

Sample preparation for hyphenated analytical techniques

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Today, most modern chromatographic and electrophoretic techniques are increasingly combined with detection systems based on mass spectrometry (MS) or tandem MS. In principle, this combination ensures more reliable detection, identity confirmation and quantification of target analytes as well as the (provisional) identification of unknowns. However, even with such a powerful instrumental techniques, the risk of interference increases with the complexity of the matrix studied, and sample preparation before instrumental analysis is still mandatory in many application areas. The efforts made in the sample preparation field in the past 15 years have led to the adaptation of existing methods and the development of new techniques in order to save time and chemicals, improve the overall performance and, if possible, hyphenate the various steps of the analytical process. For obvious reasons, miniaturization has been a key factor in designing integrated analytical systems to provide higher sample throughput and/or unattended operation, and simultaneously solve the problems associated with the analysis of small size samples. However, these new achievements also contribute to enhance the versatility of the analytical procedures developed, and that occasionally leads to the successful application of closely related analytical approaches in different working areas, as this book shows. Because it is impossible for a single book to cover comprehensively all sample-treatment techniques currently in use, this volume focuses its attention on selected relevant aspects of sample preparation for hyphenated techniques in three working fields (i.e. the analysis of biological macromolecules, the determination of small molecules such as drugs and pollutants, and single cell and in vivo analyses). Most of the attention is devoted to the first topic, which represents almost half the total volume and is covered in chapters dealing with genomics, proteomics and glycomics.

The first of these chapters reviews the principles and the practice of genomic analysis in molecular genetic pathology (i.e. in the analysis of nucleic acids with the aim of diagnosing or managing disease), covering from basic methods of molecular biology to the most sophisticated dedicated robotic instruments used in this field. The second of these chapters describes the methods currently in use for sample preparation, chromatographic isolation and accurate identification and quantification of oxidatively modified nucleosides in DNA with MS-based techniques. Using a more practical approach, the feasibility of selected peptide chemical derivatization procedures to facilitate peptide mass fingerprints that allow identifying the membrane proteins of an aquatic bacterium, *Caulobacter crescentus*, is discussed in Chapter 4. The most commonly used methods for isolation, purification and derivatization of oligosaccharides from different sources are reviewed in Chapter 5. Interestingly, the last part of this chapter describes some sample treatment procedures for carbohydrate analysis based on the instrumental technique selected for final determination (i.e. either electrophoresis or liquid chromatography), instead of following the (somehow) more usual criterion of analyte class.

The second part of the book focuses on two analytical fields nowadays characterized by the pressing demands of high throughput, cost-effectiveness and strict regulatory requirements, drug discovery and environmental analysis. Chapter 6 is divided into three sections. The first reviews the analytical techniques used within the pharmaceutical industry to support high-throughput library synthesis, with special attention devoted to the efforts undertaken to develop generic analytical methods and to discussion of the advantages and shortcomings of the different hyphenated and instrumental techniques used in this context. The characterization of the pharmacokinetic and pharmacodynamic properties of the test compounds will follow these investigations. The most relevant analytical requirements to be considered in this part of a drug-discovery study are therefore discussed in the next section, in which both conventional and automated approaches used today for sample treatment are described and critically evaluated. The different approaches used to obtain metabolite information from in vitro and in vivo samples are reviewed in the third section of this chapter. Not unexpectedly, a number of the sample-preparation and hyphenated techniques reviewed in Chapter 6 are also evaluated in Chapter 7 for the determination of trace microcontaminants in environmental samples, a field in which there is increasing demand for faster, simpler and more economic analytical methods that represent a real alternative to the highly manipulative multi-step procedures still in use in many laboratories, while still fulfilling the sensitivity, selectivity, accuracy and robustness criteria of such determinations. The last two chapters of the book review the evolution of the sampling and instrumental techniques used, respectively, for the analysis of single cells and biologically relevant compounds, such as drugs and neurotransmitters, in living organisms, including humans. Special attention is dedicated to the most recent achievements in the field of miniaturization that allow sampling on the nano-scale and the pico-scale and the development of hyphenated sampling–analysis system for near real-time measurements in live animals. Ethical considerations and regulatory restrictions for these latter studies are also stated and there is a real discussion on future trends and perspectives, something missing from some of the previous chapters. Another striking feature of this book is that, of the references collected by the authors, 726 in total, most correspond to scientific contributions published within the last six years in well-known scientific journals. Thereby, although the scope of the chapters sometimes does not allow authors to write exhaustive review articles and/or go into much detail as regards the techniques reviewed, a number of references of a more specialized nature are mentioned for interested readers. It is also important to highlight the remarkably few typos and other mistakes found in the book.

This book represents a good starting point for readers who want to obtain a general overview of the main sample-preparation and instrumental techniques used in the selected application areas reviewed as well as of the many novel analytical developments achieved in these dynamic fields of research.

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