ENDOBIOTICS QUANTIFICATION AND STRATEGIES FOR BIOANALYTICAL METHOD VALIDATION: THE CASE OF THE HIPPURIC ACID

Giulia De Simone[1,2*], Alessia Giannetta[1], Mirco L. Randazzo[1], Aurelia Morabito[1,3], Roberta Pastorelli[1], Alice Passoni[1] and Laura Brunelli[1]

[1] *Department of Environmental Health Sciences, Mario Negri Institute, Milan, 20156 (Italy)*
[2] *Department of Biotechnology and Bioscience, University of Milan Bicocca, Milan, 20126 (Italy)*
[3] *Department of Bioengineering, Politecnico di Milano, Milan, 20133 (Italy)*
**Email:* giulia.desimone@marionegri.it - g.desimone10@campus.unimib.it

Accurate quantification of endogenous analytes plays a critical role in both clinical and pre-clinical studies. The European Medicinal Agency (EMA) and the Food and Drug Administration (FDA) guidelines [1], define the procedure for validating bioanalytical methods for the quantification of xenobiotic by mass spectrometry (MS) or other hyphenated techniques. The guideline assures the robustness and reproducibility of the proposed analytical strategy. Having analyte-free biological matrix when dealing with endogenous compounds detection/measurement is challenging, thus the validation of a quantitative method become a complex analytical issue. Hippuric acid, an endogenous molecule with a potential as biomarker of frailty in the elderly [2], takes center stage in this work. We evaluated different strategies to enhance the accuracy, precision, and reliability of MS quantitative analyses for endogenous compounds.

To mitigate the absence of blank matrices, four distinct strategies have been suggested in literature such as background subtraction, standard addition, the matrix surrogate, and the analyte surrogate [3]. In this study, we examinate two suitable approaches: the use of the matrix surrogate and the use of the analyte surrogate. Results indicate the successful implementation of our targeted MS methods, leading to a fully validated method for the quantitation of hippuric acid in plasma samples. The method demonstrated an excellent linearity (0.1–40 ng/ml in human plasma) in terms of accuracy (mean concentration of quality controls, N = 6, within ±15%) and precision (CV < 15%), while sample preparation was validated for recovery, matrix effect (CV < 15%), and stability (within ±15%) using the limits proposed by EMA guidelines. This study demonstrated the feasibility of establishing a comprehensive method validation for the analysis of endogenous compounds, exemplified by the case of hippuric acid, within the field of clinical studies.

References:

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