Chemosphere 168 (2017) 676–684

Contents lists available at ScienceDirect

Chemosphere

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

Biomonitoring of 21 endocrine disrupting chemicals in human hair samples using ultra-high performance liquid chromatography–tandem mass spectrometry

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HIGHLIGHTS

- The analysis of 21 endocrine disrupting chemicals (EDCs) in human hair is proposed.
- The target analytes belong to four different families of compounds.
- A very simple 2-step extraction procedure prior to UHPLC-MS/MS is optimized.
- This is the first validated method that allows the biomonitoring of this large number of EDCs.
- The method is a potent analytical tool for further human exposure studies to EDCs.

ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form
9 October 2016
Accepted 2 November 2016
Available online 10 November 2016

Handling Editor: Andreas Sjodin

Keywords:
Endocrine disrupting chemicals
Biomonitoring
Human hair analysis
UHPLC–MS/MS

ABSTRACT

Rapid industrial growth has increased human exposure to a large variety of chemicals with adverse health effects. These industrial chemicals are usually present in the environment, foods, beverages, clothes and personal care products. Among these compounds, endocrine disrupting chemicals (EDCs) have raised concern over the last years. In the present work, the determination of 21 EDCs in human hair samples is proposed. An analytical method based on the digestion of the samples with a mixture of acetic acid/methanol (20:80, v/v) followed by a solid-liquid microextraction and analysis by ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) was developed and validated. The most influential parameters affecting the extraction method were optimized. The method was validated using matrix-matched calibration and recovery assays. Limits of detection ranged from 0.2 to 4 ng g^{-1}, limits of quantification from 0.5 to 12 ng g^{-1}, and inter- and intra-day variability was under 15% in all cases. Recovery rates for spiked samples ranged from 92.1 to 113.8%. The method was applied for the determination of the selected compounds in human hair. Samples were collected weekly from six randomly selected volunteers (three men and three women) over a three-month period. All the analyzed samples tested positive for at least one of the analyzed compounds.

1. Introduction

Humans are constantly exposed to new chemicals with adverse health effects. These potentially hazardous compounds are ubiquitous in the environment, foods, beverages, and personal care products. Among these emerging contaminants, endocrine disrupting chemicals (EDCs) have raised concern over the last years. EDCs are synthetic and natural substances that can interfere with the hormonal systems in wildlife and humans. The biological effects of EDCs are related to their ability to mimic/antagonize endogenous hormones, or alter the synthesis and metabolism of endogenous hormones and receptors (Sonnenschein and Soto, 1998). Bisphenol A (BPA) and its chlorinated derivatives, parabens (PBs), benzophenone-UV filters (BPs) and perfluoroalkyl compounds (PFCs) are included in this group of compounds (Fei et al., 2009; Kavlock et al., 1996; Liao et al., 2009; Liu et al., 2007; Paris et al., 2002; Rivas et al., 1997; U.S.

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http://dx.doi.org/10.1016/j.chemosphere.2016.11.008
0045-6535/

Environmental Protection Agency, 2003).

BPA is mainly used for the manufacturing of epoxy resin and polycarbonate plastics, with an annual production of over 2–3 million tons. BPA has attracted considerable attention of governments and the scientific and medical community (Bisphenol A website). The European Food Safety Authority (EFSA) and the US-Environmental Protection Agency (US-EPA) have established the maximum acceptable level of exposure to BPA of 50 μg kg^{-1} body weight/day (Nicolucci et al., 2013). There are many sources of human exposure to BPA, with diet considered the main source (Calafat et al., 2008). Interestingly, literature has reported the formation of chlorinated derivatives of BPA during the chlorination process of drinking water before consumption (Yamamoto and Yasuhara, 2002). PBs are the alkyl esters of *p*-hydroxybenzoic acid. They are used as antimicrobial preservatives, mainly against mold and yeast. PBs are used as additives in the manufacture of cosmetic products, pharmaceuticals, food and beverages. In the last years, several studies have demonstrated the ability of PBs to disrupt physiological functions in both in vitro (van Meeuwen et al., 2008), and in vivo models (Boberg et al., 2010; Soni et al., 2005). Inhalation, dermal contact and ingestion are the main sources of exposure to PBs (El Houssein et al., 2007). The existing European Union (EU) limit for total PB concentration in cosmetics is 0.8% (w/w) and 0.4% (w/w) as acid for mixtures or individually, respectively (European Union Regulation No. 1223/2009). BPs are one of the most common UV filters used in sunscreens because they absorb UVA (320–400 nm) and UVB (290–320 nm) radiation. BPs have been reported to enter the human body through the food chain (Cuderman and Heath, 2007) and skin absorption (Jiang et al., 1999). Finally, PFCs are commonly used in the manufacture of finished products such as paints, adhesives, waxes, polishes, electronics, fire-fighting foams and caulks, as well as grease-proof coatings for food packaging (Corsini et al., 2014; Giesy and Kannan, 2001; Hekster et al., 2003; Rivière et al., 2014). Although food is the major exposure route for PFCs, drinking water, indoor air and house dust can also be potential sources of PFC exposure (Domingo, 2011).

