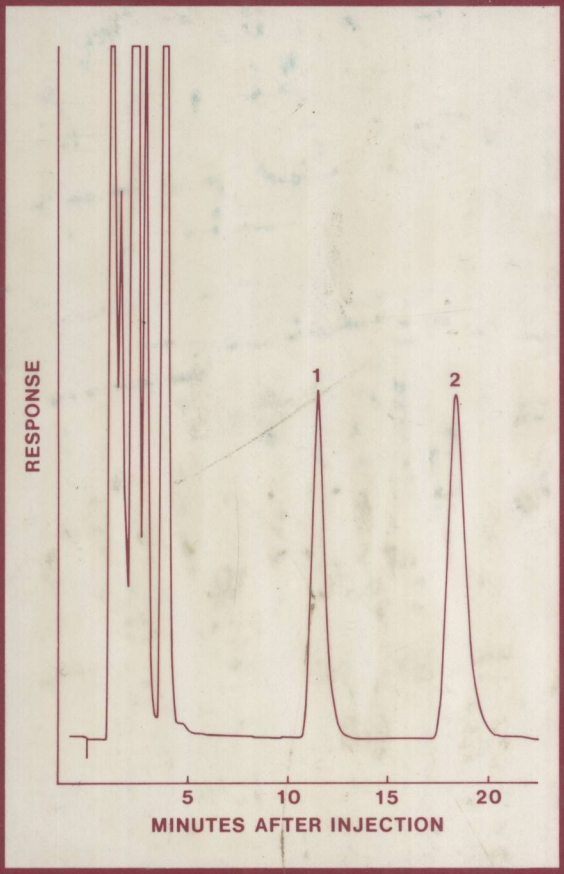
DRUGS AND THE PHARMACEUTICAL SCIENCES VOLUME 11

Pharmaceutical Analysis

Modern Methods

Part A



edited by James W. Munson

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Modern Methods

(in two parts) **Part A**

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Pharmaceutical Analysis

Modern Methods

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A Series of Textbooks and Monographs

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PREFACE

The pharmaceutical sciences are, by their nature, applied sciences. Whether examining the physical, chemical, or biological properties of a drug molecule, an investigator must bring many aspects of the basic sciences into play for the correct experimental design and data interpretation. Regardless of the purpose of the investigation, the need for chemical analysis of a drug molecule, degradation products, or metabolites is almost always evident. Since few pharmaceutical scientists are experts in all fields of chemical analysis, we must rely on the literature for information on any given analytical method. However, we often find that these reference materials fall into the category of undergraduate texts or appear as a highly specific work on a very limited subject area.

The purpose of Pharmaceutical Analysis: Modern Methods is to provide an intermediate level of coverage. This work is intended to fill the gap between basic texts and highly specific journal articles. The material presented here is designed for the graduate student studying pharmaceutical analysis and for the researcher in another field of the pharmaceutical sciences who wishes to increase his personal awareness and understanding of the modern techniques of pharmaceutical analysis. Each chapter in this work consists of a fairly comprehensive discussion of applicable theory, instrumentation, and pharmaceutical applications. Pertinent literature references are provided so that the reader may pursue a given topic further.

The first three chapters deal with the important technique of gas chromatography. Chapter 1 develops the basic theory necessary for an understanding of the mechanisms involved in gas chromatography (GC). Stationary phases, instrumentation, derivatization reactions, and methods of quantitation are described from a very pragmatic standpoint. Practical applications are given in each section. Finally a comprehensive survey of recent gas chromatographic methods for drugs is presented in tabular form. Chapter 2 deals with a modification of gas chromatography, namely pyrolysis-gas chromatography. Since this technique deals mainly with the treatment of the sample prior to chromatographic separation,

most of the emphasis is placed on the pyrolysis process and pyrolyzing units for GC. Once again references are provided to show applications of this technique to pharmaceutical problems. Examples are given which show how pyrolysis GC can give considerable information as to the identity of the analyte. While pyrolysis GC focuses primarily on sample treatment prior to chromatography, the third chapter explores the use of a mass spectrometer as a detector in GC. The first section of this chapter describes the mechanisms of the important ionization methods and the most widely used mass spectral analyzers. By far the most important section of this chapter is the discussion (with examples) of how the mass spectrometer can be used as a highly selective and sensitive detector for monitoring drugs and metabolites in biological samples. A comprehensive discussion of the use of gas chromatography-mass spectroscopy in studies of drug metabolism, toxicological and forensic work, and the diagnosis of disease states is also given.

