# Modern Methods of Pharmaceutical Analysis

## Volume III

Editor

Roger E. Schirmer

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Roger E. Schirmer
Group Leader
Analytical Chemistry Group
Biology Department
Battelle/Pacific Northwest Laboratories
Richland, Washington


CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data

Schirmer, R. E. (Roger E.)
Modern methods of pharmaceutical analysis.

Vols. 2-3: Edited by Roger E. Schirmer.
Includes bibliographies and indexes.
1. Drugs--Analysis. I. Title. [DNLM:
1. Drugs--Analysis. 2. Chemistry, Analytical.
3. Chemistry, Pharmaceutical. QV 25 M6894]
RS189.S29 615'.1901 81-9976
ISBN 0-8493-5244-4 (v. 1) AACR2



Direct all inquiries to CRC Press, Inc., 2000 N.W. 24th Street, Boca Raton, Florida 33431.



International Standard Book Number 0-8493-5244-4 (Volume I)
International Standard Book Number 0-8493-5245-2 (Volume II)
International Standard Book Number 0-8493-5246-0 (Volume III)

Library of Congress Card Number 81-9976
Printed in the United States

# FOREWORD

The analysis of pharmaceuticals can be subdivided into several distinct classes of analytical problems. The classes are listed in Table 1. Each class of problems imposes a unique set of constraints on the analyst. For example, the determination of the purity of a drug substance usually requires an analysis of high specificity and precision, but does not require high sensitivity or preliminary isolation of the analyte from a sample matrix. Analysis of a potent drug in a low dose formulation on the other hand requires selectivity, precision, high sensitivity, and (usually) a preliminary separation of the drug from excipients. Evaluation of reference standards requires use of absolute methods such as coulometry, differential scanning calorimetry, or NMR that do not need a standard of the test substance for calibration, and so on. As a consequence of the diverse requirements of the different classes of problems, all common organic analytical techniques are routinely used in pharmaceutical analysis. Instrumental methods are particularly important in modern pharmaceutical analysis, but classical procedures are often used in conjunction with them. The classical methods remain very important in routine quality control of pharmaceuticals.

The objective of "Modern Methods of Pharmaceutical Analysis" is to review the major methods in current use in pharmaceutical analysis. The review covers principles, special instrumentation, experimental techniques, and a survey of pharmaceutical applications for each method. The discussion of principles is intended to provide insight into important experimental variables, possible sources of error in applications of the technique, and factors that should be taken into account when adapting the method to solve new analytical problems. Tables of buffer compositions, characteristics of chromatographic column packings, physical properties of solvents, positions of UV, IR, and NMR absorption bands, and other frequently used reference data are also included in the text. The literature surveyed in each section has been selected to demonstrate the range of applications of the technique in pharmaceutical analysis and to provide essential details of specific applications that can serve as a guide in related analytical development efforts. More comprehensive reviews of the applications literature are referenced whenever they are available.

**Table 1**
**Classes of Problems in Pharmaceutical Analysis**

- Detection, isolation, and identification of impurities in a drug substance or formulation
- Evaluation of the purity of reference standards of drug substances
- Purity determination of a drug substance for routine quality control
- Identification tests for quality control of a drug substance
- Determination of the potency of a formulation for routine quality control
- Identification of the drug substances in a formulation for routine quality control
- Evaluation of content uniformity for low dose formulations
- Analysis of other materials such as moisture, residual solvents, heavy metals, preservatives, specific impurities, etc. in drug substances and their formulations
- Determination of the chemical and physical stability of drug substances and their formulations
- Measurement of physical properties such as crystalline form, dissolution rate, disintegration times, hardness, pH, color, etc. for drug substances and formulations

# INTRODUCTION

During the development of a new drug product, detailed chemical studies must be made of raw materials, synthetic intermediates, the drug substance itself, and the final formulated product. These studies must identify types and levels of impurities, degradation products, degradation rates, and analytical methods suitable for monitoring these factors. The information resulting from these studies is used to identify potential sources of safety problems in the product, to meet the requirements of foreign and domestic regulatory agencies, and as a basis for establishing quality control procedures and specifications for the product. The analytical effort required to provide this information can be divided into a number of tasks as shown in Table 1. The requirements for specificity, precision, accuracy, and the degree of complexity acceptable in the analytical procedure vary considerably from task to task and therefore require a variety of analytical techniques to satisfy them.

