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Determination of parabens in urine samples by microextraction using packed sorbent and ultra-performance liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT

A simple, sensitive, and selective method using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS) was developed and validated for simultaneous determination of parabens [methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP), butyl paraben (BuP), and benzyl paraben (BzP)] in human urine samples. After microextraction by packed sorbent (MEPS) using a C18 phase, the parabens were separated on a Kinetex C18 column (100 mm × 2.1 mm × 1.7 μm) within 4.6 min using isocratic elution. These compounds were detected on a triple quadrupole tandem mass spectrometer using the multiple reactions monitoring (MRM) mode via an electrospray ionization source operating in the negative ionization mode. Important factors that influence MEPS performance were evaluated, such as the sample pH, draw–eject sample volume, clean-up step, and desorption conditions. The proposed MEPS/UPLC–MS/MS method presented a linear range from 0.5 ng mL^{-1} (limit of quantification – LOQ) to 50 ng mL^{-1}, and interassay precision with coefficients of variation lower than 15%, and relative standard error values of the accuracy ranged from −8.8% to 15%. The MEPS/UPLC–MS/MS method was applied successfully to determine parabens in urine samples from 30 postpartum volunteers, enabling assessment of human exposure to these compounds.

1. Introduction

Parabens, such as methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP), butyl paraben (BuP), and benzyl paraben (BzP), are esters of *p*-hydroxybenzoic acid. These compounds are widely used as antimicrobial preservatives, especially against molds and yeast, in cosmetic and pharmaceuticals products as well as in processed food and beverage. The antimicrobial activity of parabens increases, but water solubility decreases with the length of the alkyl chain. Consequently, methyl and propyl parabens are the most extensively used in cosmetics and food processing [1–3].

The widespread application of parabens stemmed from their low toxicity, inertness, worldwide regulatory acceptance, and low cost. However, some parabens may exert estrogenic action, which can disrupt endocrine processes in wildlife as well as in humans, leading to reproductive problems and susceptibility to cancer [1,4,5]. Kang et al. [6] have reported that exposure of pregnant rats to butyl paraben produces male offspring with adversely affected reproductive organs. Hence, the large-scale use of parabens has raised concerns about their safe levels, and their toxic effects on humans.

Humans are exposed to parabens via absorption through skin, and via the gastrointestinal tract after oral intake. Parabens are rapidly metabolized into mainly the harmless p-hydroxybenzoic acid and its respective glucuronic and sulfuric acid conjugates [7]. However, measuring *p*-hydroxybenzoic acid and its conjugates in urine may not be the best approach for assessing human exposure to parabens, because *p*-hydroxybenzoic acid measurements are not specific, and different parabens can possess quite different estrogenic bioactivities. Therefore, measuring the unchanged precursor parabens may be a viable alternative [1,2,8].

Sample preparation of biological matrices is an important step in analytical processes: it removes endogenous components and pre-concentrates the analytes that exist at trace levels in the sample. Proteins and other interferences may negatively affect the

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performance of separation columns, increase column backpressure [9–12], and suppress electrospray ionization (ESI) during LC–MS/MS analysis [13]. Asimakopoulos et al. [14] have developed a method based on liquid–liquid extraction (ethyl acetate) protocol, followed by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI–MS/MS) to determine 19 compounds, including parabens, in human urine.

Solid phase extraction (SPE) [15] or an online SPE (RP18 ADS)-LC–MS/MS system are the most commonly used techniques to analyze parabens in biological samples [1,2,7,16–18]. Frederiksen et al. [7] correlated the parabens concentrations in urine to their concentrations in both serum and seminal plasma of a group of 60 young healthy Danish men. Meeker et al. [16] have determined the distribution, variability, and predictors of urinary biomarkers of environmental phenols and parabens measured at multiple times during pregnancy among women living in Northern Puerto Rico. Kang et al. [2] have assessed urinary parabens levels in pregnant women and their matched newborn infants; they associated parabens levels with stress markers. Berglund et al. [17] estimated the exposure to parabens in Swedish mothers and their children. The levels of parabens were higher among the children in the urban area than in the rural area. However, mother's levels of parabens were higher than the children's levels of parabens. The levels of parabens were associated with use of cosmetics and personal care products.

MEPS is a relatively new sample preparation technique based on the miniaturization of the conventional SPE device from milliliter bed volumes to microliter volumes [11]. MEPS can be fully automated, the sample processing, extraction and injection steps are performed online using the same syringe. MEPS significantly reduces the volume of solvents and sample needed [12,19].

