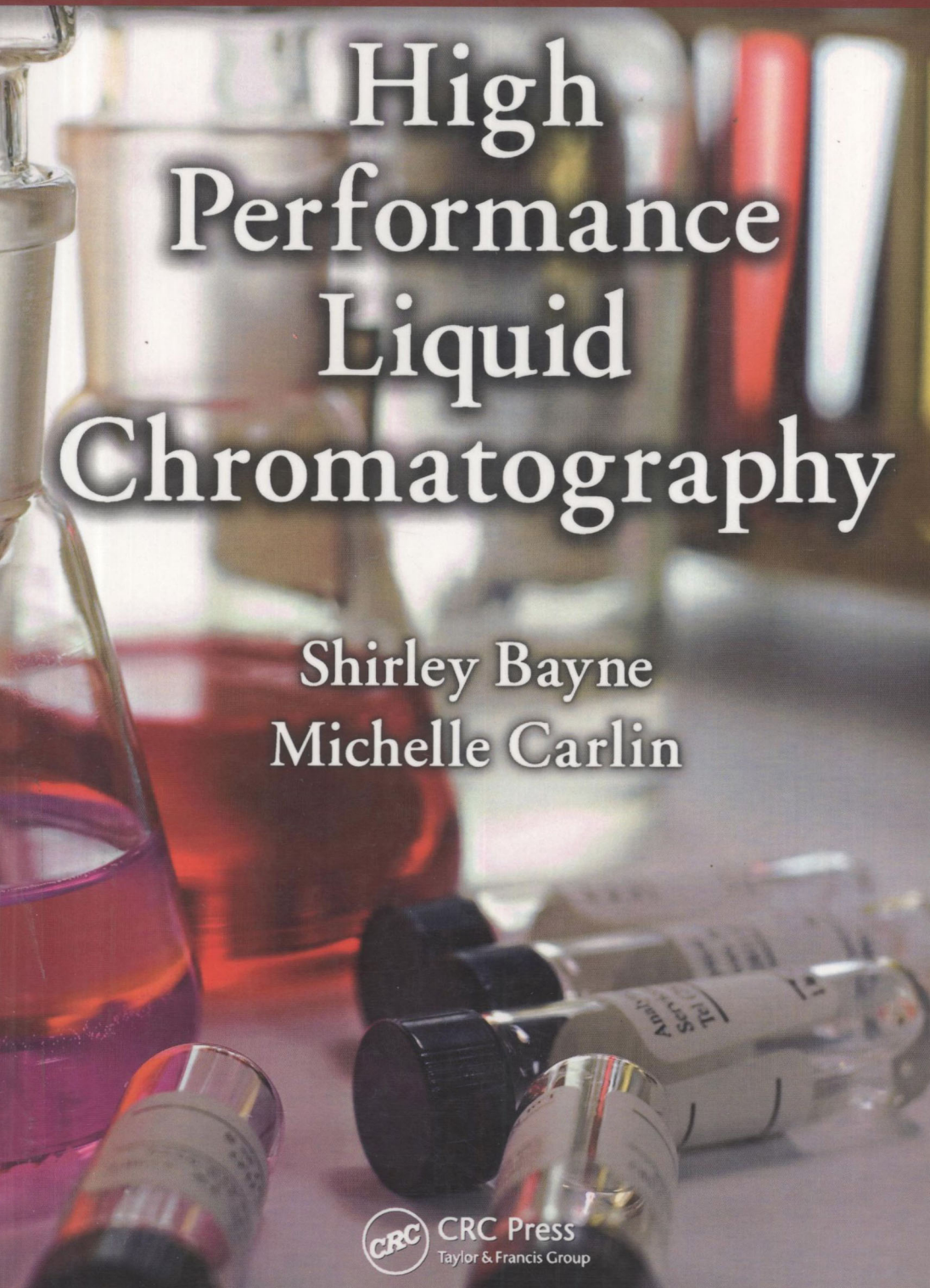
Forensic Applications of High Performance Liquid Chromatography



