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Determination of personal care products –benzophenones and parabens– in human menstrual blood

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ABSTRACT

Benzophenones and parabens are synthetic chemicals used in many personal care products, foods and pharmaceuticals. Benzophenones are used to protect the skin and materials from the adverse effects of UV-radiation, and parabens are used as preservatives. Despite their widespread occurrence and proven endocrine disrupting activity, relatively little is known about human exposure to these compounds. In the present work, an analytical method based on sample treatment using dispersive liquid-liquid microextraction (DLLME) for the extraction of six benzophenones (benzophenone-1, −2, −3, −6, −8 and 4-hydroxybenzophenone) and four parabens (methyl-, ethyl-, propyl- and butyl- paraben) from human menstrual blood samples, followed by ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) analysis, is proposed and validated. The method was validated using matrix-matched standard calibration followed by a recovery assay with spiked samples. The limits of detection ranged from 0.1 to 0.3 ng mL^{-1}, with recoveries of 93.8% to 108.9%, and precision (evaluated as relative standard deviation) lower than 14% for all selected compounds. This method was successfully applied for the determination of the target compounds in 25 samples of human menstrual blood. Methylparaben and benzophenone-3 were the most frequently detected compounds (96%).

1. Introduction

Personal care products (PCPs) include cosmetics, household items, foods and pharmaceuticals, among others. Considerable amounts of PCPs are used in every day human actions so they are produced in large quantities (thousands of tons per year). Synthetic compounds such as some benzophenones (BPs) and parabens (PBs) are largely used in the formulation of PCPs to provide protection against UV radiation and to prevent decomposition by microbial growth [1,2].

BPs comprise approximately 29 compounds [1], including benzophenone-1 (BP-1) to benzophenone-12 (BP-12) and other less documented such as 4-hydroxybenzophenone (4-OH-BP) and 2-hydroxybenzophenone (2-OH-BP). The EU allows the use of BP-3, BP-4 and BP-5 as UV filters in cosmetics [2] and the use of unsubstituted benzophenone (BP), BP-1, BP-3, BP-8 and 4,4′-dihydroxibenzophenone in the formulation of plastics or food-packing materials [3]. Increasing concern has arisen about the implications of BPs for human health since some *in vivo* studies have demonstrated their potential ability to act as endocrine disrupting compounds (EDCs) [4,5]. Furthermore, a recent epidemiological study conducted on 600 women reported an association between BP-1 exposure and endometriosis [6].

PBs are widely used as antimicrobial preservatives, especially against mold and yeast, in cosmetic and pharmaceuticals products, as well as in foods and beverages [7]. Methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB) and butylparaben (BPB) are the most commonly used parabens [8]. The widespread use of these compounds as preservatives arises from their low toxicity, broad inertness, worldwide regulatory acceptance and low cost [9]. However, there is an increasing tendency to limit their use because of growing evidence of adverse human health effects. In this regard, PBs have demonstrated estrogenic and antiandrogenic properties both *in vitro* and *in vivo* [12–15]. Furthermore, human epidemiological studies have associated PBs exposure with sperm DNA damage

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[16], allergen sensitization [17] and reproductive tract disorders [18]. As a result, the European Commission has recently banned the use of isopropyl-, isobutyl-, phenyl-, benzyl- and pentyl- paraben [10] and has limited the lower maximum concentrations of PPB and BPB in cosmetics [11].

Human biotransformation of PBs and BPs depends on the exposure pathway, but it is known that they are mainly transformed into β-D-glucuronide and sulfate derivatives, which can be easily excreted in urine [19]. Nevertheless, given that the metabolic-excretory system is not completely effective, PBs and BPs may accumulate in body compartments [20,21].

In this context, it seems particularly important to develop fast, accurate and sensitive analytical methods to measure exposure to PBs and BPs in different human matrices. Urinary measurements of PBs and BPs are most preferred in estimating human exposure, but other matrices such as serum, adipose tissue, placenta, breast milk [20–23] and hair [24] have also been investigated. To our knowledge, there is no published literature on the determination of these compounds in human menstrual blood. Sample preparation is a critical step in the analysis of complex biological matrices like menstrual blood and usually an extraction technique is required to purify and isolate the target compounds. Moreover, because of the usually low levels of these contaminants, these extraction techniques must be able to concentrate the analytes.

