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Simultaneous determination of some phthalate metabolites, parabens and benzophenone-3 in urine by ultra high pressure liquid chromatography tandem mass spectrometry

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ABSTRACT

Phthalates, parabens and 2-hydroxy-4-methoxybenzophenone or benzophone-3 are thought to act as endocrine disrupting chemicals, being able to disrupt the endocrine balance and therefore able to lead to some hormonal diseases. Numerous large-scale biomonitoring studies have detected the biomarkers of these compounds in more than 75% of the general population. To assess the exposure to these chemicals, we developed an analytical method based on a Solid Phase Extraction (SPE) prior to ultra high pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for the simultaneous measurement of seven phthalate metabolites (monobenzyl phthalate, mono-n-butyl phthalate, mono-iso-butyl phthalate, mono-2-ethylhexyl phthalate, mono-2-ethyl-5-hydroxyhexyl phthalate, mono-2-ethyl-5-oxohexyl phthalate, monoethyl phthalate), four parabens (methyl paraben, ethyl paraben, n-propyl paraben, n-butyl parabens) and benzophenone-3 in human urine. The distinction between unconjugated, glucuro- and sulfoconjugated forms was achieved using different enzymatic hydrolyses. The whole procedure was validated according to the total error approach, and was demonstrated to be linear (regression coefficient ranging from 0.987 to 0.998) and accurate (inter and intra assay precision <17.71%, relative bias <5.87%) in the dosing range of concentrations. The limits of quantification (LOQs) obtained ranged between 0.30 and 1.23 ng/ml depending on the analyte. The reliability of the method was proven in passing successfully the German External Quality Assessment Scheme (G-EQUAS). Moreover, the urine from 25 volunteers were analyzed for the determination of glucuro-, sulfo- and free species separately. Phthalate metabolites, parabens and benzophenone-3 were positively detected in almost all urine samples, with detection rates ranging from 40 to 100%. Levels measured ranged from <LOQ to 2207 ng/ml varying widely depending on the compound and the individual. In our small participating population, most of the phthalate metabolites were excreted predominately as glucuroconjugated forms while parabens and benzophenone-3 were detected as glucuro- and sulfoconjugated species in variable proportions according to the target compound.

1. Introduction

Phthalates, esters of p-hydroxybenzoic acid (parabens) and 2-hydroxy-4-methoxybenzophenone or benzophenone-3 (BP3) are high-production chemicals [1–3] present in a broad range of everyday life products (Fig. 1). The phthalates are mostly used as plasticizer in plastics and more particularly in polyvinyl chloride (PVC). They are commonly found in many household products, construction materials, solvents, lubricants, personals care products, textiles, food contact materials, etc. [1]. Parabens are widely employed as antimicrobial conservator in some cosmetics, sunscreens, foodstuffs and pharmaceutical preparations [4]. Benzophenone-3 is a broadband UV filter found in 59% of sunscreens in the United States [3] and also present in plastic surface coatings of food packaging as an UV stabilizer [5]. All these compounds are suspected to exhibit endocrine disruptive properties: they would be able to interact with the endocrine system causing alteration of the endocrine homeostasis and potentially lead to adverse health effects [2,6,7].

The endocrine disrupting chemicals (EDC) have been linked to various endocrine diseases such as some reproductive disorders,

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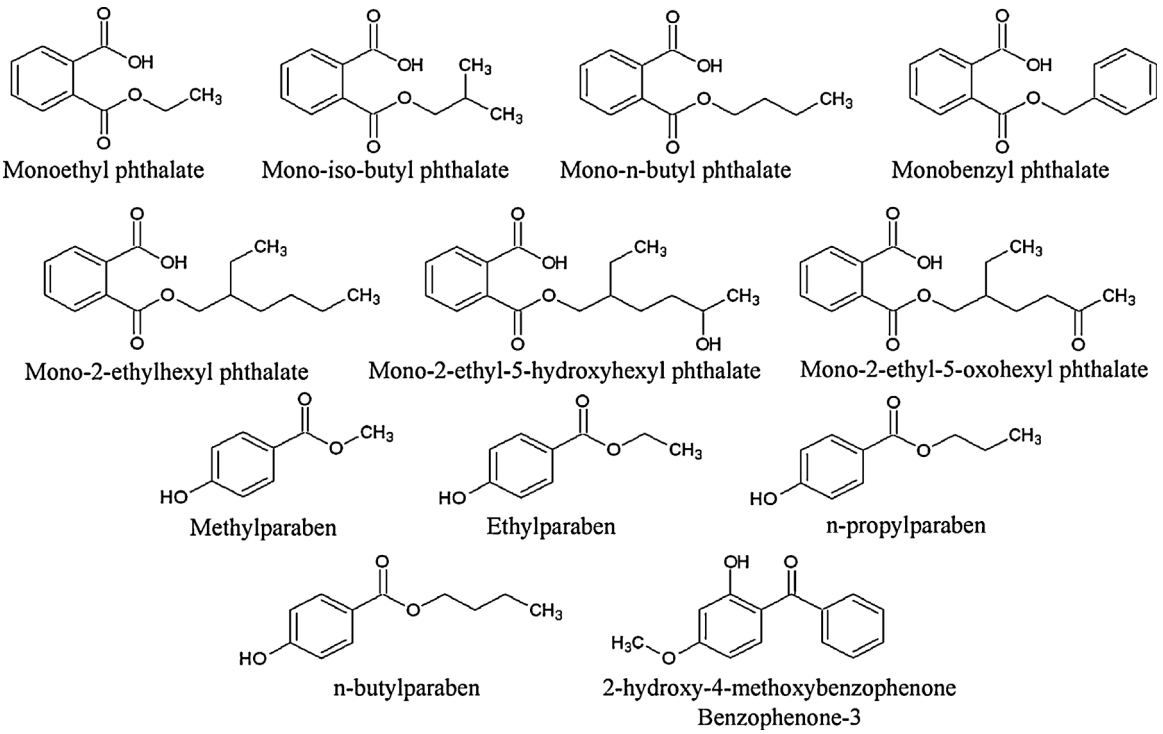

