Tackling the challenges of untargeted microbiome research using multidimensional chromatography

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In Omics research setting, access to sample is usually a key factor of the experimental design. In some cases, samples can be abundant and readily available. However, in the case of biological matrices, samples can be difficult to obtain and the chemical integrity difficult to maintain. This is particularly true for microbiome research. Stool samples are difficult to obtain, difficult to homogenized, and difficult to store. Unstable samples make difficult potential combinations of different analytical techniques without introducing bias.

In this study, we have developed a complete analytical workflow for volatilomics profiling of microbiome samples. This workflow was design to maximize the number of replicates without compromising the sample integrity.

For the sampling, we evaluated simultaneous multiple SPME fibers sampling. Using three fibers simultaneously, we generated three technical replicates from one biological sample. New generation of nitinol-core fibers were tested. Each fiber was then analyzed by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) in separated runs. The robustness of the procedure was tested on a 24-standards mixture, human whole stool matrix, and human whole stool samples from the National Institute of Standards and Technology (NIST). Next, the separation conditions were optimized. We focused mostly on the columns' combination and the influence of the stationary phase thickness and composition on the separation of volatile molecules. Finally, the generated data were used for the development of a data processing workflow. Different alignment and modeling strategies were tested and compared in order to evaluate the user-impact on the data processing. The resulting data will be shared openly in order to provide the first fully controlled microbiome data set based on GC×GC-TOFMS. We aim to make this data set a reference for the evaluation of new data processing strategies.