The last three chapters, while not as closely related as the first three, closely follow each other in that many forms of radioimmunoassay depend upon measurements by liquid scintillation counting which in turn depends upon the measurement of fluorescence processes.

Fluorescence and phosphorescence spectroscopy are well known and widely used techniques in chemical analysis. However, in many texts the fluorescence and phosphorescence processes are only superficially explained. Frequently, the relationships of these processes to structural and solvent effects are almost entirely neglected. In Chapter 4, the authors spend considerable effort in developing the theoretical background for a more thorough understanding of the molecular processes that lead to fluorescence and phosphorescence spectra. Instrumentation and techniques for measurement of the fluorescence and phosphorescence phenomena in terms of efficiencies, concentration determinations, and interferences are extensively covered. Finally a comprehensive review of fluorescence and phosphorescence methods for drug molecules is given in tabular form.

Liquid scintillation counting is a technique used by many pharmaceutical scientists but rarely studied in a formal setting. The purpose of Chapter 5 is to provide a basic description of the scintillation process, sample preparation, instrumentation, and quantitation techniques. This chapter is intended as an introduction to this technique for scientists who wish to expand their knowledge. Finally, a description of how liquid scintillation counting is used to study problems of pharmaceutical interest is given with pertinent literature citations. Principal emphasis is placed on how this technique is used in the study of drug metabolism and disposition.

Radioimmunoassay (RIA), the subject of Chapter 6, promises to be one of the more powerful analytical techniques for determining trace levels of organic compounds in complex biological matrices. Since this technique is relatively new and is expanding rapidly, few textbook treatments are available. This chapter serves to provide a comprehensive description of

theory of RIA, methods used in RIA, procedures, and data reduction. Finally, a broad review of the use of RIA in the analysis of drugs is given. The author gives a thorough description of the strengths of RIA while fully recognizing its shortcomings.

The second part of this work will cover such topics as high-performance liquid chromatography, quantitative thin-layer chromatography, electronic absorption spectroscopy, molecular (ultraviolet and visible) spectroscopy, functional group analysis, flow-injection analysis, and electrochemical methods of analysis. In each case, primary emphasis will be placed on applications of pharmaceutical importance.

I would like to express my appreciation to my employers (at the University of Kentucky and The Upjohn Company) for allowing time for me to pursue this project. I would also like to thank my contributing authors for their fine work. Finally, I express my sincerest gratitude to my family for their understanding, patience, and confidence.

James W. Munson

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CONTENTS OF PART B

CONTENTS

1

GAS CHROMATOGRAPHY: THEORY, INSTRUMENTATION, AND PHARMACEUTICAL APPLICATIONS

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I. INTRODUCTION

Gas chromatography is a separation technique whereby a vaporized sample is carried by a flowing stream of (usually) inert gas through a tube which is either filled with fine particles or is wall-coated with a low-volatility liquid. The tube is termed the column. If the column is filled with dry particles, the technique is called gas-solid chromatography. In gas-liquid chromatography, the particles or the inside walls are coated with a low-volatility liquid. Gas chromatography can be used for the analysis of gas, liquid, and solid samples, providing that the latter two can be thermally vaporized without significant decomposition.

