Regulatory Toxicology and Pharmacology 79 (2016) 42–48

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Determination of parabens in serum by liquid chromatography-tandem mass spectrometry: Correlation with lipstick use

Gabriella Padovani Tahan, Nayara de Kássia Souza Santos, Ana Carolina Albuquerque, Isarita Martins*

Laboratory of Toxicants and Drugs Analysis, Faculty of Pharmaceutical Sciences, Federal University of Alfenas – Unifal-MG, Gabriel Monteiro da Silva Street, 700, CEP 37130-000, Alfenas, MG, Brazil

ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form
30 April 2016
Accepted 2 May 2016
Available online 3 May 2016

Keywords:
Liquid chromatography-tandem mass spectrometry
Parabens in serum
Lipstick use
Exposure assessment
Toxicology of cosmetics

ABSTRACT

Parabens are the most widely used preservative and are considered to be relatively safe compounds. However, studies have demonstrated that they may have estrogenic activity, and there is ongoing debate regarding the safety and potential cancer risk of using products containing these compounds. In the present work, liquid chromatography-tandem mass spectrometry was applied to determine methylparaben and propylparaben concentrations in serum, and the results were correlated with lipstick application. Samples were analyzed using liquid-liquid extraction, followed by liquid chromatography-tandem mass spectrometry. The validation results demonstrated the linearity of the method over a range of 1–20 ng/mL, in addition to the method's precision and accuracy. A statistically significant difference was demonstrated between serum parabens in women who used lipstick containing these substances compared with those not using this cosmetic ($p = 0.0005$ and 0.0016, respectively), and a strong association was observed between serum parabens and lipstick use (Spearman correlation = 0.7202).

1. Introduction

Cosmetic products are applied on a daily basis. Although external human contact with a substance rarely results in its penetration through the skin and significant systemic exposure, there is local exposure. When a product is used in the oral cavity, e.g., lipstick, systemic exposure to its ingredients cannot be completely excluded (Nohynek et al., 2010). Methylparaben and propylparaben are the most commonly used parabens in foods and cosmetics to prevent microbial contamination because of their broad-spectrum antimicrobial activity, low volatility and effectiveness over a wide pH range (Cao et al., 2013; Fei et al., 2011; Guo and Kannan, 2013; Guo et al., 2014; Liao et al., 2013a, 2013b; Soni et al., 2005; Zotou et al., 2010). Products containing these preservatives may contact the skin, hair, scalp, lips, mucosae (oral, ocular and vaginal), axillae and nails, and they may be used on an occasional or consistent basis. Their use may extend over a period of years. In some instances, application frequency and duration may be continuous. Human exposure to parabens is estimated at 76 mg/day, which consists of approximately 1 mg/day from food, 50 mg/day from cosmetics and personal care items, and 25 mg/day from pharmaceutical formulations (Soni et al., 2005). Based on the paraben concentrations measured in food and the per capita daily ingestion rates of food, a recent estimated daily intake of total parabens in the USA was calculated at 307 ng/kg body weight for adults (Darbre and Harvey, 2014; Liao et al., 2013b). Based on the amount and frequency of use of personal care products, the total dermal intake was calculated to be 31 μg/kg body weight for women (Darbre and Harvey, 2014; Guo and Kannan, 2013). The results of *in vitro* skin penetration studies suggested that parabens penetrate into or through human skin at a rate of >20%, and, considering their hydrolysis by skin esterases, as little as 1% of topically applied parabens is estimated to reach the organism intact (Nohynek et al., 2010). High levels of free parabens were detected in plasma shortly after whole-body topical application (rapid skin absorption with peak levels in serum only 3 h after application to the whole body) (Vela-Soria et al., 2013).

To reduce the harm to human health from parabens, they are regulated in several countries. In Brazil, the list of preservatives allowed for personal care products and cosmetics is regulated by

* Corresponding author.
E-mail address: isarita.sakakibara@unifal-mg.edu.br (I. Martins).

http://dx.doi.org/10.1016/j.yrtph.2016.05.001
0273-2300/

the Brazilian Health Surveillance Agency (ANVISA), which has established a maximum allowable concentration for 4-hydroxybenzoic acid and its salts and esters (parabens) of 0.4% in individual use and 0.8% for mixtures, similar to the European Union (ANVISA, 2001; Piao et al., 2014).

Although parabens are the most widely used preservative and are considered relatively safe compounds, recent studies have demonstrated that parabens may have estrogenic activity and have detected these compounds in human breast tumors. There is an ongoing debate regarding the safety and potential cancer risk of using products containing these compounds (Darbre et al., 2004; Ye et al., 2006; Zotou et al., 2010). According to Prusakiewicz et al. (2007), butylparaben blocks the skin cytosol's sulfation of estradiol and estrone but not the androgen dehydroepiandrosterone, suggesting that chronic topical application of parabens may lead to prolonged estrogenic effects in skin as a result of the inhibition of estrogen sulfotransferase activity. Accordingly, the anti-aging benefits to the skin of many topical cosmetics and pharmaceuticals could be derived, in part, from the estrogenicity of parabens.