The most commonly used methods for sample treatment for the determination of BPs and PBs in human samples are liquid-liquid extraction (LLE) and solid-phase extraction (SPE) [20,21]. In order to increase concentration factors and reduce analysis time and solvent, numerous microextraction techniques have also been proposed [20,21], such as dispersive liquid-liquid microextraction (DLLME). DLLME was developed by Reazee and co-workers in 2006 [25] for the extraction and preconcentration of organic compounds from water samples. Since then, this technique has been widely used in the analysis of many types of organic compounds in environmental matrices, especially waters [26]. More recently, new applications for the analysis of different families of EDCs in human urine [27–29] and serum samples [30,31] have been reported.

The aim of the present work was to develop a sensitive method for the simultaneous determination of six BPs (benzophenone-1, −2, −3, −6, −8 and 4-hidroxybenzophenone) and four PBs (methyl-, ethyl-, propyl- and butyl- paraben) in samples of human menstrual blood, using DLLME and UHPLC–ESI–MS/MS. Fig. 1 shows the chemical structure of the target compounds. The proposed method was satisfactorily validated and applied for the determination of above selected compounds in menstrual blood samples collected from 25 volunteers living in Southern Spain.

2. Experimental

2.1. Chemicals and reagents

All reagents were analytical grade unless otherwise specified. Methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB), butylparaben (BPB), benzophenone-1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3), benzophenone-6 (BP-6), benzophenone-8 (BP-8), 4-hydroxybenzophenone (4-OH-BP), ethylparaben ring $^{13}C_6$ labelled (EPB-$^{13}C_6$) and labelled deuterium benzophenone (BP-d_{10}) were supplied by Sigma-Aldrich (Madrid, Spain). Stock standard solutions of compounds (100 mg L^{-1}) were prepared in methanol and stored at 4 °C in the dark. The solutions were stable for at least four months. Working standard solutions were prepared by dilution in methanol immediately before use.

β-glucuronidase/sulfatase (*Helix pomatia*, H1) was purchased from Sigma-Aldrich. The enzyme solution was prepared daily

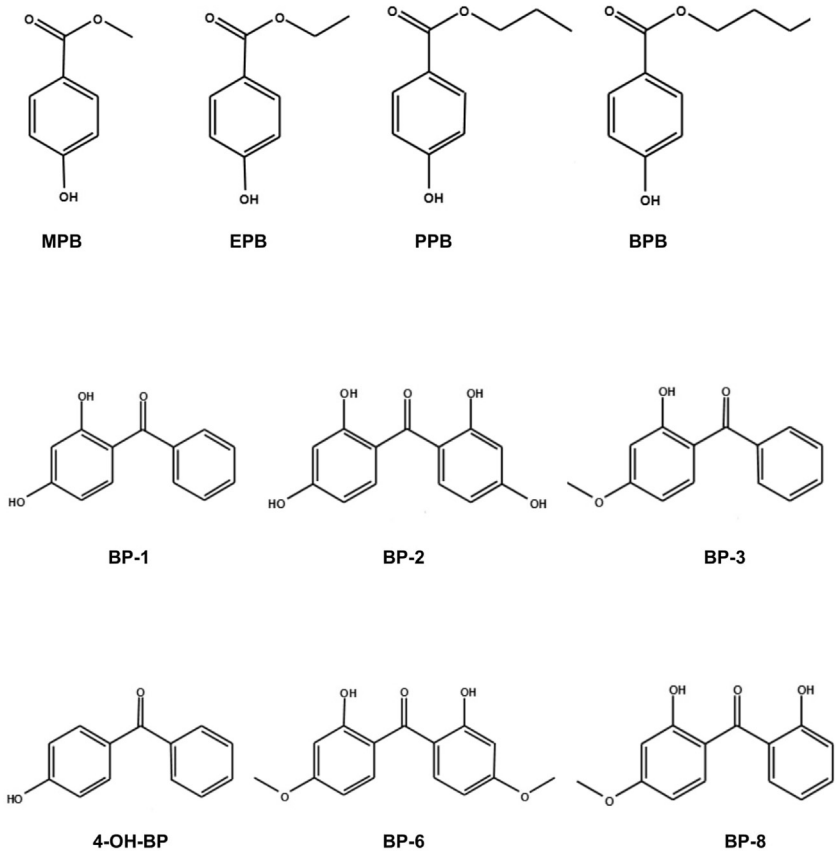

Fig. 1. Chemical structure of the target compounds.