Ultrasound-Enhanced Enzymatic Hydrolysis of Paraben Conjugates in Urine for Human Biomonitoring Studies

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Nowadays, humans are exposed to a wide spectrum of environmental chemicals. Human Biomonotoring (HBM) studies allow to asses the exposure of humans to chemicals. One class of substances with estrogenic acitivty are para-hydroxybenzoic acid esters (parabens).

Parabens are extensively used in personal care products as well as in food and pharmaceuticals for preservation. Today the EU permit cosmetic products with concentrations above 0.8% for total parabens ("Commission Regulation (EU) No 358/2014," 2014). The exposure of humans was accounted for approximately 76 mg total parabens per day in the US (SONI, BURDOCK, TAYLOR & GREENBERG, 2001). Water solubility, antimicrobial activity and estrogenic acitvity depends on the length and the structure of the alkyl chain of the ester group (OKUBO, YOKOYAMA, KANO & KANO, 2001). Therefore, analytical methods used for HBM of parabens need to distinguish between isomers of propyl- and butyl parabens (*n*-, *iso*- and *sec*-isomers). Additinally, methods used for HBM should offer a short sample preparation and analysis times, minimized sample volumes, simple application and automation to allow for large numbers of samples to be processed.

Here, we present a quick and simple method for the quantification of parabens in human urine by isotope-dilution UPLC-MS/MS. Cleavage of paraben conjugates (glucuronide as well as sulfates) is completed within 30 min enzymatic incubation by the support of ultrasonication. Moreover, the sample preparation procedure is simplified by the use of centrifugation devices (with molecular cut-off at 3 kDa) instead of time and effort intensive extraction methods. The new method shows high accuracy (relative method recoveries 91-130%), high precision (intra-day as well as inter-day standard deviation 0.6-12%), is sensitive enough for non-occupationally exposed individuals and covers a wide range of concentrations (linear calibration ranges 0.1-100/250 µg/L).

A total of 39 urine samples (62% female and 48% male adults) from two different locations (Leipzig and Berlin) were analysed and quantified. Methyl-, ethyl and *n*-propyl paraben were the predominante compounds in both gender groups with detection frequencies ranging from 90-100%. Median concentration of the sum of 8 parabens from female and male adults were 25.3 µg/L and 7.1 µg/L, respectively. Further, concentrations of methyl- and *n*-propyl paraben showed strong correlation within female adults (R^2=0.94) after correction by creatinine excretion compared to males (R^2=0.03). These variations between female and male samples may be due to differences in the lifestyle.

Results are in agreement with previous studies in Germany (MOOS et al., 2014) demonstrating the feasibility of the new method and potential applications to HBM studies. When UPLC-QTOF is used the method is sensitive enough to determine and to quantify parabens in target and non-target screening approaches.

Commission Regulation (EU) No 358/2014 (2014).

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