For example, the identification of impurities and degradation products requires the extensive use of chemical separations followed by qualitative analysis of the isolated product. Gas chromatography(GC) and high pressure liquid chromatography (HPLC) are frequently used to separate trace impurities, but older techniques such as thin layer chromatography (TLC), fractional crystallization, fractional distillation, and solvent extraction are still very important. However, the classical methods of identification by preparation and characterization of derivatives have been almost completely replaced by modern spectral methods of analysis. Elemental analysis, nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), and mass spectroscopy (MS) — especially GC/MS — are used almost exclusively for identification of unknown products.

In addition to identification of degradation products, it is necessary to measure the rates of degradation of the drug and its formulations under a variety of conditions. This information is needed to define conditions for storage and handling that will assure potency and safety throughout the expected shelf life of the product. Stability studies are especially demanding of analytical precision and accuracy because changes of a few percent over a period of 3 to 5 years are significant and must be accurately quantitated. In order to be able to detect such small changes reliably with a limited number of replicate assays at each time point, the analysis must be very precise, free of interference from the degradation products, and free of "drift" due to changes in instruments, standards, operators, etc. over a period of years. Gas chromatography, high pressure liquid chromatography, spectrophotometric, titrimetric, and electrochemical metbods are all capable of adequate precision. Regardless of the method of measurement, the procedure must be carefully designed to avoid chemical interferences and the method precision must be determined experimentally for use in designing the stability study.

The set of analytical procedures developed to control the quality of the final marketed product must include both qualitative and quantitative methods in order to assure the identity and purity of the product. Several categories of tests are usually included in a product specification and these are summarized in Table 2. All the procedures called out in the specification must be amenable to routine use in a quality control laboratory and must therefore be as simple and rapid as possible. The procedures used during product development can often be simplified without loss of essential specificity or accuracy because the real problems associated with the product have been defined by the developmental work. However, control limits on both the purity of the drug substance and the drug content of the finished formulation are usually very tight, thus requiring very precise quantitative procedures for testing compliance. The preferred solution to the problem of quantitative control assays is therefore to use

HPLC or GC methods which afford simplicity, high speed, good specificity, and excellent precision and accuracy. An alternate that may be chosen when suitable GC or HPLC systems cannot be found, or for facilities where these instruments are not readily available (a common problem in some foreign countries), is to combine a precise but nonspecific quantitative assay with a qualitative chromatographic test that shows the absence of interfering impurities. This approach is widely used in the compendia and in control procedures for older products. The quantitative analysis in these cases is often a titrimetric or spectrophotometric method, and the qualitative test a thin layer or paper chromatogram. Regardless of the methods chosen for the final measurement, quantitative analysis of formulated products almost always requires a preliminary separation of the drug from excipients. This separation is frequently accomplished by extraction, solvent partitioning, filtration, or column chromatography, but many other techniques find occasional application. In spite of the multiplicity of methods available, finding a reasonably simple procedure that gives a clean, quantitative separation of the drug from the excipients is often the most difficult step in development of procedures for analyzing formulations.

In many cases, quantitative analysis of drugs also requires the use of an analytical reference standard of well defined purity. A reference standard is required whenever a relative technique such as GC, HPLC, ultraviolet, visible or infrared spectrophotometry, fluorometry, or polarography is used for the analysis. Standards are also required for some qualitative tests such as identification by retention time, retention volume, $R_f$ value. The evaluation of the reference standard is accomplished using a series of tests similar to those listed in Table 2 for analysis of the drug substance. However, relative analytical techniques cannot be used to obtain a purity value because of their requirement for a reference standard (area normalization is used to estimate purity from GC and HPLC traces, but the values obtained must be treated as rough approximations to the time purity). The purity value of the standard must instead be derived from absolute methods, i.e., methods which do not require a standard of the same substance. The available absolute methods are titrations and gravimetric procedures (including methods used for elemental analysis), NMR, coulometry, differential scanning calorimetry, and phase solubility analysis. The requirement for reference standards in analysis with a wide spectrum of applications in pharmaceutical development and control makes the absolute methods especially important in modern pharmaceutical analysis.

