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Assessment of parabens and ultraviolet filters in human placenta tissue by ultrasound-assisted extraction and ultra-high performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

Increasing concerns have been raised over recent decades about human exposure to Endocrine Disrupting Chemicals (EDCs), especially about their possible effects on embryo, foetus, newborn, and child. Parabens (PBs) and ultraviolet filters (UV-filters) are prevalent EDCs widely used as additives in cosmetics and personal care products (PCPs). The objective of this study was to determine the presence of four PBs and ten UV-filters in placental tissue samples using a novel analytical method based on ultrasound-assisted extraction (UAE) and ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS). Multivariate optimization strategies were used to accurately optimize extraction and clean-up parameters. Limits of quantification ranged from 0.15 to 0.5 $\mu g\,kg^{-1}$, and inter-day variability (evaluated as relative standard deviation) ranged from 3.6% to 14%. The method was validated using matrix-matched standard calibration followed by a recovery assay with spiked samples. Recovery percents ranged from 94.5% to 112%. The method was satisfactorily applied for the determination of the target compounds in human placental tissue samples collected at delivery from 15 randomly selected women. This new analytical procedure can provide information on foetal exposure to compounds, which has been little studied.

1. Introduction

Increasing concerns have been raised over recent decades about human exposure to so-called Endocrine Disrupting Chemicals (EDCs), which have been associated with several reproductive health disorders including poor semen quality, hypospadias, cryptorchidism, testicular cancer, uterine and ovarian diseases, breast cancer, and early menarche [1–7]. Exposure to endocrine-disrupting xenobiotics may be of particular concern during development (embryo, foetus and newborn) due to the hormonal regulation of maturation processes [8].

Several EDCs are used in cosmetics and personal care products (PCPs), including parabens (PBs) and ultraviolet filters (UV-Filters). PBs such as methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB) and butylparaben (BPB), are widely used as preservatives in PCPs, processed foods and beverages [9,10]. Evidence of the endocrine disrupting effects of these chemicals led Denmark to ban PBs in PCPs for children [11]. More recently, the European Union (EU) has restricted the use of long alkyl chain PBs PPB and BPB[12]. UV-filters are sunscreen agents widely used in a variety of PCPs to protect the skin against UV radiation. Currently, EU regulations allow the use of 26 compounds as UV-filters in cosmetics [13], including compounds with different chemical structures such as benzophenone-3 (BP-3), ethylhexyl methoxy cinnamate (EMC), 3-benzylidene camphor (3-BC), and octocrylene (OCR). The endocrine disrupting properties of these chemicals have been demonstrated in multiple *in vitro* and *in vivo* studies [14–16].

EDCs are rapidly metabolised by endogenous enzymes into more hydrophilic compounds (glucuronide derivatives) that are readily excreted in urine [17]. However, the biotransforma-

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tion process is not always fully effective, and residual EDCs may accumulate in some organs and tissues [18]. Furthermore, oxidizing/hydrolyzing metabolic reactions can sometimes transform EDCs into compounds with greater disrupting capacity. For example, BP-3 is transformed into benzophenone-1 (BP-1) and benzophenone-8 (BP-8), which are more estrogenic than BP-3 [19].

Numerous biomonitoring studies in urine, serum and breast milk samples have demonstrated the high levels of human exposure to PBs and UV-filters[20–24]. However, very few data are available on the presence of PBs and UV-filters in placental tissue, which may provide a more accurate measure of direct foetal exposure [25,26]. To our knowledge, placental exposure to these compounds has been assessed only using liquid–liquid extraction (LLE) [27–29] or matrix solid phase extraction (MSPD) [30,31] for sample preparation followed by UHPLC–MS/MS as analytical technique. Given the complexity of this biological matrix, there is a need to explore other analytical techniques that may yield reliable results quickly and at a reasonable cost. Ultrasound-assisted extraction (UAE) appears to be a promising approach because the acoustic cavitation produced in the solvent results in better mass transfer ratios and good extraction efficiency without excessive solvent consumption or long extraction times. UAE is widely used for environmental analysis of many organic compounds and inorganic elements and for the analysis of food contaminants [32,33], but few studies have used UAE to assess the most prevalent EDCs such as parabens and UV-filters in human matrices [34,35], in particular in placental tissue.

The main purpose of the present study was to develop a new method for the determination of four parabens and ten UV-filters in human placental tissue. The method involves three steps: lyophilization, UAE and clean-up with PSA and C18 as dispersive solid phase extraction (d-SPE) sorbents. This sample preparation, which was followed by instrumental determination with ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS), was validated and applied to 15 human placenta samples from anonymous donors.

