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Book review

High Performance Liquid Chromatography: Principles and Methods in Biotechnology
Edited by E.D. Katz, 1996; John Wiley & Sons, Chichester, New York, Brisbane. X+522 pp.; price. £50.00 0-471-93444-5

The target audience for this book is practicing scientists in the biotechnology field who are not HPLC experts, but who are currently using or planning to use HPLC. The book discusses both theoretical and practical aspects of analytical and preparative HPLC and also includes a chapter on alternative, principally electrophoretic, separations. Chapter 1 discusses the role of HPLC in biotechnology and includes a brief introduction to the structural features of biomolecules. The next three chapters discuss the principles of HPLC separations, column selection and detection/identification approaches, respectively. Sample preparation techniques are discussed in Chapter 5 and preparative HPLC in Chapter 6. Additional chapters related to HPLC include discussions of nucleic acid analysis, analysis of the carbohydrate portion of glycoproteins and applications to proteins. The concluding chapter discusses alternative separation techniques, including various forms of electrophoresis as well as ultracentrifugation.

This book covers a wide range of topics and provides some useful information. Chapter 2 provides a particularly thorough description of the chromatographic separation process and the historical development of HPLC. Unfortunately this chapter misses the opportunity to bridge the discussion between chromatographic theory and practical aspects of the separation of biomolecules. Only a single paragraph on biomolecules, discussing the steep elution isotherm observed for proteins in reversed-phase HPLC is provided. It is stated that the fundamental reasons for the steep elution isotherm are not fully known, but the possible explanation provided, shrinking and swelling of polystyrene upon changes in solvent composition, cannot be valid, since this phenomenon is also observed for rigid stationary phases such as silica gel. The discussion of the Van Deemter equation, while thorough, does not include a discussion of the relative importance of the various terms for macromolecules, in contrast to small molecule separations. Such a discussion would have been quite useful to the target audience.

The discussion and tabulations of available column types provided in Chapter 3 is of considerable value to the reader, since column suppliers don't always provide sufficient information on column characteristics. Detection of biomolecules is thoroughly discussed in Chapter 4, and although disproportionate emphasis is placed on electrochemical and LALLS (low angle laser light scattering) detection (in contrast to the frequency of use), the information provided serves the reader extremely well. Chapters 5 and 6 provide a valuable discussion of practical aspects of sample preparation and preparative HPLC, respectively. While it is virtually impossible to address every potential need that the target audience may have in this area, the information provided in these chapters is adequate in most respects and references are provided to get the reader started in the right direction. One major omission in the sample preparation discussion is the frequent use of reduction and alkylation (or mixed disulfide formation) as a sample preparation step. This omission is probably due to the overemphasis placed on retaining biological activity during sample

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preparation. In practice, retention of biological activity is most frequently not a requirement since sample preparation is most commonly used for analytical characterization methods and even in the case of preparative applications, mechanisms for reversible inactivation, such as sulfitolysis, are available in many instances.

Chapter 7 discusses nucleic acid HPLC, with particular emphasis on the role of HPLC in separating products of the polymerase chain reaction (PCR). In view of the recent widespread use of PCR this emphasis is appropriate. Chapter 8 discusses one aspect of carbohydrate separations, namely the use of high pH anion-exchange chromatography (HPAE) with electrochemical detection for the determination of monosaccharides. This technique is now widely used, in conjunction with appropriate sample preparation techniques, for the determination of the carbohydrate composition of glycoproteins. The discussion is well written and covers the topic is good detail. Chapter 9 provides some additional information concerning applications of HPLC for protein analysis. While the information provided in this chapter is useful, it would be more effective if the information was aligned with the information provided in Chapter 2 (Principles of HPLC). Some of the separation theory provided in Chapter 2 was repeated in Chapter 9, which may tend to confuse the reader, and at a minimum makes the two chapters unduly long and less effective.

The last chapter is a useful discussion of electrophoretic separations, but does fit very well with the rest of the book. This chapter also illustrates a significant problem with the book as a whole, i.e., the inclusion of a few factually incorrect statements. On p. 471 the following statement appears: "In fact, in the case of human growth hormone (hGH), a protein which is able to stimulate protein synthesis, the dose administered to athletes is 40 ng/kg each day". hGH is not currently approved for use in athletes and the basis for this statement is not given. The author then goes on to say "... in the case of recombinant proteins of therapeutic interest, a purity grade of 99.9% is not adequate. In such cases it is absolutely necessary to have the protein required in a pure form without any contamination". These statements are not factual, since therapeutic proteins generally have purities of less than 99.9% and are used very effectively in a wide variety of clinical applications. A similarly incorrect statement occurs in another chapter, on p. 4, wherein the author states "Proteins always are associated with at least one sugar molecule".

Perhaps the most important application of HPLC in biotechnology is in peptide mapping. Curiously, this book does not provide much information on this subject. Another less significant deficiency is the lack of discussion on alternative carbohydrate separation/analysis approaches (e.g. the use of derivatization and RP-HPLC).

Overall this book includes some significant and useful information and, provided the reader is aware of the deficiencies cited in this review, the book may be helpful to the target audience.

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