The range of problems encountered in pharmaceutical analysis coupled with the importance of achieving the highest specificity, precision, and accuracy possible result in new techniques for organic analysis being adopted quickly in the pharmaceutical industry. The purpose of this book is to review several of the newer methods that now find wide application in pharmaceutical analysis, as well as several older methods (e.g., phase solubility analysis and ultraviolet/visible spectroscopy) of unique importance. The principle of each technique is discussed with emphasis on factors that directly affect its proper application to analytical problems. A thorough understanding of these principles is essential when selecting instruments, operating conditions, and sample preparation procedures to optimize the performance of an analysis, or when trying to identify the cause of a failure encountered in an existing procedure. Tabulations of data useful in method development and applications are also presented, including tables of characteristic ir, nmr, and uv band positions; composition of standard buffer solutions; properties of solvents; and properties of column packings for GC and HPLC. Finally, selected applications of each technique to problems in pharmaceutical analysis are reviewed. It is hoped that the broad coverage given each of the selected techniques will make *Modern Methods of Pharmaceutical Analysis* useful as a source of ideas and guidance in developing practical solutions to problems in pharmaceutical analysis.

## Table 1
## ANALYTICAL TASKS IN DEVELOPMENT AND MARKETING OF A DRUG

Determination of identity and purity of starting materials and intermediates used in manufacturing the drug substance
Determination of the identity and purity of the drug substance
Isolation and identification of trace impurities in the drug substance
Determination of degradation rates and products for the drug substance
Determination of identity and purity of excipients used in manufacturing formulated products
Determination of degradation rates and products for the formulated drug
Establishment of an analytical reference standard for the drug substance

## Table 2
## OUTLINE OF QUALITY CONTROL TESTS FOR DRUG PRODUCTS

### Identification Tests

Purpose: to confirm the identity of the principal component of a lot of raw material or formulation
Types of tests: color tests, melting points of the drug or derivative of the drug, formation of precipitates, ir or nmr spectrum, mass spectrum, X-ray powder pattern, chromatographic mobility, optical rotation, refractive index, density

### Quantitative Analysis of the Drug Substance

Purpose: to determine the percent purity of the drug substance or the content of the active ingredient(s) in a formulation
Types of tests: *Absolute methods* — titrations, gravimetric procedures, differential scanning calorimetry, coulometry, nuclear magnetic resonance spectrometry, phase solubility analysis
*Relative methods* — gas chromatography, high pressure liquid chromatography, spectrometry (ultraviolet, visible, or infrared), fluorometry, polarography, microbiological assays

### Tests for Specific Impurities

Purpose: to control the quantity of a specific impurity or group of inpurities in the drug product, such as water, solvents, metals, and trace organic impurities
Types of Tests: any test listed in Quantitative Analysis of the Drug Substance, atomic absorption, atomic emission, or semiquantitative limit tests using relative size of spots on thin layer chromatograms, spot tests with visual color comparison, etc.

### Chromatographic Screen

Purpose: qualitative examination of the product for impurities, including contaminants not previously encountered
Types of tests: paper, thin layer, gas or high pressure liquid chromatography; electrophoresis; bioautography

### Miscellaneous

Purpose: control of specific properties known to affect product performance or required by regulatory agencies
Types of tests: crystal form (X-ray or infrared spectroscopy), sterility, pyrogens, particle size, foreign matter, density, color, odor, etc.

