

Preface

Metabolomics is a rapidly growing and expanding subdiscipline in systems biology (Figure 1). The latter attempts to look at biological systems in a holistic manner and tries to analyze the entirety of complex molecular systems in a dynamically comprehensive way. The metabolome is downstream to the genome, transcriptome, and proteome and thus provides a great deal of information on the functional status of a cell or organism. Often it is assumed that the low molecular complement of genes and proteins is easier to analyse, and recent developments of powerful analytical techniques such as nuclear magnetic resonance spectroscopy (NMR) and, in particular, mass spectrometry (MS), hyphenated with separation technologies like liquid chromatography (LC) or gas chromatography (GC) have greatly contributed to the wide popularity of metabolomics technologies in analyzing biological systems. While NMR techniques are still preferentially used by chemists, MS, LC-MS, and GC-MS are more favoured for use by biologists as well. Developments, in particular, in MS technology are extremely rapid and continuous training is needed. Users of metabolomics techniques are seldom specialists in all methodologies and techniques that are needed or are helpful and utilized to address metabolomics questions. This may be related either to separation technologies, mass spectrometry, chemometric data processing or simply to the design of metabolomic studies. Some researchers are experts in a certain application field but are less interested in spending a lot of time to optimize analytical technologies. On the other hand, analytical chemists are sometimes not as familiar with biological background as it would be desirable. Thus, it may occur that one or the other mentioned aspect of significant relevance in metabolomics research is treated more like a black box due to lack of knowledge and user-friendly tools that are currently available. Critical issues may be overlooked and this may lead to suboptimal results. This book aims at presenting current methodologies from an analytical chemist's point of view, with the goal to raise the awareness for strengths, weaknesses, pitfalls, shortcomings, problems of methodologies, and solutions how to overcome them. Usually, methodological shortcomings and problems can only be recognized if suitable quality control measures are implemented in workflows. This book also attempts to draw emphasis on quality assurance and validation of analytical methods in metabolomics research. It should support the idea of implementing method validation as a tool to improve the quality and reliability of the produced

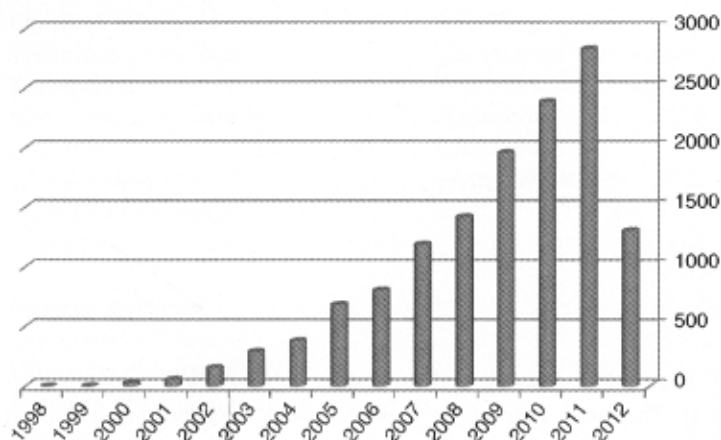


Figure 1 Gain in popularity of metabolomics research as measured by no. of publications with key word "metabolomics" in SciFinder database (as of May 2012).

data, and ascertain that conclusions from metabolomics data are significant. We hope that this book is of significant interest for readers who are new in this field but also for those performing metabolomics analyses for a while.

We have sub-structured the book in two parts. The first part deals with the most common methods utilized in metabolomics and targeted metabolite analysis. The second part focuses on the application of those methods in the major fields of metabolomics.

Inadequate sample preparation can destroy everything in metabolomics research and make results meaningless. To stress their importance, the book starts with a chapter on sampling and sample preparation in microbial metabolomics (Chapter 1). While this chapter specifically focuses on samples from microbial cultivations, it deals with important basic and general ideas that may be of relevance for other samples as well. It is clear that sampling and sample preparation has to be developed specifically for each type of sample, and that questions addressed are certainly different for plant metabolomics, microbial metabolomics, and red biotechnology. Specifics in these fields will be dealt with in the application part. Metabolomics research aims at measuring variances of substances over time, space or populations. The results should not be blurred by excessive analytical variances. It is well known that variances of individual steps in the entire analytical process are additive. Those of early steps in analytical process are usually much larger. This holds, in particular, for sampling and sample preparation. Hence, the importance of this step must not be underestimated and the first chapter emphasises this. The following chapters in the book are dedicated to targeted metabolomics (Chapter 2). This subdiscipline is often thought to be not a real metabolomics approach because only a preselected set of metabolites and not the entirety of the metabolome is analyzed comprehensively. However, we included it in this book because, on the one hand, issues and errors related to analytical methodologies can be better pinpointed by quantitative measurements, for example, ion suppression effects,

chemical, isomeric, and isotopic interferences, and so forth. On the other hand, at the end, accurate metabolite quantitation is the final goal of most metabolomics studies. To convey how errors propagate in individual steps to the total uncertainty in the entire metabolomics process, the concept of measurement uncertainty and of uncertainty budgets is presented in the next chapter (Chapter 3). Chapters dealing with methodological aspects of non-targeted metabolomics by GC-MS, GCxGC-MS and LC-MS/MS follow (Chapters 4–6). These techniques are the current workhorses in metabolomics research. Some of these chapters focus more on technological aspects and others on quality assurance issues of analytical measurements. In one chapter, methods employed in lipidomics, a subdiscipline of metabolomics, are described in more detail (Chapter 7). The lipidome is particularly complex and thus it was deemed justified to deal with this topic in more detail. Techniques less broadly used, such as capillary electrophoresis, hyphenated with MS and miniaturized technologies such as microfluidics (micro-metabolomics) as well as NMR based metabolomics follow in the next chapters (Chapters 8–10). All the above techniques, in particular those being utilized in untargeted profiling methods generate a huge body of data. It needs appropriate statistical tools such as multivariate analysis techniques to filter useful information from a noisy background. The most common statistical methods utilized in metabolomics are described in an illustrative manner in the next chapter (Chapter 11). Metabolism is a highly dynamic process with rapid turnover of metabolic intermediates. While most of the above described methods focus on analysis of metabolic samples where the metabolic state is frozen by quenching, metabolic flux analysis deals with the dynamic change of metabolism. Considerations of primary importance in “fluxomics” are described in a separate chapter (Chapter 12). There are certainly many more techniques of relevance in metabolomics research such as imaging methods but are beyond the primary scope of this book.

The second part of the book deals with applications. The application fields covered comprise plant metabolomics (green metabolomics) (Chapter 13), mammalian metabolomics (red metabolomics) (Chapter 14), and metabolomics in biotechnology and food science (white metabolomics) (Chapters 15 and 16). Peculiarities in different application fields are pointed out in these chapters including sample preparation aspects and design considerations of metabolomics experiments and studies. They should make users aware of certain problems, outline which methods are most appropriate and provide an insight to what can be achieved.

The book should give a state-of-the-art picture of the most important techniques written from the analytical chemist's point of view, thus focusing on methodological aspects and technical details, and last but not least quality assurance aspects. We hope that the book is useful and enjoyable to read for both beginners and experts in the field. We greatly appreciate the efforts of all contributing authors and would like to thank them for their excellent articles. Without their valuable contributions this book would not have been possible.

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