The practical aspects of gas chromatography can best be described by a general instrument diagram (Fig. 1). In the figure, the carrier gas module is simply a pressurized source of the inert carrier gas, usually a compressed gas cylinder. The sample introduction module encompasses the sample injection system, which is usually a heated block and may include associated sampling valves for gas samples. The vaporized sample then proceeds to the column module where it is separated into its various components. The components then emerge individually into the detection module, where their presence is sensed by the appropriate detector. The detector signal is then fed into a data acquisition module, which can be a simple recorder, an integrator, a computer, or some combination of the three. A more thorough discussion of the various modules can be found in Section III.

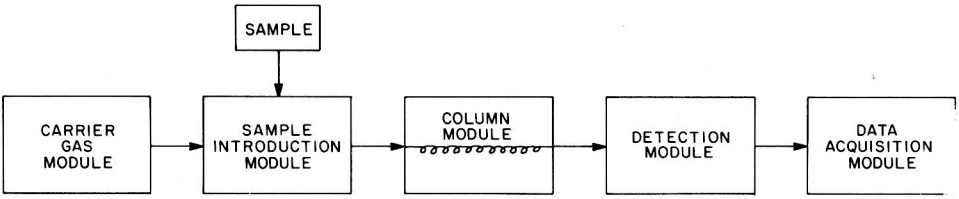

FIGURE 1. Modular diagram of a gas chromatograph.

A sample gas chromatogram is illustrated in Figure 2 for the separation of three anticonvulsant drugs, some of their metabolites, and two internal standard compounds.

The graphic presentation of a gas chromatogram is relative detector response plotted on the y axis versus time plotted on the x axis. The relative position of the individual peaks along the time axis is determined by the separating capability of the column for that mixture, and the size of the peaks (height or area) is a measure of the amount of component present in the mixture.

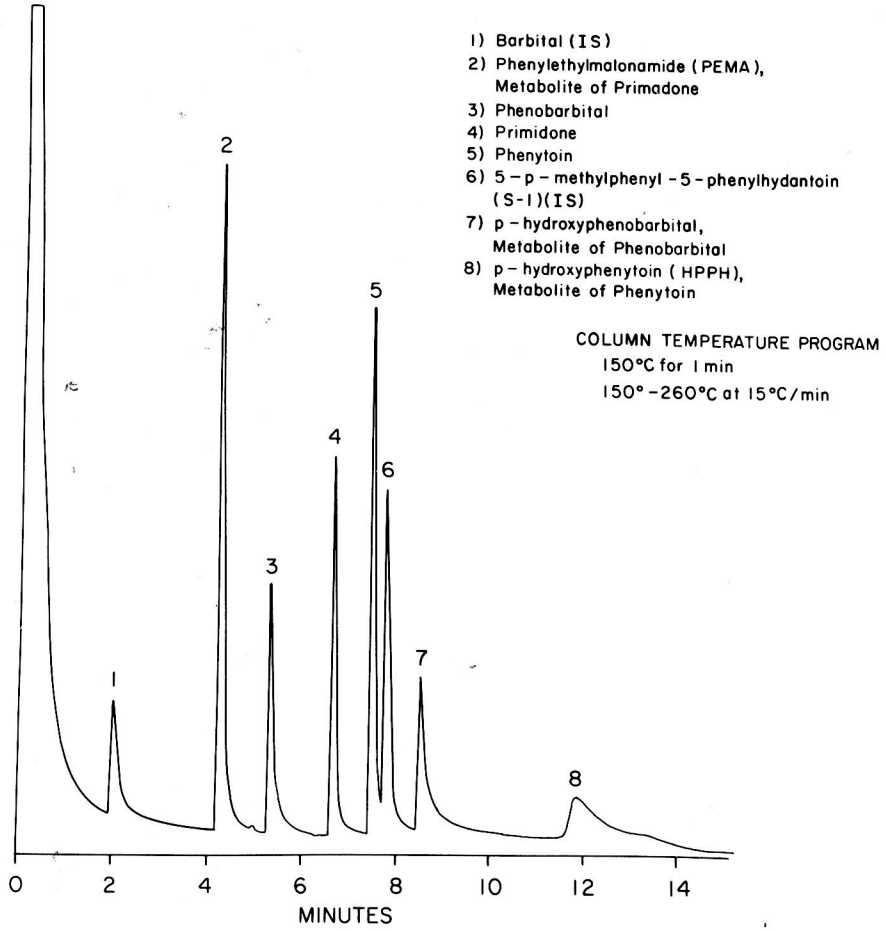

FIGURE 2. A sample gas chromatogram illustrating the separation of three anticonvulsant drugs, a metabolite of each, and two internal standard compounds. Column: 3 ft x 2 mm I. D. glass, GP 2% SP-2510 DA. Carrier gas: N_2 at 50 ml/min.

