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Review

Liquid chromatography–mass spectrometry in metabolomics research: Mass analyzers in ultra high pressure liquid chromatography coupling

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ABSTRACT

The present review gives an introduction into the concept of metabolomics and provides an overview of the analytical tools applied in non-targeted metabolomics with a focus on liquid chromatography (LC). LC is a powerful analytical tool in the study of complex sample matrices. A further development and configuration employing Ultra-High Pressure Liquid Chromatography (UHPLC) is optimized to provide the largest known liquid chromatographic resolution and peak capacity. Reasonably UHPLC plays an important role in separation and consequent metabolite identification of complex molecular mixtures such as bio-fluids. The most sensitive detectors for these purposes are mass spectrometers. Almost any mass analyzer can be optimized to identify and quantify small pre-defined sets of targets; however, the number of analytes in metabolomics is far greater. Optimized protocols for quantification of large sets of targets may be rendered inapplicable. Results on small target set analyses on different sample matrices are easily comparable with each other. In non-targeted metabolomics there is almost no analytical method which is applicable to all different matrices due to limitations pertaining to mass analyzers and chromatographic tools. The specifications of the most important interfaces and mass analyzers are discussed. We additionally provide an exemplary application in order to demonstrate the level of complexity which remains intractable up to date. The potential of coupling a high field Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (ICR-FT/MS), the mass analyzer with the largest known mass resolving power, to UHPLC is given with an example of one human pre-treated plasma sample. This experimental example illustrates one way of overcoming the necessity of faster scanning rates in the coupling with UHPLC. The experiment enabled the extraction of thousands of features (analytical signals). A small subset of this compositional space could be mapped into a mass difference network whose topology shows specificity toward putative metabolite classes and retention time.

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1. Introduction

The successes of gas chromatography mass spectrometry (GC–MS) during the sixties lead to the idea of coupling liquid chromatography to mass spectrometry (LC–MS). The attempt of merging started in the seventies but was met with skepticism due to the task's complexity and alleged limitations. Despite initial hesitations, the technique managed to revolutionize the analytical scenario by enabling the analysis of non-volatile or thermally labile high molecular compounds for which the GC–MS approach was not suitable. Several optimizations followed in the field of LC–MS and different interfaces were developed in order to guarantee an easy and robust analysis, something that brought this hyphenated technique to a huge success.

To date, many applications in different fields are being developed in many laboratories on a routine base. In the pharmaceutical field the application of LC–MS is used in order to develop analytical procedures (quality control) necessary for the identification of impurities in drugs. Different combinations of interfaces as electrospray ionization (ESI), atmospheric pressure photon ionization (APPI) and atmospheric pressure chemical ionization (APCI) are being applied [1]. The identification of compounds has become easier and more efficient thanks to the coupling of LC to mass analyzers which (compared to UV detectors) eliminates interferences from other peaks [2]. LC–MS was used in the study of the mechanisms involved in drug- induced metabolism [3] allowing high throughput analysis relative to the previous GC–MS, where derivatization of analytes was required in order to improve resolution and sensitivity [4]. The application of LC–MS, next to GC–MS, isotope ratio mass spectrometry (IR-MS) and inductively coupled plasma mass spectrometry (ICP-MS), gained popularity in forensic science where precise protocols are necessary in order to detect traces of specific chemical compounds in complex matrices [5]. These techniques were applied on the investigation of catabolites from Chemical Warfare Agents (CWAs) [6], the analysis of trace levels of explosives [7,8], the detection of dyes from textile fibers [9] and food [10], and the detection of drugs in biofluids [11] and hair [12]. Another field where LC–MS implementation is of wide interest is doping control analysis where the detection of various steroids was not possible via GC–MS [13]. Different mass analyzers such as triple quadrupole (QqQ), time of flight (TOF) and quadrupole time of flight (QTOF) were compared with respect to coupling to LC [14] for the detection of anabolic steroids in urine with qualitative, quantitative and post-targeted approaches. Qualitative and quantitative analysis via QqQ using Selected Reaction Monitoring (SRM) gave the best results in the detection of model compounds showing high linearity and accuracy. TOF and QTOF showed a limitation in the sensitivity level necessary for the detection of all above-mentioned compounds but achieved good results in the post-targeted approach.

Hard ionization techniques such as inductively coupled plasma ionization (ICP) were coupled to LC for speciation studies [15–17] and were applied to the elemental characterization of gold nanoparticles [18].

Additional hyphenated techniques, such as capillary electrophoresis (CE), are being commonly used for the complementary analysis of polar and charged molecules. Coupling of the widely used TOF-MS to CE was combined with the commonly applied UV in order to gain higher sensitivity and to enable the separation of co-migrating analytes [19,20]. The most popular on-line interfaces in CE-MS were electrospray ionization (ESI) [21] followed by ICP [22] and matrix assisted laser desorption ionization (MALDI) [23]. Non-targeted metabolomics studies via CE-TOF-MS were reported for studies on transgenic maize [24], human urine [25,26], saliva samples [27] and red blood cell lysates [28]. Studies of screened biofluids for different pathologies have been reviewed within this context [29].

In the last decade the advent of holistic approaches, such as metabolomics, vastly increased the amount of analytes to be identified and quantified in one single LC–MS analysis. As scientific demands increased, previously common detection methods such as UV–vis detection or fluorescence detection were proved inadequate in terms of selectivity, specificity and sensitivity. Mass spectrometric detection has over the years become the standard detection method; however, optimal performance of LC–MS calls for basic knowledge on LC–MS interfaces and on the advantages and disadvantages of different mass spectrometers. The contemporary analysis of the metabolome requires high resolution techniques in every aspect. The possibilities to combine separation techniques with different mass analyzers are vast. Fig. 1 gives a schematic overview on the queue of methods and their resolving powers.

This review starts with a brief introduction into the concepts of metabolomics followed by an excursus on LC–MS interfaces. Section 3 discusses the suitability of LC coupling to Fourier-transform-ion-cyclotron-resonance (FT-ICR), Orbitrap™ and time-of-flight (TOF). An application example on the at-line coupling of UHPLC and ICR-FT/MS is proposed as a solution for combining high chromatographic resolution while preserving resolving power.

1.1. Introduction into metabolomics

1.1.1. Metabolomics: the concept

Along with the numerous improvements accomplished in modern molecular biology during the past decades, the necessity of a fundamental integration of different disciplines such as transcriptomics, proteomics and metabolomics arise. The holistic integration of the different "-omics" disciplines with the support of mathematical modeling (i.e. "top-down" system biology [30,31]) represents the key for understanding regulated responses toward genetic variation, environmental factors and possible diseases [32] throughout different biological levels (subcellular, cellular, tissues, organs). In all organisms, different kinds of bio-transformations are