Gas chromatography mass spectrometry: key technology in metabolomics
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Citation

Version: Corrected Publisher’s Version
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Downloaded from: https://hdl.handle.net/1887/14328

Note: To cite this publication please use the final published version (if applicable).
Summary

Metabolomics involves the unbiased quantitative and qualitative analysis of the complete set of metabolites present in cells, body fluids and tissues. Gas chromatography coupled to mass spectrometry (GC-MS) is very suitable for metabolomics analysis, as it combines high separation power with sensitive and selective mass detection. This thesis is dedicated to the development of reliable quantitative GC-MS based methods for metabolomics analysis. A general introduction to the subject is given in Chapter 1.

In Chapter 2 the challenges in metabolomics analysis are discussed and recommendations are given on how to optimize and validate GC-MS based methods with a comprehensive non-targeted approach from sample workup up to data preprocessing. Furthermore, a strategy for quality control during metabolomics studies is proposed. In addition, the current state of method validation and data preprocessing methods used in contemporary literature are discussed and a perspective is given on the future research necessary to obtain accurate quantitative data from comprehensive GC-MS data.

In Chapter 3 the development and validation of a GC-MS method is described, consisting of oximation and silylation derivatization reactions and subsequent analysis with GC-MS. The method was set up for microbial metabolomics, but it has also been successfully applied to mammalian (blood plasma and serum, urine and tissue) and plant metabolomics studies. Despite the non-targeted character of the method and complex microbial matrix, analytical performance for most metabolites fit the requirements for target analysis in bioanalysis (FDA regulation).

The method performance of different metabolites can vary depending on their physicochemical properties. During the development of the one-dimensional GC-MS method (Chapter 3) three performance classes were defined. In Chapter 4 the performance of metabolites from the three different classes is further investigated, to identify the most critical factors influencing the method performance. Besides the compound class, the sample matrix and the inertness of the analytical system proved to be most critical. Several ‘tips and tricks’ to improve the repeatability of analysis are proposed and demonstrated.

Comprehensive two-dimensional gas chromatography (GC×GC) offers several advantages over one-dimensional gas chromatography, i.e. higher peak capacity, a broader dynamic range and lower detection limits. In Chapter 5 the development and method performance of a comprehensive GC×GC-MS metabolomics method is described. A polar × apolar column combination was used and the second dimension column had a larger internal diameter and thicker film than conventionally used narrow bore thin film second dimension columns, to improve the mass loadability and inertness of the analytical system. The developed GC×GC-MS method is particularly
suitable for metabolomic studies, as the differences in composition within and between samples are usually quite large.

Data processing in GC×GC-MS analysis is still a major challenge due to the complexity of metabolomics samples and, moreover, because all metabolites are of interest and need to be quantified. In Chapter 6 the feasibility of using commercially available software for automated non-target processing of GC×GC-MS data is assessed. For this purpose a set of mouse-liver samples were measured with GC-MS and GC×GC-MS and the results obtained with both methods were compared. The samples were a part of a larger study investigating type-2 diabetes (insulin resistance). The added value of GC×GC-MS was clearly demonstrated. Although the RSDs in the GC×GC-MS data were somewhat higher than in the GC-MS data, the biological information was preserved and additional possible biomarkers for the development of insulin resistance were found in the GC×GC-MS data set. Besides, with the GC×GC-MS method, over four times more peaks were quantified and the superior separation efficiency resulted in cleaner mass spectra, facilitating the identification of metabolites.

The developed GC-MS (Chapter 3) and GC×GC-MS (Chapter 5) methods are both very suitable for the analysis of metabolomics samples when reasonable amounts of sample are available, i.e. approximately 50 - 100 µL of cell extract or body fluids or 5-10 mg of tissue. However, these amounts of sample are not always available. For example, in studies with small animals the available amounts of biofluids can be very limited. Another even more demanding drive behind miniaturization is the growing interest in differences in metabolite concentrations at the level of individual cells. In Chapter 7 the optimization and method performance of an in-liner derivatization method coupled on-line to a GC-MS system is described. The method was suitable for metabolic profiling in ultra-small sample volumes of 2 µL down to aliquots of 10 nL of serum sample. With the optimized method the first comprehensive GC-MS metabolite profiles could be obtained from an aliquot of intracellular content of a single *Xenopus laevis* cell.

In Chapter 8 general conclusions are drawn and future perspectives are given.