Analytica Chimica Acta 853 (2015) 625–636

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Feasibility of ultra-high performance liquid and gas chromatography coupled to mass spectrometry for accurate determination of primary and secondary phthalate metabolites in urine samples

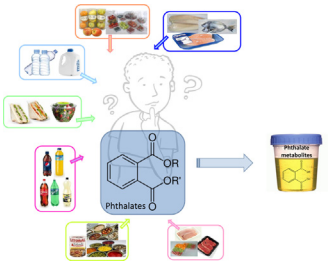
Laura Herrero, Sagrario Calvarro, Mario A. Fernández, Jesús Eduardo Quintanilla-López, María José González, Belén Gómara *

General Organic Chemistry Institute, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

HIGHLIGHTS

- GC-EI-ITD(SIM) has been validated to determine 9 MPAEs in urine samples.
- UHPLC-ESI(−)QqQ MS^2 has been validated to determine 9 MPAEs in urine samples.
- 9 MPAEs were determined in normal Spanish human urine samples.
- Differences in MPAE urine levels were found between Spanish men and women.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form 23 September 2014
Accepted 25 September 2014
Available online 28 September 2014

Keywords:
Phthalate metabolites
Urine
Gas chromatography
Ultra-high performance liquid chromatography
Mass spectrometry

ABSTRACT

Phthalates (PAEs) are ubiquitous toxic chemical compounds. During the last few years, some phthalate metabolites (MPAEs) have been proposed as appropriate biomarkers in human urine samples to determine PAE human intake and exposure. So, it is necessary to have fast, easy, robust and validated analytical methods to determine selected MPAEs in urine human samples. Two different instrumental methods based on gas (GC) and ultra-high performance liquid (UHPLC) chromatography coupled to mass spectrometry (MS) have been optimized, characterized and validated for the simultaneous determination of nine primary and secondary phthalate metabolites in urine samples. Both instrumental methods have similar sensitivity (detection limits ranged from 0.03 to 8.89 pg μL^{-1} and from 0.06 to 0.49 pg μL^{-1} in GC–MS and UHPLC–MS^2, respectively), precision (repeatability, expressed as relative standard deviation, which was lower than 8.4% in both systems, except for 5OH-MEHP in the case of GC–MS) and accuracy. But some advantages of the UHPLC–MS^2 method, such as more selectivity and lower time in the chromatographic runs (6.8 min vs. 28.5 min), have caused the UHPLC–MS^2 method to be chosen to analyze the twenty one human urine samples from the general Spanish population. Regarding these samples, MEP showed the highest median concentration (68.6 $\mu g L^{-1}$), followed by MiBP (23.3 $\mu g L^{-1}$), 5cx-MEPP (22.5 $\mu g L^{-1}$) and MBP (19.3 $\mu g L^{-1}$). MMP (6.99 $\mu g L^{-1}$), 5oxo-MEHP (6.15 $\mu g L^{-1}$), 5OH-MEHP (5.30 $\mu g L^{-1}$) and MEHP (4.40 $\mu g L^{-1}$) showed intermediate levels. Finally, the lowest levels were found for MBzP (2.55 $\mu g L^{-1}$). These data are within the same order of magnitude as those found in other similar populations.

* Corresponding author. Tel.: +34 915622900; fax: +34 915644853.
E-mail address: bgomara@iqog.csic.es (B. Gómara).

http://dx.doi.org/10.1016/j.aca.2014.09.043
0003-2670/

1. Introduction

Phthalate acid esters (PAEs) are well known chemical compounds. In fact, they began to be used in the plastics industry more than 80 years ago. PAEs are mainly used as plasticizers, but they are also used as solubilizing or stabilizing agents in other applications such as detergents, building products (flooring, sheeting, and films), lubricating oils, carriers in pesticide formulations, solvents, personal care products, cosmetics, toys, some medical devices, etc. [1]. During these years their presences in the environment in large quantities have been confirmed, but the results of different studies about their toxicity and persistence concluded that phthalates were not dangerous for human health. However, during the last few years, their activity as endocrine disruptors, their impact on the normal development of living organisms, as well as their teratogenic activity in both human and animal studies have caused them to be considered new emerging contaminants [2,3]. As a result, a high amount of research is now being conducted in order to know their levels and behavior in the environment and in humans, their main pollution sources as well as the way to decrease their levels around us [4,5]. They are now included on the priority list of dangerous substances in the legislation of most industrialized countries, where the use of plastic materials containing food or anything child-related is limited. Besides, maximum permitted levels in the water environment and ambient workplaces have been established [6–9].

For the general population, the oral exposure has been considered the major route, including inhalation of air (indoors and outdoors), ingestion of food, incidental ingestion of soil, and ingestion of dust (indoors), as well as direct contact with products that contain phthalates. Some studies suggested [10,11] that, among them, food represents the most important source of exposure to dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP) and diethylhexyl phthalate (DEHP). Once ingested, phthalate diesters are metabolized, initially hydrolyzed in the intestine to their corresponding monoester (primary metabolites), which are then absorbed, and could be further oxidized in the body (secondary metabolites), and excreted quickly via urine [12,13].

