A HANDBOOK OF BIOANALYSIS AND DRUG METABOLISM

EDITED BY

Gary Evans

CRC PRESS

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Contents

Editor's preface

Originally this book was to be called 'Grieves Harnby's Guide to Bioanalysis and Drug Metabolism', as the project conceived by David Scales was going to be produced by Grieves. Unfortunately Grieves died following a heart attack shortly after he retired. We decided to complete this book and dedicate it to his memory. Grieves had worked in Research and Development in Glaxo in all its various guises from 1961 until he retired in 1996. The majority of that time he was involved in the formation of a new discipline – Drug Metabolism arising out of the regulatory changes introduced in the late 1960s. I was recruited by Grieves in 1979 and worked with him for the next 18 years. Each chapter in this book was written as an individual contribution by one or more authors, all of whom worked at Glaxo-Wellcome at the time of writing their chapters. It was decided to allow each chapter stand alone. Each chapter was the responsibility of the contributing authors. All of the chapters compliment each other and present a comprehensive picture of the breadth of functions and activities which are included in Bioanalysis, Pharmacokinetics and Drug Metabolism.

Gary Evans

Preface

I came to drug metabolism and bioanalysis rather late in life when, in 1995, I was appointed VP of the Division in GlaxoWellcome. It was the time of the merger between two great companies and I had the unenviable task of making many fine people redundant. I remember a lunch meeting with Grieves Harnby, at the beginning of, what was euphemistically called, the integration process. With only two years before retirement Grieves was convinced he would be a casualty of the reorganisation. I however wanted him to stay, for it gave me the chance to work with one of the founding fathers of industrial drug metabolism. When I told him of his promotion to position of International Director, he nearly choked on his sandwich. This was the only time I ever saw Grieves lost for the right words.

Grieves acted as a mentor and guide to many. I was fortunate that he was mine, if only for two brief years. His task was to convert me from a toxicologist into what he called 'a real scientist'. The new company had many outstanding individuals who devoted time and effort, under the watchful eye of Grieves, in getting me up to speed. It was from this teaching programme that the idea for a book grew. It was envisaged that it would cover the necessary information to work in a pharmaceutical drug metabolism and bioanalysis function. Staff from the company based in the USA, Italy and the UK agreed to contribute. Grieves would work on the project, post-retirement, on those few rare occasions when he was not improving his golf handicap.

To our great sadness, Grieves died shortly after retiring. It is a tribute to the man that his loss created a void in so many lives. His friends and colleagues still toast his memory and mourn his passing. Mike Tarbit, who knew him for many years, has captured, in an anecdotal reminiscence given below, some of Grieves' humour and humanity.

The book was still very much at a preliminary stage when we lost our Editor-in-Chief. We decided, however, to continue with the project. Gary Evans took over the herculean task of acting as editor, as well as cajoling the contributors to finish their chapters. It is a great credit to Gary and all the authors that, despite yet another merger, the book has finally been completed. It is, of course, dedicated to Grieves Harnby.

David Scales
Henley-on-Thames
England

Grieves Harnby: In memoriam

I had the pleasure and privilege to know Grieves for nearly twenty years before his untimely death. He was a rare person: a true, loyal, humorous, but candid, friend. Humour, wit and sagacity oozed from Grieves at all times. 'Candid' meant, with Grieves, that he would always tell you what he really thought with characteristic northern bluntness, whether it was what you wanted to hear, or not! He could kill with a sentence! If you have the courage to treasure such friendship, it always serves you well, and Grieves gave me immeasurable guidance and counsel over the years of our shared time in GlaxoWellcome.

