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Analytical method for the determination and a survey of parabens and their derivatives in pharmaceuticals

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ABSTRACT

Exposure of humans to parabens is a concern due to the estrogenic activity of these compounds. Parabens are widely used as preservatives in some personal care products, foodstuffs and pharmaceuticals owing to their low cost, high water solubility and broad spectrum antimicrobial properties. Despite this, little is known on the occurrence of parabens in pharmaceutical products. In this study, a method based on solid–liquid or liquid–liquid extraction (SLE or LLE), and high performance liquid chromatography (HPLC) coupled with triple quadrupole tandem mass spectrometry (QqQ or MS/MS) was developed for the determination of six most frequently used parabens and four paraben derivatives (methyl- and ethyl-protocatechuates, and mono- and di-hydroxybenzoic acids) in pharmaceuticals. A sample-purification step involving solid phase extraction (SPE) was optimized for the analysis of solid and lipid-rich pharmaceuticals. To our knowledge, this is the first comprehensive report on the occurrence of parabens in pharmaceuticals. The developed method was applied for the analysis of 128 liquid/syrup, cream, solid, prescription or over-the counter (OTC) drugs collected from the USA and a few other countries in Europe and Asia. Although majority of the drugs analyzed in the study did not contain parabens, concentrations as high as 2 mg/g were found in some drugs. Methyl- and propyl- parabens were the frequently detected compounds. 4-Hydroxybenzoic acid was the major metabolite found in pharmaceutical products.

1. Introduction

Parabens are alkyl esters of 4-hydroxybenzoic acid (4-HB) and are widely used as preservatives in consumer products including processed foodstuffs (Liao et al., 2013a,b), cosmetics (Guo and Kannan, 2013; Guo et al., 2014; Baranowska et al., 2014), toiletries (Guo and Kannan, 2013; Guo et al., 2014; Baranowska et al., 2014), paper products (Liao and Kannan, 2014) and pharmaceuticals (Baranowska et al., 2014) due to their low cost, effectiveness over a wide range of pH, high stability, water solubility and a broad spectrum antimicrobial activity. In particular, preservatives are added to pharmaceuticals to prevent any microbial growth and/or degradation of the drug (i.e., to increase the shelf-life).

Studies have shown that parabens possess a weak estrogenic activity (Routledge et al., 1998). The most commonly used parabens are methyl-, ethyl-, propyl-, butyl-, benzyl- and heptyl-parabens and the estrogenic activity of these compounds increases with the length of the alkyl chain (Routledge et al., 1998). Parabens are considered as endocrine disrupting compounds. Some studies have associated a decrease in sperm production or an increase in the incidence of breast cancer and malignant melanoma to paraben exposures (Oishi, 2002a, 2002b; Darbre et al., 2004; Darbre and Harvey, 2008; Witorsch and Thomas, 2010).

Concomitant with an increase in the understanding of toxicological properties of parabens, the European Union has lowered allowable maximum concentrations of propyl- and butyl- parabens in cosmetics from 0.4% when used individually and 0.8% when mixed with other esters, to 0.14% when used individually or in mixture (Bernauer et al., 2013). In addition, the use of propyl- and butyl- parabens is banned in cosmetics intended for children under three years of age (Angerer et al., 2011).

Human exposure to parabens is a concern, and sources of human exposure to parabens are not fully characterized. A few studies have reported the occurrence of parabens in consumer products including processed foods and personal care products (Liao and Kannan, 2014; Guo et al., 2014; Baranowska et al., 2014;

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Lokhnauth and Snow, 2005; Jaworska et al., 2005; Thomassin et al., 1997), as well as in environmental and biological samples including water, sediments, sewage sludge, soil, indoor dust, saliva, serum and urine (Piao et al., 2014; Azzouz and Ballesteros, 2014; Liao et al., 2013c; Wang et al., 2012; Wang et al., 2013). However, to our knowledge, little is known on the occurrence of parabens in pharmaceuticals (Baranowska et al., 2014; Lokhnauth and Snow, 2005; Jaworska et al., 2005; Thomassin et al., 1997). The two earlier studies that measured parabens in pharmaceuticals involved a small sample size of 2–17 liquid pharmaceuticals and these studies analyzed only for 4-HB, MeP and PrP (Baranowska et al., 2014; Jaworska et al., 2005).

Parabens have been typically analyzed by high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detector or mass spectrometry (MS) (Piao et al., 2014; Cabaleiro et al., 2014) and gas chromatography (GC) coupled with MS (Lokhnauth and Snow, 2005; Haunschmidt et al., 2011; Piao et al., 2014; Cabaleiro et al., 2014). Liquid–liquid extraction (LLE), solid–liquid extraction (SLE) and ultrasonic assisted extraction (UAE) are the common methods employed in the extraction of parabens from sample matrixes. In addition, pressurized liquid extraction (PLE), dispersive liquid–liquid microextraction (DLLME), supercritical fluid extraction (SFE) and stir bar sorptive extraction (SBSE) (Piao et al., 2014) have been reported for the extraction of parabens. LLE and UAE have been employed in the analysis of parabens in liquid syrup pharmaceuticals (Baranowska et al., 2014; Lokhnauth and Snow, 2005; Jaworska et al., 2005; Thomassin et al., 1997). However, no earlier studies have determined parabens in pharmaceutical tablets/capsules.

