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# Determination of UV filters in human breast milk using turbulent flow chromatography and babies' daily intake estimation

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ABSTRACT

UV filters (UV-Fs) are a group of hormonally active chemical compounds used to protect against the deleterious effects of UVA and UVB solar radiation, which are currently present in most consumer goods (personal care products, plastics, fabrics, paints, etc). Last years the concern about these emerging contaminants has been on the rise, and increasing efforts are being taken in order to properly asses the hazard that the exposure to these compounds in the early stages of life may pose. In this study, a new method for the analysis of 11 UV-Fs residues in human breast milk samples has been developed. The method is based on turbulent flow chromatography coupled to liquid chromatography-tandem mass spectrometry (TFC-HPLC-MS/MS). The validated method was successfully applied to 79 human breast milk samples from mothers in Barcelona (Spain). Twenty-four per cent of the samples contained UV-Fs, with major contributors being oxybenzone (benzophenone 3, BP3), its metabolite 4,4′-dihydroxybenzophenone (4DHB), and UV320 showing maximum concentrations of 779.9, 73.3, and 523.6 ng $g^{-1}$ milk, respectively. Additionally, the plastic containers of the milks were also analysed, revealing high concentrations of BP3 and 4DHB, up to 10.6 µg $g^{-1}$ plastic. The calculated mean ΣUV-Fs were useful to estimate the daily intake (EDI) by babies, which were 69.1 µg $d^{-1}kg-^{1}$ body weight.

## 1. Introduction

UV filters (UV-Fs) constitute a large group of man-made chemicals used as a protection against the harmful effects of the UV solar radiation. We can find them in numerous consumers' goods such as personal care products (PCPs) like shampoos, lotions, and sunscreens, and in a variety of industrial applications as additives in polymeric materials needing protection against sunlight, such as food-packaging materials, fabrics, protective coatings for vehicle and photography devices, as well as in many other industrial goods (Molins-Delgado et al., 2015). During the last decade, sunscreens have received increased worldwide attention and emerged as a major focus of concern, mainly because of their global occurrence in the different environmental compartments and in humans, their pseudo-persistence and eco-toxicity (Brausch and Rand, 2011; Caliman and Gavrilescu, 2009; Molins-Delgado et al., 2016). A few studies showed that the degradation of UV-Fs in wastewater treatment plants (WWTPs) is incomplete, usually < 50% (Li et al., 2007). Most UV-Fs are lipophilic, tending to accumulate in sediments (Gago-Ferrero et al., 2011a) and sewage sludge (Gago-Ferrero et al., 2011b), and to bioaccumulate in living organisms (Fent et al., 2010).

Several studies have revealed that these compounds have hormonal activity. In *in vitro* and *in vivo* tests, it has been shown that UV-Fs affect the reproductive cycle, as well as the development of both aquatic and terrestrial organisms (Weisbrod et al., 2007; Klammer et al., 2007). For instance, 4-methylbenzylidene camphor (4MBC) presented similar effects than those of 17β-estradiol in exposed mammals and amphibians (Klann et al., 2005), and oxybenzone (benzophenone 3, BP3), among others UV-Fs, showed estrogenic activity in fish (Weisbrod et al., 2007; Kunz et al., 2006). It is also noteworthy that the UV-Fs' transformation products are another matter of concern as they may also display increased hormonal activity compared to the parent compounds. Despite that, the efforts to characterise the hazard that UV-Fs pose to life are sparse, and consequently the regulation of these compounds is yet lacking. Nevertheless, two UV-Fs have already been proposed as potential future pollutants to be regulated, i.e. 4MBC, whose use was banned in child's products in Denmark (Hass et al., 2012), and 2-ethylhexyl 4-methoxycinnamate (EHMC) that is currently included in the last Water Framework Directive's Watch List, characterised as

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endocrine disrupting and bioacumulative compound (Carvalho et al., 2015).

Nowadays, it is known that various environmental factors play an important role in human health being this effect decisive in the early stages of life, including the perinatal period, because of the major development taking place. Some authors correlated limited foetal growing, and some other anthropometric values, with exposure to organochlorine compounds during the pregnancy (Lopez-Espinosa et al., 2011). Current research indicates that UV-Fs are present in human fluids and tissues such as urine and semen (León et al., 2010), and placenta (Vela-Soria et al., 2011; Valle-Sistac et al., 2016). In breast milk, concentrations of BP3, 4MBC, and ethylhexyl dimethyl-PABA (ODPABA) have been measured at concentrations as high as 102.9 ng $g^{-1}$ lipid weight (Schlumpf et al., 2010). As mentioned above, UV-Fs are used in the industry to protect the produced materials from degradation processes caused by the UV radiation (Monteiro et al., 1999; Nakajima et al., 2009), as photoionisators (Sanches-Silva et al., 2009), or simply as precursors for the synthesis of some kind of plastics (Parker et al., 2012). Previous studies have shown that some of these additives can be leached from plastic to the contained products, hence potentially exposing consumers to them (Monteiro et al., 1999; Sanches-Silva et al., 2009). Humans exposure to these compounds takes place mainly through percutaneous absorption after the application on the skin of PCPs, a group of compounds used in everyday goods (Benech-Kieffer et al., 2000; Jiang et al., 2001; Janjua et al., 2004), although oral exposure to these compounds is also feasible through water (Díaz-Cruz et al., 2012) and contaminated food consumption (Fent et al., 2010; Schlumpf et al., 2010; Johns et al., 2000).

