Hyphenated MS-Methods as a Tool for Orthogonal Metabolite Annotation in On-Line Breath Analysis with SESI-HRMS

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On-line breath analysis with secondary electrospray ionization (SESI) coupled to high-resolution mass spectrometry (HRMS) is a powerful method for rapid and non-invasive examination of the human metabolome. Various clinical research projects have shown the effectiveness of this technique. [1] However, the identification of metabolites and biomarkers using SESI-HRMS is still limited due to the lack of a hyphenated separation method prior to the MS analysis. Comparing annotated metabolites found in exhaled breath condensate (EBC) with detected features in on-line data can merge this gap and serve as a step toward building a database of metabolites for the human exhalome. For the comparison, the breath of 16 healthy adults was measured on-line for 10 days with a SESI source (*Fossil Ion Tech*, Spain) coupled to a Q-Exactive Plus Orbitrap mass spectrometer (*Thermo Fischer Scientific*, Germany) while also simultaneously condensing part of the exhaled breath. EBC was analyzed using an Acquity UPLC system (*Waters Corporation*, USA) coupled to the same mass spectrometer, employing both reverse-phase and hydrophilic interaction columns. In addition, a data-independent MS^2-acquisition method derived from PAcIFIC [2] was utilized. GC-MS analysis was carried out using dynamic headspace vacuum transfer in-trap extraction coupled to GC-MS (DHS-VTT GC-MS) [3]. On-line and EBC MS^2 data were processed by custom workflows and EBC MS^1 data by standard workflows. An immense number of features were obtained in the LC-MS analysis, especially with the MS^2 method. Employing CANOPUS, the features were assigned to chemical classes, revealing that mostly amino acids and amines were detected in positive mode, while in negative mode predominantly amino acids and carboxylic acids were detected. The GC-MS analysis revealed mostly compounds of exogenous origin, such as additives from oral hygiene products like menthol or other fragrances, and flavoring agents. Food and beverage consumption restrictions before sampling will be needed to mitigate this issue. Comparison of the detected off-line features of both methods with the detected on-line features showed partial overlap, however, the m/z range from 150-200 needs to be better covered by the off-line methods. While these results demonstrate the potential of combined LC-MS and GC-MS analysis of breath condensate as an orthogonal annotation tool for SESI-HRMS, additional on-line fragmentation is needed for annotation with higher confidence.

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