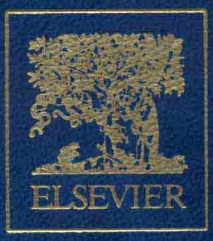

HANDBOOK OF ANALYTICAL SEPARATIONS

SERIES EDITOR: ROGER M. SMITH

VOLUME 4

BIOANALYTICAL SEPARATIONS

EDITED BY
IAN D. WILSON

HANDBOOK OF ANALYTICAL SEPARATIONS – VOLUME 4

Bioanalytical Separations

Edited by

IAN D. WILSON

AstraZeneca
Macclesfield, U.K.

2003

ELSEVIER
Amsterdam – Boston – Heidelberg – London – New York – Oxford – Paris
San Diego – San Francisco – Singapore – Sydney – Tokyo

ELSEVIER SCIENCE B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

First edition 2003

Library of Congress Cataloging in Publication Data
A catalog record from the Library of Congress has been applied for.

British Library Cataloguing in Publication Data
A catalogue record from the British Library has been applied for.

ISBN: 0-444-50658-6

♾ The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).
Printed in The Netherlands.

Preface

"Nothing tends so much as to the advancement of knowledge, as the application of a new instrument. The native intellectual powers of men in different times are not so much the causes of the different success of their labour, as the peculiar nature of the means and artificial resources in their possession"

Sir Humphrey Davy, 1840

In the context of this volume "bioanalysis" has a very specific meaning. It is the analysis of drugs and their metabolites in biological fluids. There is probably no field which has benefited from the introduction of new instrumentation and ways of analysing samples as much as this type of analysis. Today the laboratories of pharmaceutical companies, and the contract houses that serve them, are filled with sophisticated HPLC-MSMS systems devoted to the analysis of compounds at concentrations unachievable by previous generations of analysts. Thus, detection and quantification at concentrations below 1 nanogram per millilitre are now commonplace, and the pace of innovation seems still to be increasing. The bulk of these advances in bioanalysis have resulted from the development and implementation of robust and sensitive HPLC-MS interfaces. The high capital cost of much of this instrumentation has also led to increasing pressures on improving the efficiency of method development and instrument usage.

The application of these new HPLC-MS-based methods is covered in this volume in chapters on forensic bioanalysis and the role of this way of analysis in drug discovery. However, despite the success of such devices, all of the problems of bioanalysis have not been solved by their introduction, and there is a continuing need for sustained innovation. In particular the low concentrations, and the presence of large amounts of endogenous interferences in biological fluids and tissues, has meant that sample preparation techniques remain of prime importance to the bioanalyst seeking the highest sensitivities and specificity. This activity is reflected in several contributions on the theme of sample preparation. Chromatography is still important, even when the detector is as sensitive and specific as the mass spectrometer is claimed to be, and new phases of the types described in this volume, providing chiral separations or improvements in conventional chromatography, are always needed. In addition the linking of spectrometers other than mass spectrometers has been an important area of innovation, and this is reflected in contributions on HPLC-NMR and HPLC-ICPMS.

Finally, it has always to be remembered that, because of the importance of bioanalytical data in the generation of regulatory submissions, such work is subject to

very precise and challenging regulatory control. The needs of the regulators for bioanalytical separations are therefore also covered in the final contribution to the volume.

I would like to thank all of the authors who have contributed to this work for their time, patience and expertise. The collected wisdom and scholarship that these chapters reflect have been a source of considerable pleasure and education to me as I have put the volume together.

Ian D. Wilson
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CHAPTER 1

New developments in integrated sample preparation for bioanalysis

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1.1 INTRODUCTION

Increasing knowledge of the working mechanisms of drugs has led to the development of very potent drugs. Hence, the administered dosages are small, and consequently, the concentration levels in biological fluids are decreasing. Furthermore, biological samples are very complex, because they contain many endogenous substances. Blood fluids, such as serum and plasma, represent an extra problem due to the presence of proteins. Protein binding may affect the extractability of the analytes. Deproteinisation techniques can help to overcome this problem. It may, however, also give rise to even more difficulties, since analytes can be co-precipitated with the proteins. Thus, sample pretreatment techniques are required that retain the analyte(s) of interest, at the same time efficiently removing the endogenous interferences. The most common systems exist of an extraction step prior to separation and detection. A considerable gain in sensitivity and selectivity can be obtained during the extraction, as the analytes of interest are usually concentrated and separated from the matrix. An ideal extraction method should be rapid, simple, inexpensive, and give reproducible and high recoveries without the possibility of degradation of the analytes. Furthermore, the extraction method should not generate large amounts of chemical waste [1].

Sample pretreatment used to be a long step in the analysis of biological samples. Since the numbers of samples to be analysed is increasing, very rapid, but still selective and sensitive systems are required. In modern systems using advanced sample handling, the separation step may be more time-consuming. However, with the introduction of short columns in liquid chromatography (LC) and the selectivity of the mass spectrometer (MS), throughput of samples is again more and more limited by the time

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required for sample pretreatment. This is especially the case in off-line systems, which may also require extensive manual work. Therefore, various systems have been developed in order to integrate sample pretreatment with the separation and detection technique (Fig. 1.1) [2].

