**Determination of 18 phthalate metabolites and 2 alternative plasticizer metabolites in urine using high-throughput off-line SPE and LC-MS/MS**

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**Introduction**:Phthalates are ubiquitous compounds used worldwide as plasticizers. Some of them may have negative effects on human health, a few have been already forbidden in toys and food packaging materials. Specific metabolites are often measured in human urine to determine exposure to phthalates. For the purpose of epidemiologic studies high level of precision, reproducibility and high throughput are crucial factors of an analytical method. Therefore, a new method has been developed, where 18 phthalate metabolites and 2 metabolites of alternative plasticizer (Hexamoll® DINCH - 1,2-cyclohexane dicarboxylic acid diisononyl ester) are analyzed. To fulfill requirements for high throughput, precision, low sample and solvent consumption 96-well plate format was adopted. The whole sample preparation procedure including enzymatic deconjugation and clean-up/preconcentration using off-line SPE was optimized and validated.

**Materials and Methods**:Phthalate metabolites are analyzed by HPLC (Agilent 1290 – Agilent Technologies) combined with tandem MS (AB Sciex QTrap 5500 – Sciex). Zorbax Eclipse Plus C18 (2.1 x 50 mm; 1.8 μm) is used as analytical column. Deionized water and methanol, both with 0.01% formic acid are used as mobile phase. For off-line SPE Oasis HLB 96-well plates are used. For the purpose of validation 52 urine samples from pregnant women were analyzed in the past to determine phthalate metabolites. These samples were part of pilot phase in the project CELSPAC: TNG (Central European Longitudinal Study of Pregnancy and Childhood: The Next Generation), and were obtained from The University Hospital in Brno.

**Results**: Sample preparation procedure and both MS and HPLC conditions were optimized for 18 monoalkyl phthalates and 2 DINCH metabolites. Limit of detection (MDL) defined as three times the signal to noise ratio is in the range of 0.1-0.3 ng/mL. Limit of quantification (MQL) defined as ten times the signal to noise ratio is in the range of 0.3-1.0 ng/mL for all analytes. 52 urine samples from pregnant women were analyzed. The most common detected analyte was MBP (monobutyl phthalate) (in 100% of samples), MEP (monoethyl phthalate) was detected in highest concentrations (median = 69.4 ng/mL; maximum = 2817.4 ng/mL). Measured results are in agreement with results from similar studies from Czech Republic, Slovak Republic and Hungary1 and other studies from Europe. The requirements for high sensitivity, precision, reproducibility and short sample preparation time were fulfilled. Moreover, 96-well plate off-line SPE enables high throughput, precision and low consumption of sample and other materials in general. Presented method is therefore well suited for analysis of large sample sets common within the frame of epidemiologic studies.

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