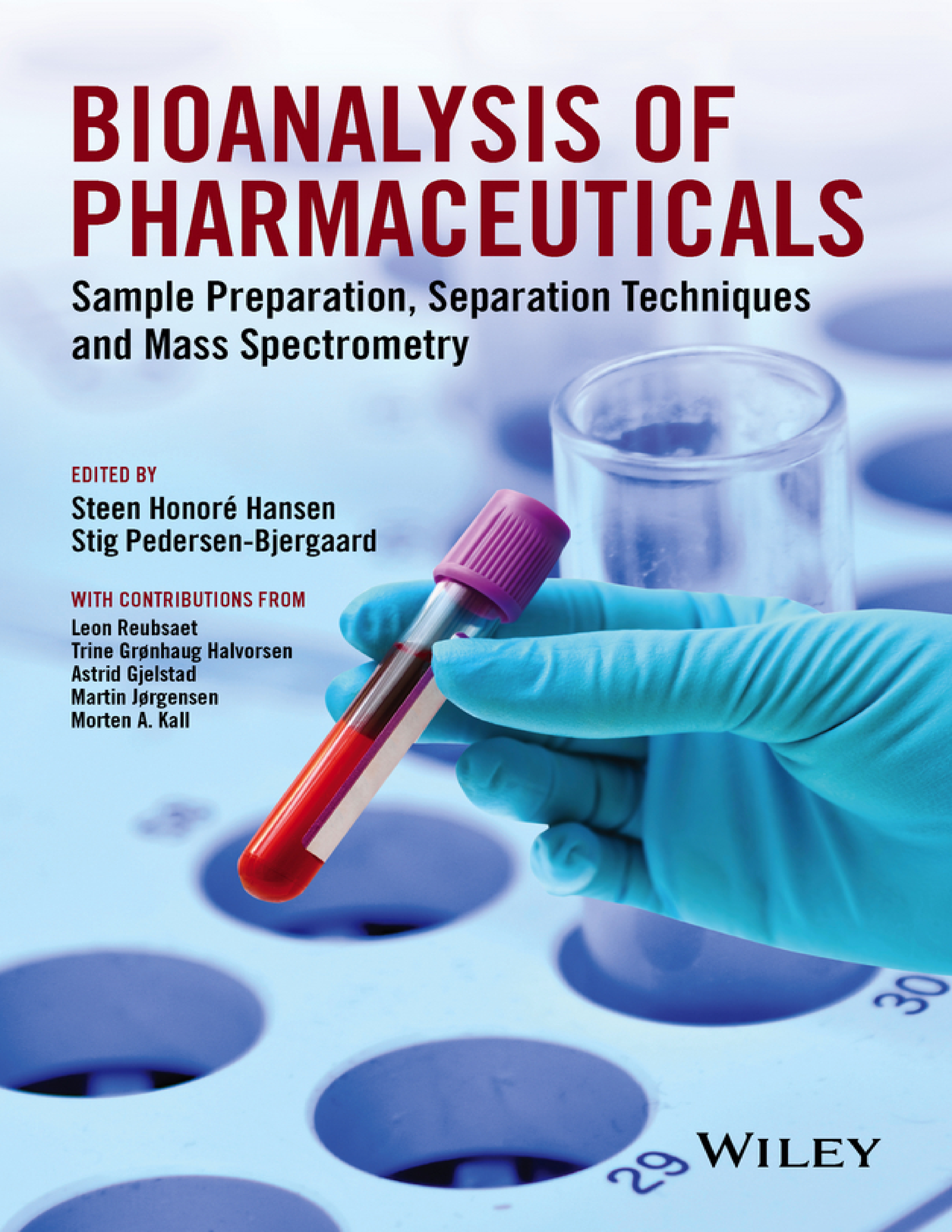

BIOANALYSIS OF PHARMACEUTICALS

Sample Preparation, Separation Techniques and Mass Spectrometry

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Table of Contents

List of Illustrations

List of Tables

Bioanalysis of Pharmaceuticals

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Chapter 11

Preface

The field of bioanalysis is very broad, complex, and challenging, and therefore writing an introductory textbook in this field is a difficult task. From our point of view, a good introductory student textbook is limited in the number of pages, discusses the different principles and concepts clearly and comprehensively, and contains many relevant and educational examples. Given these criteria, we have narrowed our focus on bioanalysis. First, we have limited our discussion to the chemical analysis of *pharmaceuticals* that are present in biological fluids. The focus is directed toward substances that are administered as human drugs, including low-molecular drug substances, peptides, and proteins. Endogenous substances are not discussed. Second, the discussion of different analytical methods has been limited to those based on *chromatography* and *mass spectrometry*. Certainly, different immunological methods are also used, but teaching all the principles and applications of chromatographic, mass spectrometric, and immunological methods was too ambitious to meet our criteria for a good introductory student textbook.