This paper reports the use of MEPS followed by UPLC–MS/MS for the simultaneous determination of parabens in urine samples from postpartum women, aiming to assess human exposure to these compounds.

2. Experimental

2.1. Reagents and analytical standards

The methyl paraben, ethyl paraben, propyl paraben, butyl paraben, benzyl paraben and propyl 4-hydroxybenzoate-ring-$^{13}C_6$ (internal standard – IS) analytical standards were acquired from Sigma–Aldrich (São Paulo, Brazil).

The standard working solutions of the five parabens and of the internal standard were prepared by diluting the parabens stock solutions (1 mg mL^{-1}) in methanol. These solutions were stable for 45 days at a temperature of −4 °C, as well as under bench top conditions at controlled room temperature (22 °C). The water used to prepare the mobile phase was previously purified in a Milli-Q system (Millipore, São Paulo, Brazil). Methanol, acetonitrile, acetic acid (HPLC grade), and monobasic and dibasic phosphates were purchased from J.T. Backer (Phillipsburg, USA). After being prepared, the mobile phase was filtered (0.45 μm in regenerated cellulose membrane, Millipore, São Paulo, Brazil) and degassed in ultrasonic bath.

2.2. Urine sample

Blank human urine was obtained from colleagues, who had consciously avoided exposure to parabens before sampling. One pool was made for the urine matrix, and it was used to calibrate standards and to control the validation of the present method. Urine samples were collected from 30 postpartum women assisted at *Centro de Referência em Saúde da Mulher* (Ribeirão Preto, SP, Brazil). The principles embodied in the Helsinki Declaration were observed, and the study was approved by the Ethics Committee of Escola de Enfermagem de Ribeirão Preto, University of São Paulo, Brazil. A written informed consent was obtained from all the participants.

Mean anthropometric data for the population group were: age, 14–32 years (average: 23 years); height, 1.49–1.78 cm (average: 1.62 cm); weight, 55–95 kg (average: 73.05 kg).

2.3. UPLC–MS/MS conditions

The UPLC–MS/MS system consisted of a Waters ACQUITY UPLC (Waters Corporation, Milford, MA, USA) equipped with ACQUITY UPLC binary solvent and ACQUITY UPLC sample manager, coupled to the XEVO TQD mass spectrometer (tandem quadrupole) with electrospray ionization (ESI).

Chromatographic separations were conducted on a Kinetex C18 column (100 mm × 2.1 mm × 1.7 μm) (Phenomenex, USA) column at 40 °C, under isocratic conditions; mobile phase was composed of acetonitrile:water (40:60, v/v) at a flow rate of 0.3 mL min^{-1}. Data were acquired the MRM mode. Two specific transitions were optimized for each molecule, to increase the selectivity and the reliability of the method (Table 1). The first MRM transition was used for quantitation while the second MRM transition was used for qualitative identification. ESI interface in the positive and negative modes was evaluated for all the compounds. The ESI negative mode was selected because it provided higher sensitivity for all the compounds.

The source and operating parameters were optimized as follows: capillary voltage, −3.50 kV; source temperature, 150 °C; desolvation temperature, 500 °C; desolvation gas flow, 700 L h^{-1} (N_2, 99.9% purity); and cone gas flow, 20 L h^{-1} (N_2, 99.9% purity); Argon (99.9999% purity) was used as the collision gas. The dwell time established for each transition was 0.022 s, and the interscan delay was set to the automatic mode. Data were acquired using MassLynx V4.1 software.

2.4. MEPS procedure

The MEPS syringe (250 μL syringe, and 2 mg of the C18 sorbent material) was donated by SGE (Melbourne, Australia). This sorbent had irregular particles with an average size of 50 μm and nominal porosity equal to 60 Å. Before being used for the first time, the

Table 1
Ion transitions, instrument settings, and retention times for each studied paraben.

Analyte	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	DP (V)	CE (eV)	Qualifier ion (*m*/*z*)	RT (min)
Methyl paraben	151.0	92.0	35	10	136.0	1.03
Ethyl paraben	165.0	91.9	33	23	136.0	1.29
Propyl paraben	179.0	91.9	30	20	136.0	1.78
Butyl paraben	193.0	92.0	30	20	136.0	2.65
Benzyl paraben	227.0	92.0	30	20	136.0	2.69
Internal standard						
Propyl 4-hydroxybenzoate-ring-$^{13}C_6$	185.0	98.0	38	20	142.0	1.78

Declustering potential (DP), collision energy (CE), and retention time (RT).