Grieves had an almost uncanny and yet completely natural gift in communicating with people, and anecdotes abound about his ability to get on with strangers in no time at all. Thus, for example, he was the only person in my experience, who could have made such an impression with the normally 'detached' and 'seen it all before' air crew on one 40 minute USAir flight that the air hostess put her arm round him and kissed him goodbye as he was leaving the flight! No concern over sexual harassment there! Indeed he specialised in 'melting' air crew, and perhaps the most famous example of this was when the normal USAir/British Airways connection from our North Carolina laboratories to Heathrow, via Philadelphia, went badly awry due to storms, and we were stranded in Philadelphia. Much to Grieves' obvious delight, we were driven to New York by British Airways and brought home on Concord. Soon after take-off, a fairly formal and slightly upper crust 'Concord class' lady Purser arrived with a wine carrier laden with vintage Dom Perignon, Chateau Beychevelle St Julien and an excellent Mersault. The basket was offered to Grieves, not someone normally viewed as a wine connoisseur, with a svelte "And which wine would you like Sir?" "All of them, Pet!" was his reply, delivered with such a twinkle that three glasses were instantly placed on our tables by a giggling Purser, along with three bottles of wine! After that we owned the plane! Grieves felt that he had enjoyed the experience of a lifetime on that flight.

Mike Tarbit

GRIEVES HARNBY GLAXO: 1961–1996, Drug Metabolism

CHAPTER 1

Introduction

Gary Evans

1.1 *Bioanalysis, pharmacokinetics and drug metabolism (BPDM)*

The prime function of the Research and Development subsidiaries of pharmaceutical companies is to discover and develop new medicines. Achieving these objectives is not easy, only a small percentage of the chemical compounds synthesised become medicines, most compounds prove unsuitable for reasons of efficacy, potency or toxicity.

Both the discovery and development phases are time-consuming processes which take between five and ten years to complete. They involve scientists from many different disciplines, working together, to identify disease targets and, to discover suitable chemical entities which have the appropriate biological, chemical and pharmacological/toxicological properties to be quality medicines.

When the potential drug is selected for development, extensive safety and clinical studies are conducted to provide sufficient data for a regulatory submission for registration of a new medicine.

One group of scientists whose contribution is particularly important in both drug discovery and drug development are those working in the discipline which may be described as bioanalysis, pharmacokinetics and drug metabolism (BPDM). Whilst other names have been used to describe this discipline, it has broad range of activities which seek the same objectives – to understand what happens to the drug after it is administered and to determine what implications a knowledge of the fate of the dosed drug has for either improving the drug or for dosage regimens and safety.

This discipline consists of three main areas which are closely related. Bioanalysis is a term generally used to describe the quantitative measurement of a compound (drug) in biological fluids primarily blood, plasma, serum, urine or tissue extracts.

Pharmacokinetics is the technique used to analyse these data and to define a number of parameters which describe the absorption, distribution, clearance (including metabolism) and excretion (often referred by the acronym ADME). The ability to monitor the presence of the drug in the body and to measure its removal is critical in understanding the safety, dosage and efficacy of any medicine.

Drug metabolism is the study of the metabolism of a drug. It can be used to discover the nature and route of metabolism of the drug and the information permits predictions to be made concerning the potential for interactions with co-administered drugs through knowledge of the enzymes involved in the metabolism of drugs. Increasingly predictions can be made about the metabolism of a putative drug using the knowledge base from existing drugs. These three areas have developed together because of the use of common techniques and knowledge. The scientists conducting these functions may be geographically separated or, increasingly, scientists may specialise in one of the functional areas. However, the functions are closely inter-related and may be considered a single scientific discipline. In this book this discipline will be referred to as BPDM.

1.2 *The role of BPDM in drug discovery and drug development*

The roles and techniques of BPDM used in supporting drug discovery and development are similar but the information is required for different purposes, hence the priorities and approaches differ. Information is used for decision-making in both phases; however, the nature of the decisions affects the quality and quantity of information required.

For discovery, the priority is to examine a large number of compounds and determine which pharmacologically active compounds are most suitable for drug development. In practice when a compound is obtained which has the required biological activity, a number of analogues or chemically similar compounds will be synthesised and tested to optimise the preferred characteristics of the compound (a process is known as lead optimisation). Figure 1.1 shows an illustration of a possible scenario in discovering drugs which are active *in vitro* and improving these by modification of the chemical structure optimised for *in vivo* activity.