Although parabens are stable, they can be metabolized by esterases (Abbas et al., 2010). In addition, hydrolytic transformation of several parabens to *p*-hydroxybenzoic acid has been reported (Aubert et al., 2012). Another degradation pathway for parabens is oxidative hydroxylation. Light–induced hydroxylation of methyl paraben to methyl protocatechuate has been reported (Okamoto et al., 2008). Therefore, hydrolysis and hydroxylation of parabens can occur in pharmaceutical formulations during production and storage.

In this study, a method comprising SLE/LLE and HPLC–MS/MS was developed for the determination of six parabens and four paraben derivatives (methyl- and ethyl-protocatechuates, and mono- and di-hydroxybenzoic acids) in pharmaceuticals (Fig. S1) encompassing liquid/syrup, cream, gel, and solid capsules (pills) collected from pharmacies in the USA and a few other countries. The method was applied in the determination of parabens in 128 pharmaceuticals.

2. Materials and methods

2.1. Chemicals and reagents

Methyl-(MeP), ethyl-(EtP), propyl-(PrP), butyl-(BuP), benzyl-(BzP), and heptyl-(HpP) parabens were purchased from AccuStandard Inc (New Haven, CT, USA) in methanol (MeOH) at 100 μg/mL (purity ≥98%). 4-Hydroxybenzoic acid (4-HB) and 3,4-dihydroxybenzoic acid (i.e., protocatechuic acid; 3,4-DHB) were also purchased from AccuStandard in acetonitrile (ACN) at 100 μg/mL (>99.5%). Methyl protocatechuate (OH–MeP; 97%) and ethyl protocatechuate (OH–EtP; 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Isotopically labeled internal standards, $^{13}C_6$-MeP, $^{13}C_6$-BuP and $^{13}C_6$-4-HB, were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA) as individual standard solutions in methanol at 1 mg/mL (99%).

Formic acid (ACS grade; 88%), hexane (ultra-residue grade; 95% n-hexane) and ethyl acetate were purchased from J.T.Baker® (Center Valley, PA, USA). Acetone and dichloromethane (DCM) (ACS grade) were purchased from Macron Fine Chemicals™ (Center Valley, PA, USA). LC–MS grade methanol (MeOH) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Milli-Q water was prepared by an ultrapure water system (Barnstead International, Dubuque, IA, USA). All standards and solutions were prepared in LC–MS grade MeOH and were stored at −20 °C until analysis.

Strata® NH_2 (55 μm, 70 Å, 200 mg/3 mL) and Oasis® HLB (3 cc, 60 mg) cartridges were used for solid phase extraction (SPE) and were obtained from Phenomenex (Torrance, CA, USA) and Waters Corporation (Milford, MA, USA), respectively.

2.2. Sample collection and preparation

A total of 128 pharmaceuticals were collected from July to November 2014. Over-the-counter (OTC) drugs were purchased in local stores and prescription drugs were obtained from volunteers who donated a small amount (<1 g) for this research. Name of the drug (commercial name), expiration date, manufacturer, and sampling location were recorded. Some of the prescription drugs analyzed had passed the expiration date.

The pharmaceutical samples originated from the USA (New York and New Jersey) (n=104), Italy (n=2), Poland (n=2), Spain (n=5), China (n=7), India (n=5), and Japan (n=3). The pharmaceuticals collected from the USA were grouped into three categories: solid samples (capsules/pills; n=58), liquid/syrup or cream samples (n=32) and softgels (n=14). Samples were also categorized as over-the-counter (OTC) and prescription medicines, as well as, by their therapeutic effects.

Solid samples (tablets, caplets or capsules) and softgels were kept at room temperature in the dark. Tablets and caplets were homogenized with a solvent-rinsed ceramic mortar prior to extraction. The exterior shell of softgels and capsules were cut into small pieces (1–2 mm^2) using scissors. Liquid/syrup, cream and homogenized samples were stored at 4 °C in polypropylene (PP) tubes until analysis.

2.3. Extraction and clean-up

Between 0.05 and 0.10 g of pharmaceutical sample was placed in a 15-mL polypropylene conical tube (PP tube), followed by the addition of 200 μL of 1 μg/mL internal standard mixture and 4.5 mL of MeOH. The extraction was performed by shaking the mixture in a reciprocal shaker for 30 min at 280 ± 5 osc/min (Eberbach Corporation, Ann Arbor, MI, USA). The sample was centrifuged at 5000 × g for 15 min (Centrifuge 5804 Eppendorf, Hamburg, Germany) and the supernatant was transferred into another PP tube. Liquid/syrup, cream and non-oily softgel samples were analyzed by HPLC–MS/MS. Solid and oily softgel (e.g., fish oil supplements) samples required further SPE clean-up before instrumental analysis. The supernatant was concentrated to 2 mL under a gentle nitrogen stream using a multivap nitrogen evaporation system (Organomation Associates Inc., Berlin, MA, USA). After vortex mixing, 6 mL of water acidified with 0.1% formic acid were added. The final mixture was vortexed and centrifuged at 5000 × g for 15 min, and the MeOH/water (25:75 v/v) mixture was transferred into another PP tube. The sample purification was accomplished by use of a 24-port solid phase glass block vacuum manifold (Burdick & Jackson, Muskegon, MI, USA). The extract was purified by passage through Oasis HLB 3 cc extraction cartridge (60 mg and 30 μm particle size), that was previously conditioned with 3 mL of MeOH and 3 mL of water. The cartridge was washed with 3 mL of 10% MeOH in water and 3 mL of water. The cartridge was dried under vacuum for 10 min and then the analytes were eluted with 3 mL of MeOH. All of the SPE steps were carried out at