2. Experimental

2.1. Chemicals and reagents

All reagents were analytical grade unless otherwise specified. Water (18.2 MΩ cm) was purified using a Milli-Q system from Millipore (Bedford, MA, USA). Ethylhexyl dimethyl p-amino benzoate (EDP), 3-benzylidene camphor (3-BC), 4-methylbenzylidene camphor (MBC), ethylhexyl *p*-methoxy cinnamate (EMC), octocrylene (OCR), benzophenone-1 (BP-1), benzophenone-3 (BP-3), benzophenone-6 (BP-6), benzophenone-8 (BP-8), 4-hydroxybenzophenone (4-OH-BP), methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB) and butylparaben (BPB) were supplied by Sigma-Aldrich (Madrid, Spain). Isopropyl p-amino benzoate (IsPP), isobutyl cinnamate (IsBC), ethyl 2-cyano-3,3-diphenylacrylate (ECDA), labelled deuterium benzophenone (BP-d_{10}) and ethylparaben ring $^{13}C_6$ labelled (EPB-$^{13}C_6$) were also purchased from Sigma-Aldrich (Madrid, Spain). The structural formula of these compounds is shown in supplementary material (Fig. S1). Stock standard solutions of compounds (100 mg L^{-1}) were prepared in acetonitrile and stored at 4 °C in the dark. The solutions were stable for at least four months. Working standards were prepared by dilution with acetonitrile immediately before use.

Ethyl acetate, acetone and acetonitrile (HPLC-grade) were purchased from Merck (Darmstadt, Germany). LC–MS grade acetonitrile and water, ammonia (25%) and formic acid were purchased from Sigma-Aldrich. Acetic acid and ammonium acetate were supplied by Panreac (Barcelona, Spain).

2.2. Instruments and software

Sample extraction was performed using a Branson digital Sonifier unit model S-450D (Danbury, CT, USA) with a standard 12.7 mm titanium disruptor horn. HPLC–MS/MS analysis was performed using an Agilent Series 1290 LC system (Agilent Technologies, Santa Clara, CA, USA) with an API 4000 (triple quadrupole) mass spectrometer (AB SCIEX). Freeze-drying of samples was performed using ScanVac CoolSafe Freeze Drying™ equipment (Labogene, Lynge, Denmark). Statgraphics Plus version 5.0 (Manugistics Inc., Rockville, MD, USA, 2000) was used for statistical analyses.

2.3. Sample collection and storage

Human placental samples were collected from 15 volunteer's mothers at delivery in San Cecilio University Hospital of Granada (Spain). All volunteers signed the informed consent form for placenta donation. The study was approved by the Institutional Ethical Committee of the San Cecilio University Hospital. Each placenta was examined and accurately weighed (placentas were not dried or drained). In order to ensure the homogeneity and representativeness of the whole placenta tissue, half of the placenta (including maternal and foetal sides and central and peripheral parts) was placed in the glass container of a mixer for homogenization (B-400 Buchi mixer). Once homogenized, aliquots of 35 g were coded and stored at −86 °C until analysis.

2.4. Preparation of spiked samples

In the absence of certified materials for these EDCs in this matrix, placental tissue was spiked with the selected chemical compounds at seven different concentrations (0.5–15 μg kg^{-1}) for calibration purposes. Validation of the method and quality control were performed using spiked matrices at 0.5, 5 and 15 μg kg^{-1}. Regarding the method set up, placental tissue was spiked at 5 μg kg^{-1}. The spiking process was done by adding 10 μL of acetonitrile standard solution for each g of placental tissue (for example, 10 μL of a 50 μg L^{-1} standard solution was added to 1 g of placenta to obtain 0.5 μg kg^{-1} of spiked placental tissue). The mixture was then gently stirred and heated at 50 °C to ensure the homogenous distribution of analytes in the sample. Aliquots (2 g) were weighed in 12.5 mL glass vials and fortified with 20 μL of the surrogate solution (500 μg L^{-1} of each internal standard), resulting in a placental tissue concentration of 5 μg kg^{-1}. Aliquots were then freeze-dried and stored at −23 °C.

2.5. Ultrasound assisted extraction procedure

After lyophilization, samples were transferred to 15 mL stainless steel tubes and 3 mL of acetonitrile was added; samples were vortexed for 30 s to ensure contact between sample and solvent. Samples were then sonicated for 10 min at 70% amplitude. The extraction cycle was run only one time. Tubes were centrifuged and extracts were transferred to a conical glass tube.

2.6. Cleaning d-SPE procedure

A clean-up procedure was performed to minimize the matrix effect associated with co-extractives. A mixture of 325 mg of PSA and 35 mg of C18 sorbents was added to the extract, and shaken manually for 1 min. After centrifugation at 2060 x g for 5 min, the supernatant was evaporated under a nitrogen stream. The residue was dissolved in 100 μL of acetonitrile–water (70:30 v/v), vortexed for 30 s and centrifuged at 16,300 × *g* for 5 min. The sample was then ready for injection into the LC system.

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