Studies on health effects of PAEs in humans have remained controversial due to limitations of the study design. Some findings in human populations are consistent with animal data, suggesting that PAEs and their metabolites (MPAEs) produce adverse effects in the reproductive system [14]. However, it is not yet possible to conclude whether phthalate exposure is harmful to human reproduction [15]. To achieve that, more results about toxicity in humans and, especially, about the daily intake for the general population are needed to estimate if the presence of phthalates involves a problem for human health and, in this case, to limit or even ban its industrial use.

Estimating human exposure to PAEs by measuring them in foodstuffs, collecting survey/questionnaire data on personal lifestyle and food consumption, is not very satisfactory because, as previously mentioned, there are other sources which contributed to the overall human exposure to PAEs (e.g., dermal contact, environmental media). Because of that, since the late 90s many studies have been conducted aiming to prove that urinary concentration of PAE metabolites could be used as biomarkers to estimate dose in risk human assessment of PAEs. After the study carried out by David in 2000 [16], who estimated daily intake levels of phthalates based on primary urinary MPAE concentrations in the US population, it was followed by similar studies, published between 2003 and 2014, in which the daily intake of PAEs based on the urinary metabolite concentrations in various populations was estimated (e.g., US, China, Italy, Germany, Denmark, Taiwan) [12,17–24]. At the same time, the reporting of levels of MPAEs in the urine of populations from different countries has been rapidly increasing during the last few years [25–28].

Everything expounded up to the moment demonstrates the need to count on well characterized and validated analytical methods, that allow the unequivocal determination of the concentrations of primary and secondary MPAEs in human urine samples, being, preferably, fast, cheap and environmental friendly methods.

Until now, high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS^2) using isotope dilution technique has been the preferred method for analyzing MPAEs in human urine samples [17–28]. Various reliable HPLC–MS^2 methods covering the most important primary [29], and secondary MPAEs [13,30–32] have been developed. However, long analysis time has been obtained when secondary metabolites are included and the use of ultra-high performance liquid chromatography (UHPLC) has not yet been reported for MPAEs analysis. In the case of gas chromatography coupled to mass spectrometry (GC–MS), there are very few studies using this technique for MPAE analysis in urine samples, and they were focused on primary [20,33] or secondary oxidized MPAEs [34], but not the simultaneous determination of primary and secondary metabolites in urine samples.

In this study the feasibility of GC and UHPLC methods, both coupled to MS, to simultaneously determine nine MPAEs (primary and secondary) in urine human samples is presented. In both cases, the development, optimization and validation have been presented and their feasibility was studied in terms of acquisition and maintenance costs, simplicity of use, accuracy, sensitivity, selectivity, precision and analysis time, by comparison of the results achieved using them, and also in comparison with the methods previously published in the literature. Regarding GC, a gas chromatograph coupled to an ion trap mass spectrometer, working in selective ion monitoring mode and equipped with an electron impact ionization source (GC-EI-ITD(SIM), GC–MS hereafter) was used. The UHPLC method was developed in an ultra-high performance liquid chromatograph coupled, by an electrospray ionization source, to a triple quadrupole mass spectrometer working in its tandem operation mode (UHPLC-ESI(−)-QqQ (MS^2), UHPLC–MS^2 hereafter). To our knowledge, this is the first time that GC–MS and UHPLC–MS^2 have been used for the simultaneous determination of primary and secondary oxidized metabolites of phthalates. Finally, the concentration levels of MPAEs in urine samples from Spanish volunteers are provided.

2. Experimental

2.1. Standards, chemicals, adsorbents and solvents

Nine individual MPAEs, (6 phthalate monoesters (mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-*iso*-butyl phthalate (MiBP), mono-*n*-butyl phthalate (MBP), mono-benzyl phthalate (MBzP) and mono(2-ethylhexyl) phthalate (MEHP) and 3 secondary oxidized metabolites of DEHP (5OH-mono(2-ethylhexyl)phthalate (5OH-MEHP), 5oxo-mono(2-ethylhexyl)phthalate (5oxo-MEHP) and 5carboxy-mono(2-ethylpentyl)phthalate (5cx-MEPP), seven $^{13}C_4$ labeled internal standards (MEP, MBP, MBzP, MEHP, 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP) and the deuterated mono benzyl butyl phthalate, BzBP-D_4 were purchased from Cambridge Isotope Laboratories (MA, USA) and AccuStandard Inc. (New Haven, CT, USA).

Florisil® and sodium chloride were purchased from Merck (Germany). OASIS® HLB SPE cartridges (6 cc, 200 mg) were provided by Waters (MA, USA). β-Glucuronidase solution (140 unit mL^{-1}) from *Escherichia coli* was purchased from Roche Diagnosis GmbH (Germany). Trimethyl silyl diazomethane (TMSDM) (2 M in