## THE EDITOR

Roger E. Schirmer, Ph.D., is Group Leader of the Analytical Chemistry Group, Biology Department, Battelle/Pacific Northwest Laboratories, Richland, Washington. Prior to joining Battelle in 1977 he was Group Leader in the Raw Materials Group, Analytical Chemical Development Department, Eli Lilly and Co., Indianapolis, Indiana.

Dr. Schirmer graduated in 1965 from Wayne State University, Detroit, Michigan, with a B.S. degree in Chemistry and Mathematics. He obtained his Ph.D. in Physical Chemistry from the University of Wisconsin, Madison, Wisconsin in 1970.

Dr. Schirmer is a member of the American Association for the Advancement of Science, American Chemical Society, Society for Applied Spectroscopy, American Physical Society, and the Academy of Pharmaceutical Sciences of the American Pharmaceutical Association. He has a number of publications in the areas of pharmaceutical analysis and applications of nmr to problems in chemical kinetics and structure, including a book, *The Nuclear Overhauser Effect* co-authored with Dr. Joseph Noggle.

## CONTRIBUTORS

**L. J. Lorenz, Ph.D.**
Analytical Development-Physical
The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, Indiana

**Roger E. Schirmer, Ph. D.**
Group Leader
Analytical Chemistry Group
Biology Department
Battelle/Pacific Northwest
Laboratories
Richland, Washington

**Rex W. Souter, Ph.D.**
Research Scientist
The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, Indiana

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Chapter 1

# GAS-LIQUID CHROMATOGRAPHY

R. W. Souter

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# I. INTRODUCTION

## A. Gas Chromatography

Gas chromatography is a method used to separate components of mixtures of volatile compounds. Normally the separations are made to identify or quantitate the constituents in a sample mixture, but in some applications the separations are made for preparative purposes.

Chromatography was first applied by Ramsey[1] in 1905 to separate gaseous mixtures. During the following year Tswett[2] obtained colored bands of plant pigments on a chromatographic column, and he coined the term "chromatography" meaning "color writing", which is a misnomer when applied to current techniques. In 1952, James and Martin introduced gas-liquid chromatography[3,4] based on the suggestion of Martin and Synge.[5] As is obvious to all those familiar with its literature, the sensitivity, selectivity, speed, accuracy, and precision of this technique for the separation, identification, and determination of volatile compounds has resulted in almost explosive growth.

Gas chromatography separates volatile substances by percolating a gas stream over a stationary phase. If the stationary phase is a solid, the technique is known as gas-solid chromatography (GSC), and the separation is based on the adsorptive properties of the column packing. When the stationary phase is a liquid, the technique is gas-liquid chromatography (GLC). The basis for separation in GLC is the partitioning of the sample in and out of a thin film of liquid spread over an inert solid. GLC is probably the most selective and versatile form of gas chromatography since there exists a wide range of liquid phases usable up to about 450°C. This chapter will deal primarily with GLC.

## B. Applications of Gas Chromatography to Pharmaceutical Analysis

GLC has found increasing application in the analysis of drugs and their metabolites. GLC methods often are more sensitive and specific than colorimetric, spectrofluorometric, and spectrometric assays, and thus may be used for assay purposes per se or to validate the simpler methods. The speed, resolution, and sensitivity of GLC separation and detection methods, especially with compounds amenable to some of the new, specific detectors, make these very attractive for drug analysis problems dealing with bioavailability, raw materials, and pharmaceutical formulations.

## C. Basic Gas Chromatography Apparatus

The relatively simple instrumentation for gas chromatography is shown in the block diagram in Figure 1. Inert carrier gas from a compressed gas cylinder passes to a controller whose purpose is to maintain a constant gas flow through the column. The gas passes next to the head of the column, at the inlet to which is an injection port through which the sample is introduced. The carrier gas then moves the sample components through the column to the far end where the detector is located. The function of the latter is to detect or sense the components as they emerge, and this is done by use of some chemical or physical property of the vapors. From the detector the signal is sent to a recorder and/or to a computer for calculation of peak areas.

