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Development of new approaches for the evaluation of the metabolism of bioactive compounds of nutritional interest

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INTRODUCTION

Desorption electrospray ionization (**DESI**) is a mass spectrometry imaging (MSI) technique that provides a visual ***in situ*** **representation of the distribution of small molecules within complex samples and biological tissues**. Detailed evaluations are needed to ensure the instrument's sensitivity to the metabolites of interest, its ability to produce consistent results, and its overall operational functionality. These assessments are crucial for establishing a reliable and accurate analytical method. To verify this, a mimetic model was developed to consider the matrix effect due to the tissue in the desorption of the compounds using DESI mass spectrometry imaging.

MATERIALS AND METHODS

Sample preparation

The mimetic model was created following the protocol published by Barry et al. (2018), with some minor modifications. Animal tissues were purchased, homogenized with an ultra-turrax homogenizer Polytron PT 10/35 with PCU-8 EU Controller (Kinematica AG, Switzerland) and aliquoted. A pool of analytical standard compounds was prepared with (+)-catechin, (−)-epicatechin-3'-sulfate, 5-(3'-hydroxyphenyl)-valerolactone-4'-sulfate, 3-(4'-hydroxy-3'-methoxyphenyl)propanoic acid, 3-(3'-hydroxyphenyl)propanoic acid-4'-glucuronide, 3',4'-dihydroxyphenylacetic acid, 3,4-dihydroxybenzoic acid, 3-methoxybenzoic acid-4-sulfate, hippuric acid, naringin, hesperetin, hesperetin-glucuronide, hesperetin 7-sulfate, 3,8-dihydroxy-urolithin, 3-hydroxy-urolithin-8-glucuronide, 3-hydroxy-urolithin, urolithin-3-glucuronide, 2-(3'-hydroxyphenyl)ethanol, hydroxytyrosol-sulfate, hydroxytyrosol-glucuronide, 4'-hydroxy-3'-methoxyphenylacetic acid, 3’-methoxy-4'-hydroxyphenylacetic acid sulfate. The pool was added to each aliquot at increasing concentrations, from 0.3 to 6 µg/g.

Tissue sectioning

The frozen samples were sectioned in 20 µm-thick slices using a Leica Reichert Jung 2800E cryostat. Prior to analysis, the slides were transferred to a vacuum desiccator and dehydrated at room temperature for 30 minutes.

DESI-MSI analysis

Mass spectrometry imaging was performed on a Synapt XS mass spectrometer equipped with a DESI-XS source (Waters Corporation, Wilmslow, United Kingdom) in negative ionization and in resolution mode. Different solvent ratios were used, with various percentages of methanol:water (Honeywell, Germany) acidified with 0.01% v/v formic acid and spiked with 200 pg/µL leucine enkephalin (Waters Corporation, Milford, MA, USA). Data were acquired using MassLynx v4.2 software at a m/z 50–600 mass range. Lock mass correction was performed on the raw data with the internal standard Leucine Enkephalin (m/z = 554.2615) and finally data were processed using High-Definition Imaging (HDI) Software (Waters Corporation, Wilmslow, UK).

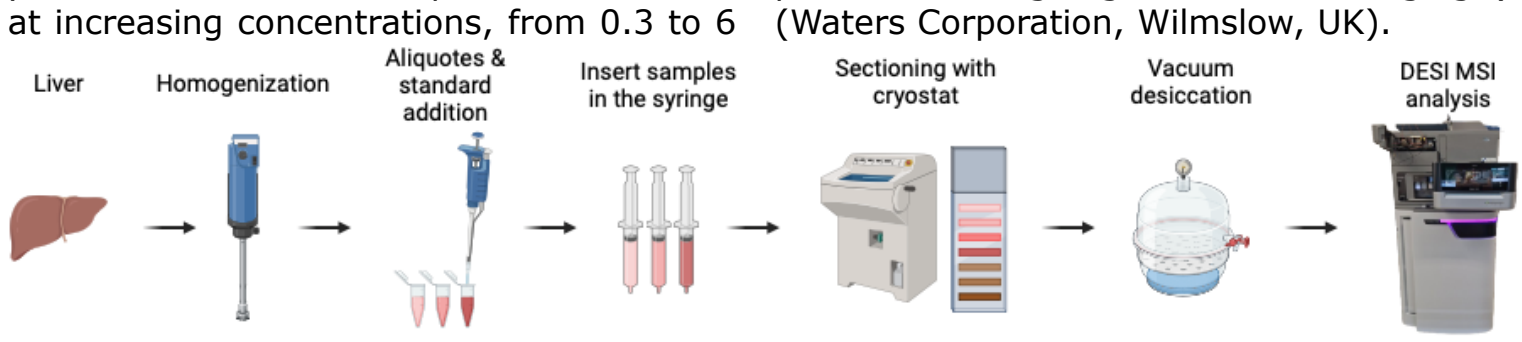

Fig 1. Workflow for sample preparation.

