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Development and application of a LC-HRMS method for the identification and quantification of CECs concern in urine

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Abstract. Chemicals are everywhere in our daily lives, and we encounter them through various pathways. Human biomonitoring (HBM) is commonly used to understand our exposure to these chemicals. Traditionally, HBM focused on a limited number of chemicals analyzed with specific instruments. However, recent advancements in high-resolution mass spectrometry (HRMS) have allowed for a more comprehensive analysis of the chemical exposome. This study aimed to validate a methodology using HRMS to accurately profile exogenous chemicals and their metabolites in urine samples. Five extraction protocols covering different chemical classes were evaluated for their effectiveness in terms of extraction recoveries, linearity, sensitivity, and reproducibility. The best protocol was then validated (e.g., recoveries generally in between 60-120%, or lower matrix effect) and applied to analyze over 2,000 chemicals in 10 real human urine samples. Using the HRMS approach, 36 chemicals (e.g., plastic additives, UV-filters or pharmaceuticals) were identified and semi-quantified, demonstrating the effectiveness of the methodology. Interestingly, the laborious deconjugation step was deemed unnecessary as HRMS yielded comparable results without it, while also successfully identifying other metabolites. **Keywords:** Human biomonitoring (HBM), Non-target, Method validation, Deconjugation, Glucuronidation.

1. Introduction

Contaminants of emerging concern (CECs) encompass various pollutants that can enter the human body through different exposure routes. Understanding their presence and levels is crucial for assessing potential risks and establishing effective regulations. Urine has been widely used for screening CECs due to its easy collection and non-invasive nature. However, conventional screening methods with low-resolution mass spectrometers (LRMS) such as triple quadrupoles (QqQ) have limitations in terms of the number of chemicals they can analyze. Additionally, they often fail to detect metabolites, necessitating deconjugation steps to hydrolyze phase II metabolites. Recent advancements in high-resolution mass spectrometry (HRMS) have opened up new possibilities for comprehensive screening of CECs in human biomonitoring (HBM). HRMS allows wide-scope target screening, suspect, and non-target strategies without preselecting specific chemicals. It enables retrospective searches and identification of metabolites without deconjugation steps, which is particularly valuable in urine samples. This study aimed to validate and implement an analytical methodology using LC-HRMS for analyzing a diverse range of CECs in urine samples. Additionally, the necessity of a deconjugation procedure for glucuronidated chemicals in HRMS-based protocols was assessed.

2. Methods

A urine pool was used for validation, evaluating five different methodologies. The best-performing methodology was thoroughly validated, assessing extraction recoveries, matrix effect, sensitivity, linearity, linear range, and precision. This validated method was then applied to 10 human urine samples and concentrations were estimated using a semiquantitative method based on ionization efficiency. Additionally, the necessity of deconjugation was examined by comparing two methods: with and without deconjugation (β-glucuronidase). Instrumental analysis: UHPLC-QTOF Impact II (Bruker).

3. Results and discussion

Method optimization and validation. The optimized methodology included filtration for endogenous substances removal and was successfully validated with a set of 90 chemicals. **Deconjugation in HRMS analysis.** Deconjugation in HRMS analysis was finally considered as not required. This study is the first to compare protocols with and without deconjugation, providing insights into their advantages and disadvantages. It also demonstrates the potential application of these protocols for quantification using HRMS instruments. **Method application.** 36 CECs, including glucuronide and hydroxylated metabolites, were identified. These chemicals belonged to various categories (i.e., food-related, personal care, tobacco-related, biocides, plastic additives, plant-growth regulators, and pharmaceuticals).

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