In drug development, a single compound is progressed and information relating to the safety of the drug and the dosage required for efficacy in man is obtained. Figure 1.2 shows the studies which are conducted on a drug under development – the exact experimental design and priorities will depend on the particular drug under development.

There is a significant overlap in the techniques and methodology used in BPDM for drug discovery and drug development and often the difference is in the experimental

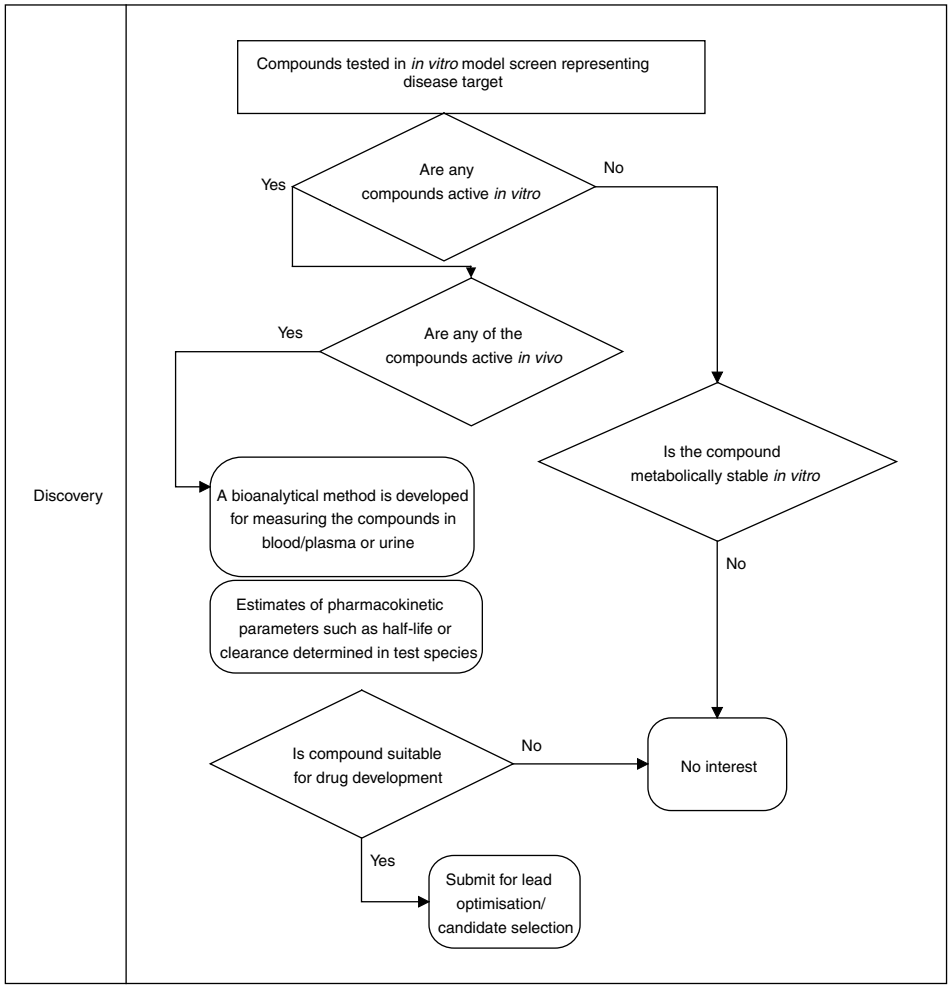

FIGURE 1.1 *Process diagram representing some of the BPDM steps in drug discovery.*

design. Consequently, the chapters in this book have been organised on the basis of functional topics and where there are different approaches for discovery and development these are discussed in each chapter.