# GAS CHROMATOGRAPHY THEORY

## A. Introduction

The separation of two substances depends on the quality of the performance by the column (its efficiency) and on the relative retention or stationary phase efficiency (selectivity).

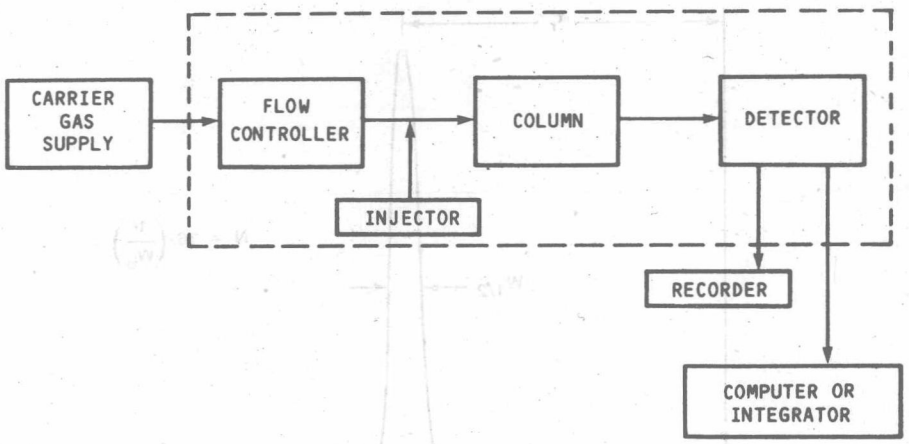



FIGURE 1. Block diagram of the apparatus for a basic gas chromatograph. The dashed line outlines the components under temperature control.

Column performance determines the width of peaks relative to the length of time they spend in the column. A zone of vapor is generally introduced into the column as quickly as possible so that it takes the form of a very narrow band. The band will broaden while being eluted through the column, and the degree of broadening may be defined and related to the factors upon which it depends. Historically, an artificial model of the chromatographic column, the "plate" model, was developed to treat the behavior of sample vapor molecules moving in and out of a stationary phase.[5,6] More useful yet is a consideration of what happens to the vapor molecules whose motion is controlled by diffusion and by column geometry.[7]

Stationary phase or solvent efficiency results from the interaction of the sample vapor (solute) and the stationary phase (solvent), and it determines the relative positions of the solute bands on the chromatogram. In a particular chromatographic run, column variables are the same for each vapor, so the separation is determined by the values of the partition coefficients; if they are sufficiently different, an adequate separation will result. The partition coefficient is the ratio of solute concentration in the liquid phase to solute concentration in the gas phase. Relative retention is the ratio of the partition coefficients for two substances, and may be predicted from a knowledge of the factors determining partition coefficients. This information may subsequently be applied to predict the best stationary phase for a separation.

## B. Column Efficiency

The concept of a "theoretical plate" is carried over from distillation processes, and is used to evaluate column performance. Plates are useful for comparison of columns or to set standards for column-packing techniques. From an experimental chromatogram it is easy to calculate the number of theoretical plates.

In a GC experiment the carrier gas flows in a tube of uniform cross-sectional area, and causes the sample zone or zones to migrate. The flow rate f is the volume swept out by a cross section of carrier gas per unit time. The interval which elapses between injecting the sample and the elution of the center of mass of the zone or peak is called retention time, $t_r$. The product of flow rate and retention time is retention volume, $V_r$. With reference to Figure 2, the number of theoretical plates, N, is defined as