The present book is the first introductory student textbook on chromatography and mass spectrometry of pharmaceuticals present in biological fluids, highlighting an educational presentation of the principles, concepts, and applications. We discuss the chemical structures and properties of low- and high-molecular pharmaceuticals, the different types of biological samples and fluids that are used, how to prepare the samples by extraction, and how to perform the final analytical measurement by use of chromatography and mass spectrometry. Many examples illustrate the theory and applications, and the examples discuss all practical aspects, including the calculations. Thus, in this textbook, you will even learn how to convert the numbers recorded by the instrument to the concentration of the actual drug substances in the biological sample.

Bioanalysis is an applied scientific discipline, and this represents another challenge in terms of writing an introductory student textbook. University professors are well trained in teaching the basic principles. However, bioanalysis is mainly performed outside the university by researchers in the pharmaceutical industry, in contract laboratories, and in hospital laboratories. Thus, the researchers outside the university have the best overview of the most important applications and techniques in practical use. To address this, both university professors and researchers from the pharmaceutical industry have authored this textbook. Hopefully, this has resulted in a textbook that reflects bioanalysis in the year 2015. The authors have been in close contact with colleagues for advice, and we would especially like to thank Elisabeth Leere Øiestad for fruitful discussions.

The present textbook is intended for the fourth- or fifth-year university pharmacy or chemistry student. Reading the textbook requires basic knowledge in organic chemistry and biochemistry, as well as in analytical chemistry. With respect to the latter, we have given priority to discuss the analytical techniques in a fundamental and educational frame, and detailed knowledge on instrumental analytical methods is not required prior to reading this textbook.

Good luck with the reading!

Oslo and Copenhagen, June 2014
Steen Honoré Hansen, Stig Pedersen-Bjergaard, Leon Reubsaet, Astrid Gjelstad,

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Chapter 1
Introduction

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Welcome to the field of bioanalysis! Through reading of this textbook, we hope you get fascinated by the world of bioanalysis, and also we hope that you learn to understand that bioanalysis is a highly important scientific discipline. In this chapter, five fundamental questions are raised and briefly discussed as an introduction to the textbook: (i) What is bioanalysis? (ii) What is the purpose of bioanalysis? (iii) Where is bioanalysis conducted? (iv) Why do you need theoretical understanding and skills in bioanalysis? And (v) how do you gain the understanding and the skills from reading this textbook?

1.1 What Is Bioanalysis?

In this textbook, we define *bioanalysis* as the chemical analysis of pharmaceutical substances in biological samples. The purpose of the chemical analysis is normally both to *identify* (identification) and to *quantify* (quantification) the pharmaceutical substance of interest in a given biological sample. This is performed by a *bioanalytical chemist* (scientist) using a *bioanalytical method*. The pharmaceutical substance of interest is often termed the *analyte*, and this term will be used throughout the textbook. Identification of the analyte implies that the exact chemical identity of the analyte is established unequivocally. Quantification of the analyte implies that the concentration of the analyte in the biological sample is measured. It is important to emphasize that quantification is associated with small inaccuracies, and the result is prone to errors. Thus, the quantitative data should be considered as an estimate of the true concentration. Based on theoretical and practical skills, and based on careful optimization and testing of the bioanalytical methods, the bioanalytical chemist tries to reduce the error level, providing concentration estimates that are very close to the true values.

Bioanalytical data are highly important in many aspects. As an example, a patient serum sample is analyzed for the antibiotic drug substance gentamicin, and gentamicin is measured in the sample at a concentration of 5 µg/ml. First, the identification of gentamicin in the blood serum sample confirms that the patient has taken the drug. This is important information because not all patients actually comply with the prescribed medication. Second, the exact concentration of gentamicin measured in the blood serum sample confirms that the amount of gentamicin taken is appropriate, as the recommended concentration level should be in the range of 4–10 µg/ml. For aminoglycoside antibiotics such as gentamicin, it is recommended to monitor the concentration in blood if the treatment is expected to continue for more than 72 hours as these antibiotics have the potential to cause severe adverse reactions, such as nephrotoxicity and ototoxicity.

As will be discussed in much more detail in this book, not only blood serum samples are used for bioanalysis. Bioanalysis can be performed on raw blood samples (whole blood) or on blood samples from which the blood cells have been removed (serum or plasma). Alternatively, bioanalysis can be performed from urine or saliva as examples, depending on the purpose of the bioanalysis. Bioanalysis is