High Performance Liquid Chromatography in Enzymatic Analysis

By E.F. Rossomando

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The methods used for assaying enzyme activity will depend on the nature of the chemical change and the ingenuity of the investigator. Biochemists have never been tardy in adopting new techniques and it is not surprising that HPLC is increasingly being employed for enzyme assay. This book is an attempt to introduce HPLC to those as yet unfamiliar with the technique.

HPLC is essentially a discontinuous method of assay in that the reaction must be stopped before the assay is carried out. In the most commonly used discontinuous method, radiochemical assay, labelled substrate and product must be separated prior to radiochemical detection. HPLC offers an important advantage in that the separation is actually part of the assay procedure. Another advantage is that HPLC can detect several or all of the reactive components of the assay simultaneously. These features and others are discussed in the first chapter which also outlines the nature and scope of HPLC and the criteria for selection of assay methods to help decide whether HPLC is a method of choice in a particular application. Details such as choice of eluting solvent and problems introduced by various termination methods are also considered here.

Chapter 2 introduces the components of an HPLC system. Useful practical hints are given: interpretation of chromatograms, factors governing choice of column type, methods for monitoring and maintaining column performance; right down to the prosaic level, e.g. 'the perils of ferrules'. There is much of value here but also some puzzling omissions. The importance of, and methods for avoiding one of the banes of the chromatographer's life, i.e. air bubbles, hardly receive a mention, nor the desirability of membrane filtration of samples.

Chapter 3 describes strategies to be used in designing HPLC assays and the effects of assay components on HPLC separation. The utility of HPLC in detecting secondary reactions and how to interpret such results is well described. Various methods for reaction termination are given but one of the most useful, ethanol or methanol addition, is not mentioned. There is some confusion regarding the meaning of K_m, i.e. a low K_m is confused with low reactivity.

The next chapter is mostly concerned with enzyme isolation. It is hard to see what this has to do with HPLC and indeed this is a very superficial treatment of a subject which is well covered elsewhere. The space taken by this chapter would have been better used, e.g. to present tables of commercially available columns and their applications.

The final chapters are devoted to examples of HPLC assay of individual enzymes and multienzyme systems. Many reaction types are covered but again there are some important omissions, notably those reactions involving sugar phosphates and carboxylic acids. The author has grouped the enzymes according to the nature of the assay component detected in the HPLC. This has some justification but also results in some curious classifications e.g. creatine kinase as an enzyme of purine metabolism.

A biochemist new to HPLC will learn much from this book, though at its price not enough to justify personal purchase. Its place is on the reference shelves alongside other works on techniques. Its informal but informative style probably results from the fact that much of the material is based on the author's own work. The value of the text is enhanced by an abundance of sample chromatograms. As a primer, it is excellent, and the general references at the end of each chapter will fill the gaps.

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