Physiochemical properties of compounds are also an important consideration in drug design as they will effect absorption and clearance. They will also be of concern in the development of an analytical method or determining a suitable drug formulation. These aspects are discussed in detail in Chapter 2. A sensitive and specific bioanalytical method is developed to allow the monitoring of drug levels in plasma (systemic circulating levels) and urine (excreted levels) in clinical studies. The assay is also used to monitor the levels of exposure in pre-clinical safety studies. Whilst the analytical methodology used in discovery and development will require

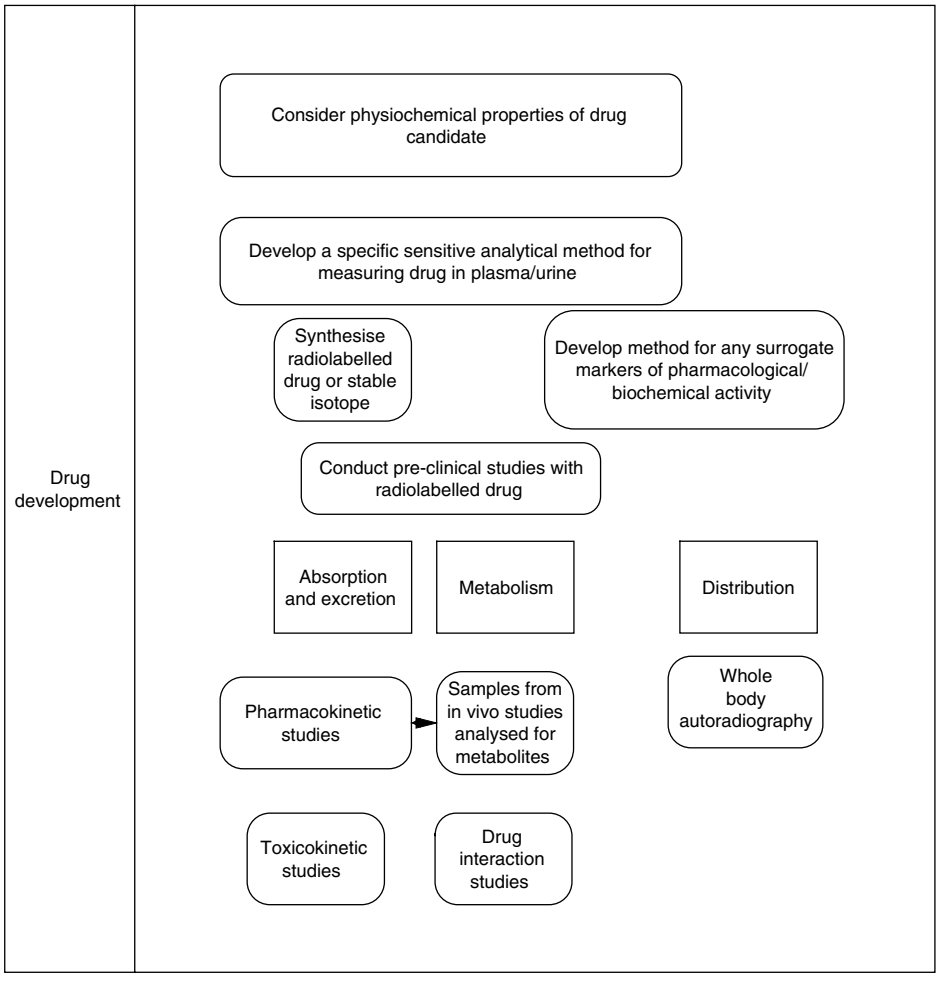

FIGURE 1.2 *Process diagram representing some of the BPDM steps in drug development.*

different levels of sensitivity and validation the basic aspects remain the same. A bioanalytical method consists of two main components:

1 Sample preparation – extraction of the drug from the biological fluid usually including a concentration step to enhance sensitivity of the method; and
2 Detection of the compound – usually following chromatographic separation from other components present in the biological extract. The detector of choice is a mass spectrometer.

These issues are discussed in Chapter 3 (Sample preparation), Chapter 4 (Chromatographic separation: HPLC) and Chapter 5 (Quantitative mass spectrometry). Whilst traditionally other chromatographic techniques have been used, the method of