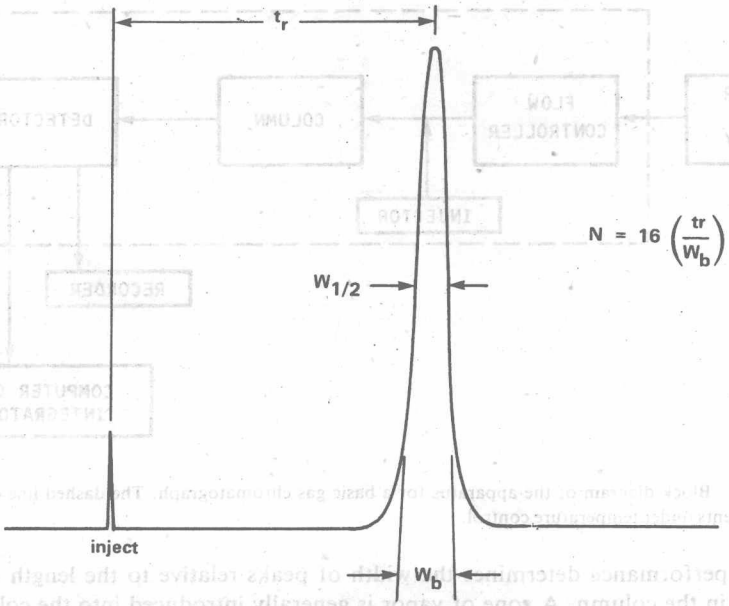



FIGURE 2. Calculation of the number of theoretical plates for a gas chromatographic column.

$$N = 16\left(\frac{t_r}{W_b}\right) \quad (1)$$

or as

$$N = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2 \quad (2)$$

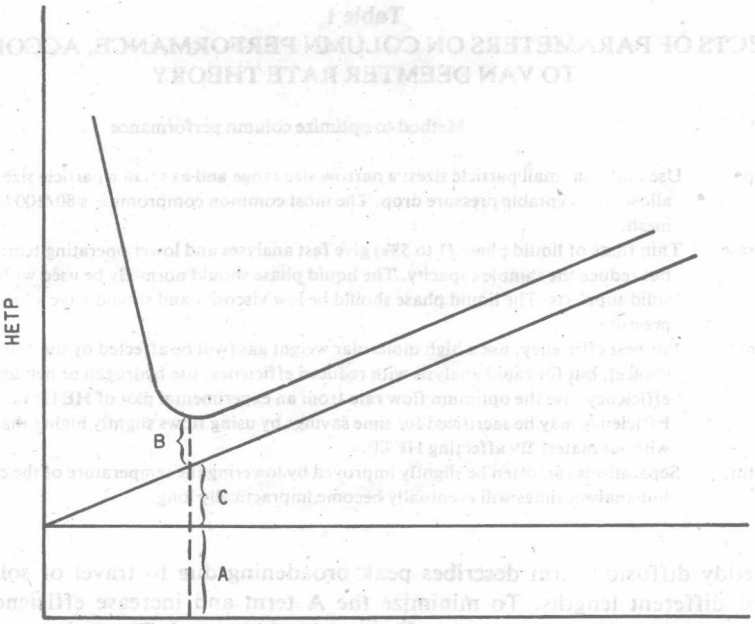
where $W_b$ is the base width of the peak and $W_{1/2}$ is the full width of the peak at half-height. The higher the value of N for a column, the more efficient it will be. Columns with high efficiency allow smaller samples to be injected onto shorter columns at lower temperatures, and consequently give greater separations in less time with reduced risk of thermal decomposition.

To compare the performance of a column under different conditions or to compare different columns, the height equivalent to a theoretical plate (HETP) may be compared:

$$HETP = \frac{L}{N} \quad (3)$$

where L is the column length, usually in centimeters.

A major development in chromatographic column theory was the rate theory of van Deemter et al.[8] which was extended by Glueckauf.[9] In its simplest form under normal conditions, the van Deemter equation relates the physical factors determining the theoretical plate height.[10]




$$HETP = A + \frac{B}{\mu} + C_{\mu}$$

FIGURE 3. Plot of HETP vs. flow velocity.