In particular, breastfed babies are exposed to the contaminants present in the milk as a consequence of the diet and life-style of the mother. So far, the occurrence in breast milk of many persistent contaminants have been extensively studied, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and perfluorinated compounds (Motas Guzmàn et al., 2016; Zanieri et al., 2007; Çok et al., 2012). However, the UV-Fs' transfer through breastfeeding has scarcely been investigated. Schlumpf et al. (Schlumpf et al., 2010, 2008a) correlated the use of cosmetics containing UV-Fs with their occurrence in human milk and suggested to abstain from their use during pregnancy and lactation to avoid transferring them to the nursling. Another study by Lee et al. (2015) determined concentrations of benzotriazole UV-Fs in breast milk from Korea, being the observed concentrations consistent with their consumption patterns.

In the scenario described, the main goals of this study were to determine the occurrence and concentration levels of most used UV-Fs in human breast milk samples from mothers resident in Catalonia (northeastern Spain). The levels measured were compared to the sparse data available in the literature. Additionally, the recipients where the milk was collected were also analysed in order to determine the potential risk of potential leaching from the containers' walls. Finally, the estimated daily intake (EDI) of UV-Fs by the nursling was calculated to assess their exposure extent to these pollutants. In order to achieve these objectives, a new method based on on-line turbulent flow chromatography (TFC) extraction followed by the analysis through high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was developed, validated and applied to the determination of UV-Fs in 79 breast milk samples.

## 2. Materials and methods

### 2.1. Standards and reagents

The CAS number, the structure and the logarithm of the partition coefficient octanol-water (Log Kow) for the target UV-Fs are shown in Table A1. 2,4-dihidroxybenzophenone (99%, BP1), BP3 (98%), ODPABA (98%), ethyl p-aminobenzoic acid (99%, EtPABA), 4-hydroxybenzophenone (98%, 4HB), 4,4′-dihydroxybenzophenone (99%, 4DHB), octocrylene (99%; OC), benzotriazole (99%; BZT), 2-(2-hydroxy-5-methylphenyl) benzotriazole (99%; UVP), 2-(2-H-benzotriazol-2-yl) – 4,6-bis(1-methyl-1-phenylethyl)phenol (99%; UV234), and 2-(2′-hydroxy-3′,5′-di-*tert*-butylphenyl)benzotriazole (98%, UV320) were obtained from Sigma-Aldrich (Munich, Germany); ethylhexyl methoxycinamate (99%; EHMC) and 4MBC (98%) were provided by Dr Ehrenstorfer (Augsburg, Germany). Methyl benzotriazole (99%; MeBZT), 2-(5-tert-butyl-2-hydroxyphenyl)benzotriazole (98%, TBHPBT), 2-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl) – 4-methylphenol (99%, UV326), 2,4-di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (99%, UV327), 2-(2H-benzotriazol-2-yl) – 4,6-di-*tert*-pentylphenol (99%, UV328), and 2-(2H-benzotriazol-2-yl) – 4-(1,1,3,3-tetramethylbutyl)phenol (98%, UV329) were obtained from TCI (Antwerp, Belgium). 2-Hydroxy-4-methoxybenzophenone-d5 (98%D), and 4-methylbenzylidene camphor-d4 (98% D), used as internal standards in the analyses, were supplied by CDN isotopes (Quebec, Canada). 2-(2H-benzotriazol-2-yl) – 4-methyl-6-(2-propenyl)phenol (99%), and benzophenone-(carbonyl-$^{13}$C) used as surrogate standards were obtained from Sigma-Aldrich. Standard solutions for single compounds were prepared in MeOH and stored in the dark at −20 °C. Hexane and acetonitrile (ACN), both HPLC-grade, were purchased from J.T. Backer (Deventer, The Netherlands). Water and methanol (MeOH), both HPLC-grade, were supplied by Merck (Darmstadt, Germany).

Z377082 Biotainer sterile PETG bottles from Sigma-Aldrich were used as breast milk containers.

### 2.2. Milk samples

The study herein presented was carried out in collaboration with the Breast Milk Bank (BLM) from the Blood and Tissue Bank of Catalonia (Spain). The objective of the BLM is to ensure the feeding with breast milk of high risk premature babies or newborns with low weight (< 1500 g) of Catalonia, who need it by medical prescription and who, due to force majeure, cannot be breastfed by their own mother. The BLM is a specialized center that performs the functions of making society aware of the value of breastfeeding and collects, analyzes processes, carries out quality controls and distributes breast milk. In addition, it ensures that the selection, extraction, acceptance and processing of milk are carried out effectively and safely. The milk samples are obtained by the BLM after getting the approval of the mothers, who were contacted and interviewed before giving birth and satisfactorily completed a detailed questionnaire providing confidential personal information. The data from the mothers included in this study is shown in Table 1 from the Supporting information and provides information on the age of the mother, allergies, medical treatments, consumption of coffee per day, consumption of homeopathic drugs or infusions, diet preferences, gestation's duration, birth procedure, birthdate of the child, gender and weight of the baby, and breastfeeding period.

All mothers in this study experienced normal pregnancy and delivery, and provided the milk samples from the first day up to 31 months after childbirth. Between April and October 2014, 79 individual breast milk samples were obtained from 71 primiparous and 8 multiparous (up to 4) nursing mothers. Most samples, 26.6%, were collected in the period 4–6 months after delivery, and followed by 1–3 months, accounting for the 25.3%. The remaining samples were collected at a later period up to 31 months after delivery. Donors were residents in Catalonia. According to the BLM protocol, potential donors are discarded if they are strict vegetarians that do not take a supplement of vitamin B12, smoke, ingest alcohol or illicit drugs and if they take > 300 mg $day^{-1}$ of caffeine, the maximum recommended ingested quantity per day.