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Application of liquid chromatography–mass spectrometry to biomonitoring of exposure to industrial chemicals

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Abstract

Recent advances on biomarker research are discussed, primarily relying on experience gained with technologies based on liquid chromatography–*tandem* mass spectrometry (LC–MS–MS). Determination of urinary metabolites of industrial chemicals (*n*-hexane, benzene, toluene, and styrene) in samples from occupationally exposed workers and controls was performed by LC–MS–MS with either electrospray (ESI) or atmospheric pressure chemical ionization (APCI), as appropriate. Both phase I and II metabolites (glucuronides, sulfates, and mercapturic acids) can be detected with little or no sample manipulation, thus allowing the identification of a number of artifacts and "new" metabolites. However, experimental evidence indicates the need for properly addressing the matrix effect, which is always associated with the analysis of biological samples. Both efficient sample preparation and the use of isotopically labeled internal standards seem to be necessary to develop validated quantitative methods.

Keywords: Biomarkers of exposure; Metabolites; Artifacts; LC–MS; Urine; Matrix effect

1. Introduction

Liquid chromatography–mass spectrometry (LC–MS) is a relatively young analytical technique. In 1982, the on-line coupling of LC and MS was depicted like a difficult courtship between a fish and a bird (Arpino, 1982), due to incompatibility of flow-rates and mobile phase composition, as well as to the difficulty in the ionization of non-volatile and thermally labile analytes. These problems in the LC–MS coupling were solved by the development of new ionization techniques and robust interfaces (Niessen, 1999). Among the number of proposed and commercialized devices, electrospray (ESI) spread owing to its wide applicability, in terms of both polarity and molecular weight of the candidate analytes. ESI ionization is obtained by applying a high electric field to the liquid flow through a capillary. A complementary technique is atmospheric pressure chemical ionization (APCI), where electrons are generated at a corona discharge needle and the solvent vapour acts as reagent gas.

The success of LC–MS can be proven by the number of publications, which is exponentially increasing year after year; at the present time, more than 3200 papers are found searching for LC–MS by PubMed (2004). The reasons for such a success are due to the fact that LC–MS kept and linked the advantages of both the parent techniques: from LC, it extended the compatibility with aqueous matrices and the high efficiency and selectivity in separation, whereas from MS, it took the peculiar

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characteristics, i.e. the high selectivity and sensitivity, as well as its wide linear dynamic range. On the other hand, the main disadvantages of LC–MS are the high costs and the requirement of highly qualified personnel. As compared to other analytical techniques, the use of LC–MS results in a little, or at least a reduced pre-analytical work, diminished risk of artifacts generation, possibility of automation, short analysis times, high sample throughput, and reliable quantitation over a wide range of concentrations.

Recently, the use of LC–MS has been extended to human biomonitoring in occupational health and it has been applied to biomarker research, although the number of applications is still limited when compared to other related fields, like pharmacokinetics (Lim and Lord, 2002), clinical chemistry, and forensic toxicology (Marquet, 2002). Although a comprehensive review of the LC–MS application is out of the scopes of this paper [for biomarkers of exposure and macromolecular adducts see (Manini et al., 2004) and (Koc and Swenberg, 2002; Törnqvist et al., 2002), respectively], some examples, primarily relying on the experience gained by our laboratory will be presented, with the aim to clearly show the potential of this technique in metabolism studies aimed at investigating minor metabolic routes and new more specific biomarkers, as well as in the quantitative determination of traditional biomarkers of exposure.

Depending on the aim of the study (identification or quantitation), the use of MS, and particularly that of *tandem* mass spectrometry (MS–MS) offers several possibilities, as shown in Fig. 1. To obtain structural information, the MS–MS can be operated in product-ion scan mode (Fig. 1a): in this case, the (de)protonated molecule, selected by the first quadrupole (Q1, select), is fragmented in the collision cell (Q2) and analyzed by the third quadrupole (Q3, scan) that acquires in full-scan mode. A complementary possibility is the precursor-ion scan mode (Fig. 1b), where the function of the quadrupoles is inverted (Q1 scan, Q3 select), to search for precursor molecules, which generated a certain fragment ion. For screening purposes, and particularly for the identification of phase II conjugated metabolites, the neutral loss scan mode (Fig. 1c) could be applied. In this case, both quadrupoles are scanning but at a fixed mass difference related to the conjugated moiety: neutral loss of 80, 176 and 275 Da are characteristic of aryl-*O*-sulfate-, *O*-glucuronide-, and aryl-*S*-glutathione-conjugates, respectively. Finally, to perform quantitative analyses, the MS–MS could be operated in the selected reaction monitoring (SRM) mode (Fig. 1d), with both the quadrupoles acquiring one (or more) fixed mass(es), that

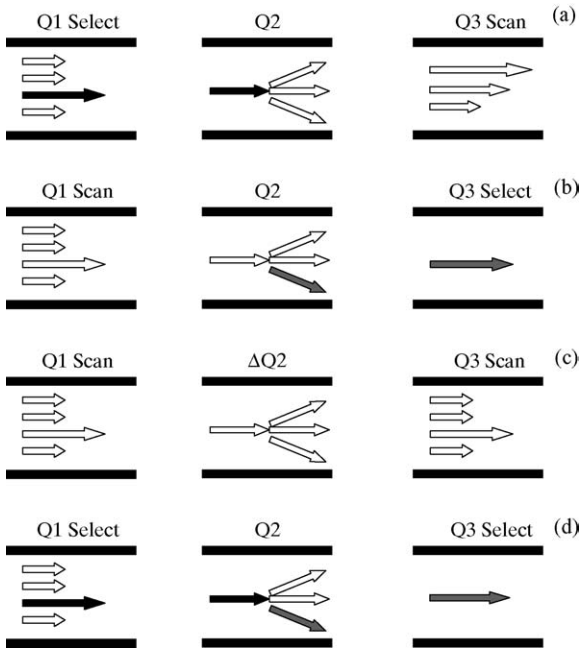

Fig. 1. Different operative set-ups in tandem mass spectrometry: (a) product-ion scan; (b) precursor-ion scan; (c) neutral loss; (d) selected-reaction monitoring (SRM) (Q1, first quadrupole; Q2, collision cell; Q3, third quadrupole).

(or those) of the precursor(s) (Q1) and fragment(s) (Q3). This operative set-up allows reaching the best sensitivity and selectivity of MS–MS.

In this paper, examples of application to authentic samples will be reported to show the unique feature of LC–MS in (a) identification of new metabolites; (b) identification of analytical artifacts; (c) quantitation of "traditional" biomarkers; and (d) quantitation of minor and "new" metabolites.

2. Experimental

2.1. Subjects

Urine samples from workers exposed to styrene were collected at the end of the work-shift. Blank urine samples were obtained from non-exposed subjects. Samples were stored at −20 °C until LC–MS–MS analysis. Before injection, samples were filtered on a 0.20-μm membrane (Chemtek Analytica, Bologna, Italy) and acidified with formic acid (0.1 M, final concentration).

2.2. Animals

Sprague–Dawley rats (200–450 g), about 6 months old, were treated with: (a) 7.3 mmol *n*-hexane/kg b.w. (s.c. injection); (b) an equimolar mixture (2.4 mmol styrene + 2.4 mmol