mass spectrometry-based metabolomics applied to the chemical safety of food

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Mass spectrometry (MS)-based metabolomics is emerging as an important field of research in many scientific areas, including chemical safety of food. A particular strength of this approach is its potential to reveal some physiological effects induced by complex mixtures of chemicals present at trace concentrations. The limitations of other analytical approaches currently employed to detect low-dose and mixture effects of chemicals make detection very problematic. Besides this basic technical challenge, numerous analytical choices have to be made at each step of a metabolomics study, and each step can have a direct impact on the final results obtained and their interpretation (i.e. sample preparation, sample introduction, ionization, signal acquisition, data processing, and data analysis). As the application of metabolomics to chemical analysis of food is still in its infancy, no consensus has yet been reached on defining many of these important parameters. In this context, the aim of the present study is to review all these aspects of MS-based approaches to metabolomics, and to give a comprehensive, critical overview of the current state of the art, possible pitfalls, and future challenges and trends linked to this emerging field.

Keywords: Chemical risk; Chemical safety; Complex mixture; Food safety; Low-dose effect; Mass spectrometry; Metabolomics; Mixture effect; Physiological effect; Trace concentration

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1. Introduction

Although not particularly that recent, the so-called ''-omic'' techniques (transcriptomic, proteomic, and metabolomic) are attracting huge interest with substantial developments and applications in various research areas in the past few years. The fundamental idea is to explore ''life complexity'' using unrestrictive descriptive methodologies. Thus, these approaches are all based on the generation of large sets of descriptors expected to characterize the biological system under investigation (cell, tissue, fluid, organ, or entire organism). These descriptors refer to genomic, proteomic or metabolic species.

With the metabolome, the monitored signals correspond to chemical substances (so-called metabolites) accessible to direct analysis, which are final products formed after complex transcription, transduction, and regulatory mechanisms. Currently, two complementary approaches are used for metabolomic investigations: metabolic profiling and metabolic fingerprinting [1]. Metabolic profiling focuses on the analysis of a group of metabolites related to a specific metabolic pathway or a class of compounds. In most cases, metabolic profiling is a hypothesis-driven approach, which depends on prior knowledge of the biological system. Initially, in metabolic fingerprinting, the intention is not to identify each observed metabolite, but to compare patterns or ''fingerprints'' of all metabolites accessible to the analysis that change in response to disease, toxin exposure, environmental or genetic alterations. Metabolic fingerprinting is the topic of this review.

In the specific field of chemical risk, an important conceptual issue linked to metabolomics is that the generated biological signatures are expected to reflect the presence of chemical pollutants (including their possible degradation and biotransformation products) and their biological impact. A particular strength of this approach is that it can potentially reveal

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some physiological effects induced by complex mixtures of chemicals present at trace concentration levels, so interrogation of the metabolome may present a new way of investigating the complex subject of chemical contamination of food [2].

The general principle of metabolomics is to characterize biological samples by the production of a chemical profile (i.e. a chemical signature or fingerprint) [3,4]. From an analytical point of view, the most widely-used technique for this purpose has been nuclear magnetic resonance (NMR) [5–7]. However, mass spectrometry (MS) is becoming more widely used in this field [8]. Indeed, MS offers higher performance in term of sensitivity, which is extremely useful for measuring species with low abundance, as that provides valuable information. Moreover, the specificity of MS (through high-resolution and/or multidimensional MS^n techniques) can help and even facilitate elucidation of the chemical structures of potential metabolites of interest (i.e. identification of biomarkers).

Besides this technical challenge, numerous analytical choices (e.g., sample preparation, sample introduction, ionization, signal acquisition, data processing, and data analysis) have to be made at each step of a metabolomic study (Fig. 1), and they influence the final results and/or interpretation. As no definitive, unique standard operating procedures currently exist, the comparative generation and analysis of data from identical samples, but acquired through different analytical conditions, must be of great value in aiding interpretation of the information generated. Such developments relating to analysis and comparisons have recently been conducted in several laboratories, leading to the formulation of in-house strategies for sample preparation, analytical tools and bioinformatics for analysis of the vast amount of data generated [9].

In this context, the aim of the present study is to review all these aspects of MS-based metabolomics approaches in the specific area of food-based chemical risk. Following each successive step usually required in this field (Fig. 1), the purpose of this review is to give a comprehensive, critical overview of the current state of the art, possible pitfalls, and future challenges and trends linked to this important emerging field.

2. Sample preparation

The extremely wide diversity of potential metabolites present in a sample in terms of chemical structures and concentrations means that it is unrealistic to have the goal of measuring them all in metabolomics. Nevertheless, the sample-preparation procedure has to be as broad as possible in order to avoid losing potentially important information. A compromise is needed between the efficiency and the reproducibility of the sample-preparation procedure applied and the scope of the analysis. Minimal sample preparation is usually preferred, especially for untargeted applications without any presupposed hypothesis. Conversely, some applications can use relatively selective sample-preparation protocols, if some preliminary data or knowledge suggest that the most useful information to look for is present in one or more particular fractions of the sample to be targeted. Typical examples are metabolomics approaches oriented toward very polar, low-molecular-weight species (e.g., amino acids) [10], or, by contrast, more lipophilic compounds [11–13] that require different analytical procedures.

Preliminary sample pre-treatments depend on the nature of the sample. For liquid samples (biological fluids), a protein-elimination step common, using precipitation or filtration to limit ion suppression when using eletrospray for liquid chromatography with MS (LC-MS) [14]. However, this approach can suffer from co-precipitation of some metabolites with such macromolecules and compounds bound to carrier proteins. A freeze-drying step followed by reconstitution at a defined dry-matter concentration can also be used for urine, as a way to avoid the dilution-factor issue typically encountered with this matrix [15,16]. Alternatively, post-acquisition normalization solutions to this problem can be envisaged (e.g., normalization of each metabolite signal with the signal intensity of creatine or normalization based on the total signal) [15]. The dilution of urine or blood (serum, plasma) samples has also been employed to limit the impact of matrix effects [17]. A preliminary quenching step is also important, especially for samples arising from cell-culture systems, in order to stabilize the sample by stopping metabolic reactions [18,19]. In research on the chemical safety of food and endocrine disruption, areas that both have a strong interest in hormonal substances or drug exposure and effects, the question of using a deconjugation step for cleaving phase II metabolites (e.g., glucuronides and sulfates) must also be considered if the detection technique to be used is not suitable for direct measurement of such very polar compounds.

For solid matrices, an extraction step is required for transferring the metabolome compounds into a liquid phase [20]. Samples can be freeze dried prior to extraction to allow for better homogeneity, repeatability, and extraction capabilities. Different solvent systems with various polarities can be used at this stage (e.g., methanol, acetonitrile, ether, acetone, hexane, cyclohexane) which leads not surprisingly to the production of different and complementary fractions of the metabolome being studied [14,18,19]. Subsequent purification steps may then be employed (e.g., liquid-liquid partitioning or solid-phase extraction), depending on degree of selectivity to be reached for the particular application.