The GC-MS tutorial to successfully carry out your metabolomics study

The term metabolite is usually restricted to small molecules that are the intermediates and products of metabolism, which represent the result of complex interactions between our genetic inheritance and multiple environmental stimuli. Characterization of the metabolome is a challenging but necessary task to be addressed to improve our knowledge on diverse disease phenotypes, efficacy of drugs or to identify diagnostic markers, among other applications.

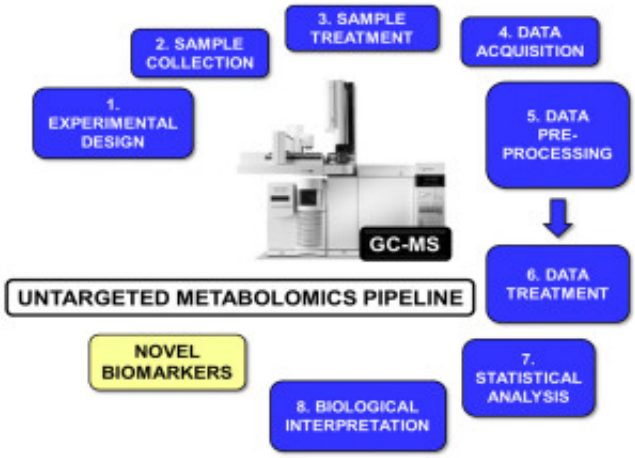

Fig. 1. Untargeted Metabolomics pipeline.

One of the ways to accomplish this goal is through what has been termed as untargeted metabolomics. The workflow consists of trying to detect as many signals as possible in the biological samples and, after pattern comparison, those compounds changing in response to a specific situation under investigation are considered potential markers. With this strategy it is possible, without a priori hypothesis, to highlight which biochemical pathways are not working efficiently and to define new and narrower directions of future study. For this purpose, gas chromatography/mass spectrometry (GC-MS) is highly efficient, sensitive and reproducible for key compounds in biological pathways such as amino acids, free fatty acids or monosaccharides. Although, as only compounds that can be made volatile after derivatization can be analyzed, sample treatment requires intensive lab work.

This tutorial is focused on the use of the GC-(EI)-Q-MS as a suitable technique for metabolomics and it intends to provide well established procedures that are fundamental to avoid biological, analytical and methodological errors. Especially in the case of metabolomics where the complexity of the workflow often hides traps and bias, clear protocols and experimental designs are pivotal.

This tutorial provides a standardized methodology with flexible and easy-to-use procedures applicable to different type of biological samples (i.e. plasma, serum, cells and different tissues, among others). All the steps in metabolomics workflow are explored and decomposed:

The first stage is the ethical approval by the local research committee, which is a key principle in clinical research.
Thereafter, sample collection and storage are also critical to reflect a real snapshot of the metabolism and to prevent contamination and metabolic degradation.
The equipment maintenance and setup are also examined and several recommendations are given to achieve the highest accuracy and reproducibility.
Another crucial step considered in this tutorial is the sample treatment which is probably one of the main sources of variability due to the intensive handling required.
We also report many recommendations to reduce to a minimum the analytical variability.
The data pre-processing and processing are undoubtedly the following stages. The main goal of the data pre-processing is to mine useful and appropriate data from those acquired during the experiment and by the data processing the results acquired are explored to remove any mystify information.
The consequent statistical analysis with general guidelines on tests and main software programs employed are depicted.
The biochemical interpretation of the results is the final step that allows obtaining the big picture of all of the metabolic changes that characterize a specific condition.

This tutorial concludes with the enumeration of the outcomes of the procedure described and its application in several metabolomics investigations led by our research group.

Altogether, all the concepts analysed are of benefit to the user and can be applied as a template for the creation of a tailored protocol applicable to every GC-MS-based metabolomics experiment.

Publication

From sample treatment to biomarker discovery: A tutorial for untargeted metabolomics based on GC-(EI)-Q-MS.
Mastrangelo A, Ferrarini A, Rey-Stolle F, García A, Barbas C.
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