The widespread use of parabens and their potential risks to human health have prompted interest in assessing human exposure to these compounds. In recent years, several methods have been proposed to measure the levels of parabens in cosmetics or personal care products (Cheng et al., 2012; Fei et al., 2011; Labat et al., 2000; Martins et al., 2011; Sanchez-Prado et al., 2011; Tzanavaras et al., 2012; Wang and Chang, 1998; Wang and Zhou, 2013), food (Cao et al., 2013; He et al., 2012), drugs (Kamble et al., 2011), plasma or serum (Vela-Soria et al., 2013; Ye et al., 2009), saliva (Zotou et al., 2010), urine (Calafat et al., 2010; Kang et al., 2013; Meeker et al., 2011; Smith et al., 2012, 2013; Ye et al., 2006), human placental tissue (Jiménez-Díáz et al., 2011), stratum corneum (Ishiwatari et al., 2007) and water (Prichodko et al., 2012). However, it is difficult to obtain data about the use of cosmetics and the biological concentrations of their active ingredients. Therefore, to evaluate whether the use of lipstick alters paraben concentrations in the body, the present work applied a liquid chromatography-tandem mass spectrometry method to determine methylparaben (MePa) and propylparaben (PrPa) concentrations in serum and correlated these results with the routine application of this oral cosmetic.

2. Experimental

2.1. Reagents, materials and solutions

MePa and PrPa working standards (purity > 99%) were kindly supplied by Sigma–Aldrich® (Steinheim, Germany). HPLC-grade methanol and acetonitrile were used during the experiments and tests (Merck, Rio de Janeiro, Brazil). Ultra-pure water (18.2 MΩ/cm) was obtained from a Millipore system. Analytical-grade reagents were purchased from Vetec (Rio de Janeiro, Brazil).

Standard stock solutions (1000 ng/mL) for each analyte were prepared in methanol and stored at 4 °C in the dark. Working standards were prepared by diluting in methanol immediately before use. Before injection into the LC system, the samples (final extracts) were filtered through 0.2 μm (4 mm diameter) Millex® filters supplied by Merck Millipore, São Paulo, Brazil.

2.2. Chromatographic instrumentation and conditions

Serum analyses were performed on a Shimadzu® LCMS-8030 triple-quadrupole liquid chromatograph-mass spectrometer (LC-MS/MS) consisting of an LC-20AD pump system, a CTO-20A column oven, and an SIL-20AHT automatic injector. Electrospray ionization was used in the negative mode. The ion source temperature was maintained at 400 °C. The IonSpray voltage was set at 4.5 kV. The flows of the drying gas and nebulizing gas were 15 L/min and 2 L/min, respectively. The collision gas was helium at 10 psi with a collision energy (CE) of 25 eV. The dwell time for each compound was 200 ms. Chromatographic separation was performed on a SHIM-PACK XR-ODS® column (100 × 3.0 mm i.d., 2.2 μm) and a SHIM-PACK G-ODS pre-column (10 × 4.0 mm i.d., 5 μm) at 35 °C. The analytes were separated at a flow rate of 0.3 mL/min with a mobile phase composed of a mixture containing 35% of a 0.1% aqueous ammoniacal solution and 65% of 0.1% ammonia in methanol (v/v). The injection volume was 25 μL, and the total run time was 8.0 min.

2.3. Study design

A group of female volunteers (n = 18) with a mean age ± standard deviation (SD) of 26.1 ± 3.4 years, a median age ± average deviation (AD) of 23.0 ± 2.2 years, a mean body mass index ± SD of 22.2 ± 2.9 kg/m2 and a median body mass index ± AD of 22.1 ± 2.3 kg/m2 were recruited from the Federal University of Alfenas-MG located in Minas Gerais, Brazil, between August and October of 2014. Blood samples (15 mL) were collected from the volunteers who participated in this study for 3 phases. In phase 1, the women used paraben-containing products according to their routine. In phase 2, they used donated lipstick containing MePa and PrPa for five days in conjunction with the routine use of paraben-containing products. The lipsticks donated to the volunteers were purchased at a market in Alfenas, MG, Brazil, and the labels indicated the presence of MePa and PrPa. To determine the concentration of total parabens an analytical HPLC-UV method was employed with a liquid-liquid extraction. Briefly, about 100 mg (accurately weighed) of lipstick sample were weighed into a 50 mL glass centrifuge tube, and 1 mL of methanol was added. The mixture was homogenized for 2 min with a vortex, sonicated for 10 min, stirred for 10 min at 1500 rpm, and centrifuged for 15 min at 2500 rpm. 0.5 mL of the underlying organic layer was transferred to a clean glass and evaporated to dryness under a nitrogen stream. The residue was dissolved in 0.5 mL of mobile phase and filtered through a 0.22 μm filter. This solution, 10 μL, was injected into the HPLC system with the same conditions previously published by Martins et al. (2011). The relative standard deviation (RSD%) and relative error (E%) for intra- and inter-assay precision and accuracy, respectively, were less than 15%. The total parabens content (MePa plus PrPa) was of 0.35% (m/m).

The lipsticks were weighed before and after five days of use. Blood was collected at the end of the fifth day of lipstick use. In phase 3, the women routinely used paraben-containing products while abstaining from lipstick for five days, and blood was collected at the end of the fifth day of no lipstick use.

After the blood collection in each phase, the blood was centrifuged (NT-811 centrifuge, Nova Técnica®) at 2000 rpm for 5 min, and the serum was stored at −80 °C until analysis. This study was approved by the Ethics Committee of the Federal University of Alfenas-MG-Brazil (n° 353.930). Informed consent was obtained from each volunteer. In addition, a completed questionnaire containing information about their habits, focusing on food ingestion and their use of hygiene/cosmetic products, was collected during the 3 phases. All of the samples and data were processed blindly.

2.4. Sample preparation and validation

Serum (0.5 mL) was added to 0.5 mL of acetone in a 50 mL glass centrifuge tube, and the mixture was stirred (tube agitator MA 162 vortex type, Marcon®) for 3 min and then centrifuged (NT-811 centrifuge, Nova Técnica®) for 10 min at 3000 rpm. Next, 0.5 mL of