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Preface

This book has been written for university students studying analytical chemistry, applied chemistry, forensic chemistry, or other such courses where there is an element of HPLC within the course curriculum. The aim of the book is to explain HPLC from a forensic science perspective, and many of the examples used here are associated with real-life samples that might be expected within a forensic science laboratory. We have tried to maintain a balance between practical solutions and the theoretical considerations involved in HPLC analysis. The book takes the reader on a journey through the world of HPLC; it is suitable for first-time users as well as those pursuing postgraduate study or in the early stages of their forensic analysis careers.

Many of the applications within forensic science adopt a reverse phase (RP) HPLC mode of separation in relation to analyses. We have chosen to use example applications of HPLC based on this particular mode of chromatography because it is the most frequently used. We have broken each chapter down into theoretical considerations with examples where appropriate, a key point summary, a series of questions where appropriate, and, finally, a list of books and journal articles that we believe will give further, thorough insight into each of the topics covered. We have attempted to keep the book as succinct as possible whilst still providing sufficient information to allow the reader to develop his or her knowledge at an effective pace.

In Chapter 1, we have included a brief history of HPLC because we believe that it is important to understand just how far the technique has advanced in its relatively short lifetime. We hope that this will inspire others to be innovative and explore a continually advancing field of study with huge opportunities. We have provided but a snapshot in time.

In Chapter 2, we move on to the theory behind the separation process. Before any judgement on an analysis can be made, it is necessary first to understand a little bit about the processes taking place. It is only by doing this that one can begin to understand when things have gone wrong and can then put them right. Forensic science requires a rigorous approach to the analysis of evidence, and the consequences of an error are far reaching. The basic chromatographic processes are discussed along with the chemistry theory that underpins this.

In Chapter 3, we concentrate on some of the basic requirements that will help to ensure a successful analysis, such as sample preparation and mobile phase preparation. We explore some of the limitations associated with these practical elements of HPLC analysis and provide information relating to current best practice.

In Chapter 4, we look at the different modes of separation that can be used in forensic science. We acknowledge that other separation chemistries do exist; however, these are beyond the scope of this primer. We have chosen to focus on reverse phase, normal phase, and ion exchange chromatography because these cover most of the mainstream applications.

In Chapter 5, we look at modes of detection, again examining those that we feel are best suited to forensic applications.

In Chapter 6, we move into the world of HPLC method development and have chosen to focus on RP-HPLC for the reasons given earlier. This chapter allows us to apply the theory from preceding chapters to more practical elements of HPLC analysis. It also allows us to explore in greater detail some of the many factors that need to be considered and the consequences of the different aspects within this field of study.

In Chapters 7 and 8, we look at how we ensure that our methodology is going to give us a true representation of what is present in the samples that we are analysing. This requires that the operator have knowledge of the other systems that are in place to support the HPLC analysis, such as the processes of validation, qualification, and estimations of error.

Chapter 9 covers the quality aspects of laboratory operation in general, and much of this theory can be applied to any analytical method. Most laboratories will operate with at least some of the elements discussed in this chapter.

Chapter 10 deals with troubleshooting HPLC systems and analyses. It is of fundamental importance that any analyst reporting results to the prosecution services in connection with an alleged offence be 100% sure of the validity of his or her analytical data. Ensuring that this happens means knowing when something is not right and being able to put it right. In this chapter, we highlight a series of common problems that can be encountered in HPLC and provide a number of possible solutions at each stage.

Chapter 11 looks at some of the applications of HPLC within the field of forensic science in greater detail. We have covered the most common areas, but we acknowledge that both HPLC and forensic science are far-reaching fields of study.

We hope that the book remains a companion throughout the reader's studies and we wish our readers well in their careers.