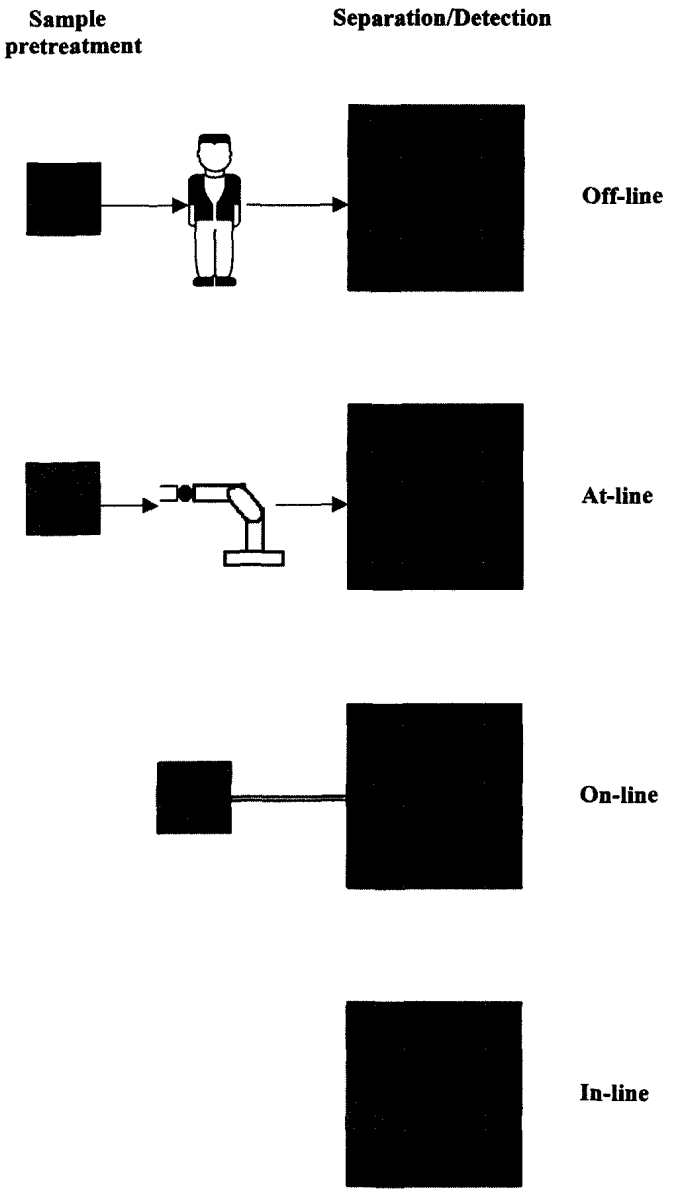

Fig. 1.1. Schematic presentation of various integration methods of the sample pretreatment step with the separation and detection technique. (Reprinted from [2, modified], with permission from Elsevier Science).

Basically, three possibilities have been proposed for integrated sample pretreatment in the analytical procedures, i.e. (1) at-line; (2) on-line; and (3) in-line. The at-line coupling involves sample preparation by a robotic device and an autoinjector to inject the extracts into the analytical instrument. No direct stream of liquid between extraction unit and analysing unit is present. Moreover, not the entire extract is transferred to the analysing instrument. Disadvantages as observed with off-line extractions, i.e. collection of the extract, evaporation and reconstitution, are not eliminated. An example of an at-line system is the 96-well plate design for solid-phase extraction (SPE). Samples can be extracted simultaneously, thus increasing the sample throughput, provided that the separation and detection can be performed very rapidly or by using simultaneous analytical instruments. With on-line systems, there is a direct transport of the entire extract to the analysing technique, and the latter is receiving the entire extract. Samples can be processed in series, i.e. samples are pretreated and analysed one after the other, or in parallel, in which one sample is being analysed while another is being extracted. The latter system offers a high sample throughput. A very prominent advantage of on-line systems is that some error-prone steps of the extraction procedure, such as evaporation and reconstitution are eliminated, hereby increasing precision and accuracy. In-line systems exist of sample pretreatment fully incorporated into the separation system, hereby creating a new device. In contrast to on-line procedures, application of in-line systems imply the direct injection of the sample into the analytical instruments. Various approaches for in-line SPE-capillary electrophoresis have been reported [2]. It should be noted that the differences in interfacing are often not as clear as mentioned above. For example, the extraction can be performed manually (off-line) or by robot (at-line), but the final step of the extraction, i.e. the desorption of the analytes may be performed on-line with the analytical step. Furthermore, dividing systems into on-line and in-line techniques is very disputable. These systems are usually closely related to each other and a distinct difference can often not be made. Therefore, in this chapter on-line and in-line systems will be considered as similar.

The goal of this chapter is to show the current status of modern sample pretreatment techniques such as SPE, solid-phase microextraction (SPME) and membrane-based extraction systems, and to outline novel trends in the bioanalytical area with regard to integrated sample preparation. It will focus not only on pretreatment techniques integrated with chromatographic separation systems, but also on their direct coupling to MS. SPE was originally designed for off-line purposes [3–5], but is now routinely used in on-line systems with LC [6–9]. The combination of SPE on-line with gas chromatography (GC) is less common, especially in the bioanalytical field. The current state of SPE–GC will be discussed here. Since an LC column can also be used as clean-up prior to GC analysis [10–13], on-line LC–GC applications without any further sample pretreatment will also be presented. Turbulent-flow chromatography (TFC) is to a certain extent similar to SPE. The use of high flow-rates offers new possibilities for sample pretreatment [14–17]. Therefore, the current state in TFC will be presented. SPME was originally designed for the analysis of volatile compounds with GC [18–22]. However, nowadays SPME is also coupled with LC for analysis of less-volatile compounds. The applicability of these SPME–LC systems in bioanalysis will be shown. Membrane-based techniques are, like SPME, diffusion-based sample pretreatment