An introductory discussion of gas chromatography would not be complete without appropriate credit to the scientists who introduced the technique to the scientific community. It is generally accepted that the first reference to the use of a vapor as a mobile phase was in a publication by Martin and Synge [1] in 1941, although the subject of that paper was liquid-liquid partition chromatography. The first publication that actually described the technique of gas chromatography did not appear until 1952 when James and Martin [2] published a gas-liquid chromatographic method for the microanalysis of fatty acids. In that same year, three additional publications followed that further described the technique [3-5]. By 1956, gas chromatography was firmly established as a valuable analytical technique, and since then, its growth has continued at a phenomenal rate despite the introduction of high-performance liquid chromatography.

II. THEORY

Before beginning the discussion of the theories used in gas chromatography, a description or definition of certain terms is necessary. The inert carrier gas first mentioned in the introduction is termed the mobile phase. The stationary phase refers to either the liquid coating the particles or column walls in the case of gas-liquid chromatography, or to the solid particles in the case of gas-solid chromatography. Since solution theory is frequently utilized in theoretical discussions, the term solute is applied to the molecular species being separated in the column.

There are three theories that have found wide acceptance in describing a gas chromatographic separation. They are the plate theory [1, 6], the rate theory [6, 7], and the random walk, nonequilibrium theory [8-10].

A. Plate Theory

The plate theory, first proposed by Martin and Synge [1], compared the gas chromatographic separation to a fractional distillation. An analogy for this process would be multiple liquid-liquid extraction such as that which occurs in countercurrent distribution. The solute proceeds from one discrete extraction equilibrium to the next by the process of partitioning between two immiscible liquids. The "theoretical" plate is the portion of the column in which the solute is in complete equilibrium with the mobile and the stationary phases. This equilibrium is defined by the distribution coefficient K_D (previously called the partition coefficient), which is the concentration of the solute in the stationary phase divided by the concentration of the solute in the mobile phase. The distribution of a solute after n equilibrations (plates) is defined by the expansion of the binomial in Eq. (1),

$$(a + b)^{n-1} \tag{1}$$

in which (n - 1) is the number of transfers between the plates while a = $1/(K_D + 1)$ and b = $K_D/(K_D + 1)$. Figure 3 shows the distribution of two solutes, A and B, after 10 and 20 equilibrations were K_D for A is 1 and K_D for B is 1/2, and the volumes of both phases are equal [6]. As the number of plates increase (n > 100), the distribution becomes approximately Gaussian. This very simple theory illustrates two points: (1) the elution peak is expected to be symmetrical; and (2) band spreading will occur and will increase as the number of plates increases. Since the distribution is Gaussian, the theory indicates that complete separation of one solute from another cannot be obtained; however, in practice this is not a serious limitation. Using this theory, a number of terms were derived to describe a chromatographic column [2] (the symbols and terminology used in this discussion may not be the same as the original authors', but follow the recommendations of the International Union of Pure and Applied Chemistry [11]). These terms include the number of theoretical plates, n, height equivalent to a theoretical plate, h (HETP), and resolution R_S. For a gas chromatogram containing one component (Fig. 4) in which the x axis is time (this axis can also be