Fig. 1. Structure of the studied compounds.

infertility, hormone-dependant cancers, obesity, diabetes, neurodevelopmental disorders [8,9]. For instance phthalate exposure was associated with insulin resistance and waist circumference [10], with DNA damages in human sperm and reduction of sperm quality [11,12], and with reduced anogenital distance [13] and premature thelarche [14]. Numerous studies have detected positively phthalates, parabens and BP3 in more than 75% of urine samples analyzed, demonstrating that the general population is widely exposed [15–17]. Since they are known to be excreted from human body as parents or metabolites in the urine, their urinary concentration would reflect their individual level exposure [15,16,18].

If the phthalate metabolites and parabens are commonly measured using liquid chromatography coupled to mass spectrometry (LC-MS) [19–22], gas chromatography-mass spectrometry (GC-MS) could also be used, but requires further derivatization steps [23]. An online solid phase extraction (SPE) coupled with LC-MS method was previously developed [24–27], providing the non-negligible advantage of saving time but requiring specific and expensive devices. Besides the LC-MS methods [28–31], conventional high-performance liquid chromatography combined to diode array detection (HPLC-UV/DAD) or GC-MS are able to detect BP3 in urine samples [32–36].

The aim of this study was to develop and validate an analytical procedure to measure 7 phthalate metabolites including monohydrolyzed and oxidized compounds, 4 alkyl parabens and the BP3 in human urine. This method consisted in an enzymatic hydrolysis followed by offline SPE and ultra high pressure liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) detection. The validation process was performed using the total error approach on the three different enzymatic conditions in order to assess accurately the proportion of the free, glucuro- and sulfoconjugated species in real urine samples. This is, to our knowledge, the first method allowing the simultaneous determination of phthalate metabolites, parabens and BP3.

2. Materials and methods

2.1. Chemicals and reagents

Monobenzyl phthalate (MBzP), monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (5-OH-MEHP), mono-2-ethyl-5-oxohexyl phthalate (5-oxo-MEHP), mono-iso-butyl phthalate (MiBP) and the isotope labeled $^{13}C_4$ monobenzyl phthalate ($^{13}C_4$ MBzP), $^{13}C_4$ mono-n-butyl phthalate ($^{13}C_4$ MnBP), $^{13}C_4$ mono-2-ethyl-5-oxohexyl phthalate ($^{13}C_4$ 5-oxo-MEHP), $^{13}C_4$ monoethyl phthalate ($^{13}C_4$ MEP), $^{13}C_4$ mono-2-ethyl-5-hydroxyhexylphthalate ($^{13}C_4$ 5-OH-MEHP), $^{13}C_4$ mono-2-ethylhexylphthalate ($^{13}C_4$ MEHP) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). The concentration of each individual commercial standard was 100 μg/ml in acetonitrile or in methyl tert-butyl ether. Methylparaben (MP), n-butylparaben (BP) and their respective labeled $^{13}C_6$ methylparaben ($^{13}C_6$ MP) and $^{13}C_6$ n-butylparaben ($^{13}C_6$ BP) were supplied in methanol at 1 mg/ml also by Cambridge Isotope Laboratories. Ethylparaben 99% (EP) and n-propylparaben >99% (PP) were bought from Sigma Aldrich (St. Louis, MO, USA), BP3 98% from Thermo Fisher Scientific (Geel, Belgium), while deuterated n-propylparaben-2,3,5,6-d4 (PP-d4), ethylparaben-2,3,5,6-d4 (EP-d4) and 2-hydroxy-4-methoxybenzophenone-2′,3′-4′,5′-6′-d5 were purchased (98% chemical purity) from C/D/N Isotopes, Inc. (Quebec, Canada), all as crystalline materials. β-glucuronidase from Helix pomatia HP-2 (H. pomatia) and β-glucuronidase from Escherichia coli IX-A (E. coli) were also purchased from Sigma Aldrich, while ammonium acetate p.a. was supplied by Merck (Darmstadt, Germany) and sodium acetate (Normapur) by VWR International (Pennsylvania, USA). The SPE cartridges Bond Elut Certify LRC (130 mg, 10 ml) were obtained from Agilent Technologies (Agilent Technologies Belgium S.A./N.V., B-1831 Diegem). Acetonitrile, water, formic acid (99%) and glacial acetic acid