Hair analysis has been traditionally used for drug testing, especially in cases of drug abuse (Oliveira et al., 2007). In addition, hair has been used as a bio-indicator of exposure to pesticides in children and exposure to organochlorine pollutants in adults. Although human milk, placental tissue, serum, blood or urine are conventionally used to determine exposure to EDCs, hair analysis could be used as a complementary tool, especially in cases of chronic exposure or when traditional matrices are not available. The main advantage of hair analysis is its large window of detection, which allows us to establish a chronological profile of exposure based on hair length (Cooper et al., 2012). Additional advantages of hair analysis include non-invasive collection and easy monitoring, difficult tampering with samples, storage and transportation at room temperature, and most contaminants are chemically stable (Caplan and Goldberger, 2001). Therefore, the analysis of hair samples might be of great interest for the assessment of exposure to different EDCs (Król et al., 2013), but not many analytical methods have been validated for the determination of the EDCs mentioned in hair samples. Moreover, the methods described in the literature focused on the individual determination of BPA (Tzatzarakis et al., 2015), PBs (Sakol et al., 2015), or both chemicals (Martín et al., 2015).

The aim of the present work was to develop a multi-class method for the determination of 21 EDCs in human hair samples. A sensitive multi-residue method based on the digestion of hair samples followed by a solid-liquid microextraction prior to ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) analysis was developed. BPA and its chlorinated derivatives mono-, di-, tri- and tetrachorobisphenol A (Cl-BPA, Cl_2-BPA, Cl_3-BPA, Cl_4-BPA), four PBs (methyl-, MPB; ethyl-, EPB; n-propyl-, PPB; and *n*-butylparaben, BPB), six BP-UV filters (benzophenone-1, BP-1; benzophenone-2, BP-2; benzophenone-3, BP-3; benzophenone-6, BP-6; benzophenone-8, BP-8; and 4-hidroxybenzophenone, 4-OH-BP) and six PFCs (perfluorobutanoic to perfluorooctanoic acid (PFBuA, PFPeA, PFHxA, PFHpA, PFOA) and perfluorooctane sulfonate (PFOS)) were selected as target analytes. After validation, the method was applied to determine the free pollutant content in samples from six randomly selected volunteers (three men and three women) from Granada, Spain.

2. Experimental

2.1. Chemicals and reagents

The reagents used for the experiments were of high analytical grade and purity. A Milli-Q system from Millipore (Bedford, MA, USA) was used for water purification (18.2 MΩ cm). PFBuA (98%), PFPeA (97%), PFHxA (≥97%), PFHpA (99%), PFOA (96%) and PFOS (≥98%), perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid (MPFOA), MPB, EPB, PPB and BPB were supplied by Alfa Aesar (Massachusetts, MA, USA). BPA, Cl_4-BPA, deuterium labelled bisphenol A (BPA-d_{16}), BP-1, BP-2, BP-3, BP-6, BP-8, 4-OH-BP, deuterium labelled benzophenone (BP-d_{10}), deuterium labelled ethylparaben (EPB-d_5), LC–MS grade methanol, water and acetonitrile, acetic acid and ammonia (25%, w/v) were supplied by Sigma-Aldrich (Madrid, Spain). Cl-BPA, Cl_2-BPA and Cl_3-BPA were synthesized in our laboratory (purity > 99%) by chlorination of BPA (Vílchez et al., 2003). Stock solutions of 1000 mg L^{-1} of each compound, prepared in methanol, were stored in a freezer at −20 °C. Working solutions were prepared fresh weekly by diluting the stock standard solutions in methanol or in the initial mobile phase. They were maintained at 4 °C in the refrigerator. All of the solutions were stored in the dark, to prevent photodegradation.

2.2. Instrumentation and software

A chromatographic system Acquity UPLC™ H-Class (Waters, Manchester, UK), provided with a binary solvent manager was used for chromatographic separation. Analyte detection was carried out using a triple quadrupole mass spectrometer (Waters) Xevo TQS with an orthogonal Z-spray™ electrospray ionization (ESI) source. The stationary phase was an Acquity UPLC® BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 μm particle size). Other laboratory equipment such as a vortex-mixer (IKA, Staufen, Germany), an ultrasound-HD bath (Selecta, Barcelona, Spain), a Spectrafuge™ 24D centrifuge from Labnet International, Inc. (New Jersey, USA) and a sample concentrator (Stuart, Staffordshire, UK) were also used. Statistical analysis of data was performed with Statgraphics Plus version 5.1 (Statpoint Technologies Inc., Virginia, USA).

2.3. Sample collection and storage

Hair samples were collected from six healthy volunteers (three men and three women) aged 18 or older from the city of Granada, Spain. Hair samples were collected weekly for a three-month period. All volunteers were informed about the scope and nature of the study. Hair specimens were cut from the posterior vertex region of the head, as this region is associated with the smallest variation in growth rate, and as close as possible to the scalp. Sample length was of roughly 3–5 cm. Samples were anonymized and stored in aluminum foil, at room (ambient) temperature until further processing and analysis. One of the concerns in hair analysis is the need to differentiate between external contamination (from air or dust) and internally incorporated chemicals. The ideal decontamination procedure should remove external contamination