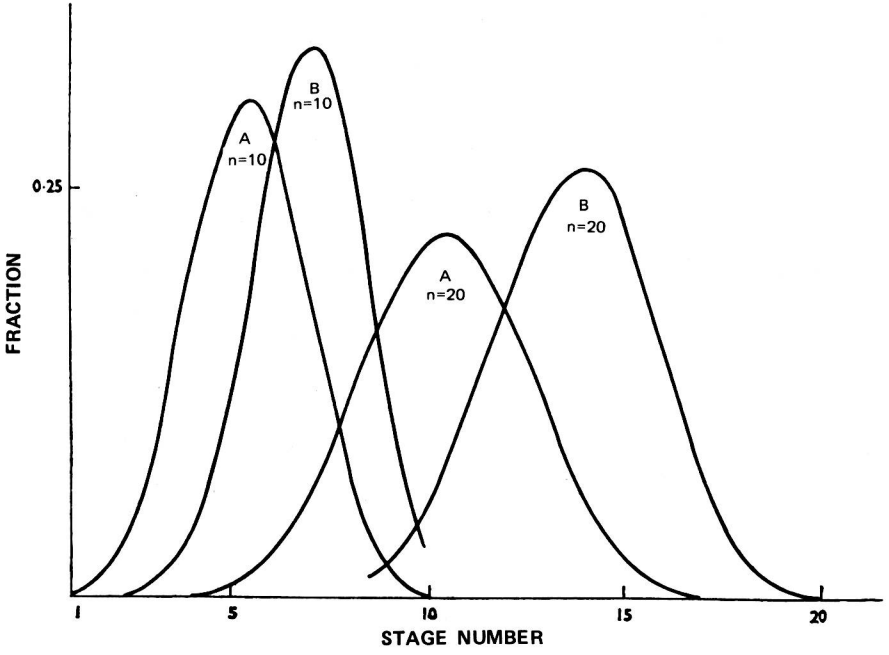

FIGURE 3. Distribution curves for solutes A and B after 10 and 20 equilibrations. (From Ref. 6; courtesy of Elsevier Publishing Company.)

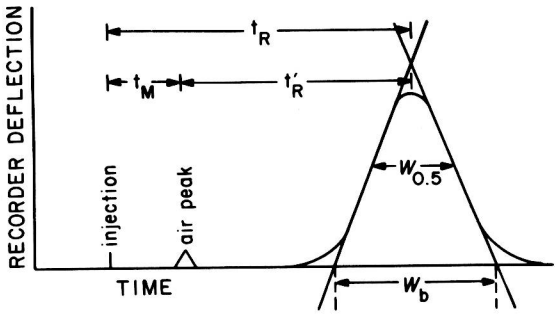

FIGURE 4. Parameters necessary for the calculation of column efficiency (plate number n) from Eq. (2).

volume of mobile phase or chart distance), n is calculated by Eq. (2), where t_R is the retention time of the peak, W_b is the width of the peak at baseline, and $W_{0.5}$ is the width of the peak at half-height. Basically, the plate number is a measure of the extent of band broadening that a solute undergoes as it passes along the column and is used as a measure of the efficiency of the column.

$$n = 16\left(\frac{t_R}{W_b}\right)^2 = 5.54\left(\frac{t_R}{W_{0.5}}\right)^2 \tag{2}$$

By definition, each component separated on a column will have a different n value for the column. This number is useful when comparing the efficiency of the same or similar columns for the same compound; however, when different columns of different composition and length are being compared, the h (HETP) is used. The h is obtained by dividing the column length L by the number of theoretical plates:

$$h = \frac{L}{n} \tag{3}$$

Therefore, the smaller the h, the more efficient the column. Because these numbers assume discontinuous flow and total equilibration whereas in reality there is continuous flow, n underestimates the real number of theoretical plates for a column. The last major term derived from plate theory is resolution. When there are two peaks, A and B, as shown in Figure 5, the peak resolution (R_S) is described by Eq. (4):

$$R_S = \frac{2\left(t_{R_B} - t_{R_A}\right)}{W_{b_A} + W_{b_B}} = \frac{t_{R_B} - t_{R_A}}{W_{0.5_A} + W_{0.5_B}} \tag{4}$$