We would like to thank our families and friends, who have provided vision and commitment and been supportive throughout the writing process. We would also like to thank the following people for their valuable time and expertise during the review process; their insightful comments were much appreciated: Dr Joseph McGinnis, Mrs Helen Hodgson, Mr Douglas McLellan, Mr Ed Ludkin, and Mr Stephen Sole. Our project student, Mr Olivier Weiss, is thanked for his valuable contribution. Lastly, we would like to give special thanks to Dr Liam O'Hare for his contribution, skills, and knowledge.

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The Authors

Shirley Bayne studied applied chemistry in Newcastle upon Tyne and graduated with an honours degree. She started her career with the Medicines Testing Laboratory (MTL; part of the Royal Pharmaceutical Society of Great Britain) in Edinburgh, where she was given the opportunity to use a variety of different analytical techniques. Her responsibility for aspects of HPLC systems within the lab sparked her interest in this technique in particular. On leaving MTL, Shirley joined Lothian and Border Police Forensic Science Laboratory as a reporting analyst in the chemistry and drugs department. After a short spell leading the chemistry team, Shirley changed police forces and continued her forensic science career.

In 1998, Shirley joined the contract research company Quintiles Ltd. in its Pharmaceutical Method Development Department. She supervised a team responsible for the development and validation of HPLC assays for new drug entities. A number of years later, Shirley moved on to join BioReliance Ltd as analytical services manager. Part of her role involved leading a team of analysts responsible for the method development, validation, and transfer of release testing methods for biopharmaceutical products. In 2005, Shirley moved into academia at Teesside University as a senior lecturer and course leader in forensic science.

Michelle Carlin studied at Heriot-Watt University on the honours programme in colour chemistry, with a spell in a dyehouse in the Scottish Borders before embarking on a career in analytical chemistry. After some time spent in a contract research organisation in Edinburgh, Michelle went on to continue her education with an MSc in forensic science from Strathclyde University. She carried out a research project in the toxicology department of the Institut de Recherche Criminelle de la Gendarmerie Nationale (IRCGN) in Paris, using LC–ESI–MS.

After this, Michelle became the manager of a workplace drug testing laboratory in northeastern England before taking up a teaching position as lecturer in forensic science at Teesside University for 3 years. In 2009, she moved to Northumbria University as a junior lecturer in forensic chemistry, where she carries out research in analytical toxicology.

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Introduction to HPLC 1

Introduction

High performance liquid chromatography is the topic of this primer, but the starting point for this goes much further back than modern times. Chromatography is a technique that has a number of forms, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas liquid chromatography (GLC, although more commonly known as GC). Each of these forms of chromatography has a variety of uses in the analytical sciences.

In forensic science, chromatography is used in the analysis of drugs of abuse, toxicology, fire debris analysis, environmental analysis, and explosives analysis, to name but a few. To understand each of the chromatographic techniques, especially HPLC as the topic of this primer, it is necessary first to explain what chromatography is and the basic principles of chromatography.

The History of Chromatography

Chromatography seems to have been around for a long time in its current state, so it is difficult to believe that the first reported work naming and using chromatography was just over 100 years ago. Mikhail Semenovich Tswett (1872–1919) was born in a small town in Italy but grew up in Switzerland with his father. In later life, he went on to study botany at university in Geneva and, by 1896, he had completed the work and write-up for his doctoral thesis, for which he carried out work on the structure of the plant cell, chloroplasts, and the movement of protoplasm.

In 1896, he moved to Russia to join his father, but Tswett had difficulty finding the academic position for which he had wished. He accepted a temporary position in a laboratory and completed work for a magisters degree in order to gain the qualification to apply for academic positions in Russia. During this research, he started building the foundations to develop chromatography as a technique.

Tswett's research involved the isolation of chlorophyll from plant material. Whilst carrying out his work, he found a difference in polar and non-polar solvents as to how well the isolation occurred. He concluded that this