RESULTS AND DISCUSSION

The section with the highest concentration was first analyzed to optimize the analysis parameters. The heat transfer line temperature (HTL) and the capillary voltage (Cap V) were the first parameters considered (table 1). The optimal value for each factor was identified as the highest average and total intensity of the signal observed for most of the compounds of the phenolic class examined. Afterwards, a series of solvent ratios were evaluated (table 1). The optimal solvent ratio was selected again based on the maximum desorption from the tissue of most of the compounds of interest. For ellagitannins the optimal parameters for their desorption from liver tissue are HTL 250°C, Cap V 0.55V and 92:8 as MeOH:H_2O solvent ratio, while for flavan-3-ols metabolites are HTL 350°C, Cap V 0.65V and 98:8 solvent ratio.

Heat Transfer Line (°C)	150	200	250	300	350
Capillary Voltage (V)	0.35	0.45	0.55	0.65	0.75
Solvent (MeOH:H_2O)	90:10	92:8	95:5	98:2	100:0

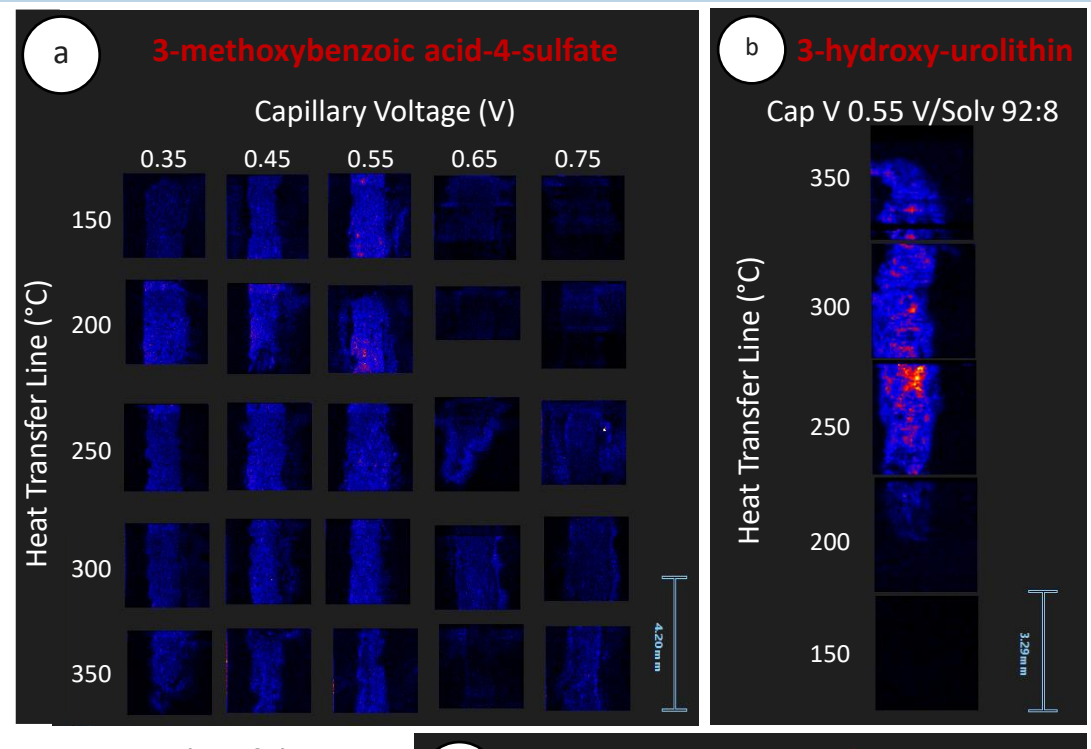

Fig 2. Examples of the optimization process with a) vanillic acid-sulfate, b) urolithin B and c) hippuric acid.

Table 1. Parameters used for the optimization.

REFERENCES

Barry, J. A., Groseclose, M. R., Fraser, D. D., & Castellino, S. (2018). Revised preparation of a mimetic tissue model for quantitative imaging mass spectrometry. *Protocol Exchange.*

ACKNOWLEDGEMENTS

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