$$HETP = 2\lambda d_p + \frac{2\gamma D_g}{\mu} + \frac{8 k' d_f^2}{\pi^2 (1 + k')^2 D_l} \mu \qquad (4)$$

where $\lambda$ is a constant related to the geometry of the column packing particles, $d_p$ is the average diameter of the solid support particles, $\gamma$ is a factor to correct for the "tortuosity" of the column's gas channels, $D_g$ and $D_l$ are solute diffusion coefficients in the gas and liquid phases, respectively, $d_f$ is the liquid film (stationary phase) thickness, $k'$ is the partition coefficient of the solute, and $\mu$ is the linear gas velocity. For a particular column, all the parameters in Equation 4 are fixed except $\mu$, and the equation may be rewritten in simpler form:

$$HETP = A + \frac{B}{\mu} + C\mu \qquad (5)$$

where A is the eddy diffusion term, B is the molecular diffusion term, and C reflects resistance to mass transfer in the liquid phase. The linear gas velocity, $\mu$, is calculated from

$$\mu = \frac{\text{length of column, cm}}{\text{retention time of air, sec}} \qquad (6)$$

An isothermal plot of observed HETP vs. $\mu$ for a single solute yields a chracteristic hyperbola with a minimum HETP. The minimum is the flow rate at which the column is operating most efficiently. The plot is shown in Figure 3 and shows how the A, B, and C terms contribute to the HETP.

**Table 1**
**EFFECTS OF PARAMETERS ON COLUMN PERFORMANCE, ACCORDING TO VAN DEEMTER RATE THEORY**

| Variable | Method to optimize column performance |
|---|---|
| Solid support | Use uniform small particle sizes: a narrow size range and as small a particle size as will allow an acceptable pressure drop. The most common compromise is 80/100 or 100/120 mesh. |
| Liquid phase | Thin films of liquid phase (1 to 5%) give fast analyses and lower operating temperature, but reduce the sample capacity. The liquid phase should normally be used with inactive solid supports. The liquid phase should be low viscosity and should have a low vapor pressure. |
| Carrier gas | For best efficiency, use a high molecular weight gas (will be affected by the detector choice), but for rapid analysis with reduced efficiency, use hydrogen or helium. For best efficiency, use the optimum flow rate from an experimental plot of HETP vs. flow rate. Efficiency may be sacrificed for time savings by using flows slightly higher than optimum without materially affecting HETP. |
| Temperature | Separations can often be slightly improved by lowering the temperature of the column, but analysis times will eventually become impractically long. |

The eddy diffusion term describes peak broadening due to travel of solute along paths of different lengths. To minimize the A term and increase efficiency, small, uniform particles and small diameter columns should be used. The molecular diffusion term is proportional to the diffusivity in the carrier gas. If diffusivity increases, solute bands broaden. Diffusivity in the liquid phase is negligible. The "tortuosity" correction, $\gamma$, adjusts linear gas velocity to actual velocity in a packed column. The resistance to mass transfer term represents transfer of solute vapor along several paths, including from liquid to gas and from gas to liquid in the flow stream. Several conclusions which may be drawn from van Deemter's theory and which are of practical value in improving column performance are summarized in Table 1.

Column performance may also be described in terms of the factors that contribute to broadening a zone as it elutes. Broadening factors each introduce a component of variance $\sigma_i^2$ into the width of the peak eluted after instantaneous injection of the sample. The components of variance are combined by the usual rule. For addition of the effects of independent sources of random error on a distribution,

$$\sigma^2_{\text{total}} = \sum_i \sigma_i^2 \qquad (7)$$

where $\sigma$ is the standard deviation of a Gaussian peak. The observed variance of the chromatographic peak — $\sigma^2_{total}$ — may be envisioned as being composed of variances due to the column partition process and to the band spreading outside the column. The former was discussed already in terms of column efficiency while the latter are summarized in Table 2.

Column efficiency and variance has been treated in detail by Grubner[11] and by Littlewood.[7] In addition, the latter author presents an excellent discussion of the relationships of plate height (HETP) and the broadening of a peak expressed as a variance.[12] HETP is proportional to peak variance; therefore, each broadening factor may be regarded as adding a term to HETP,[7] thus decreasing the efficiency of the chromatographic system.

### C. Stationary Phase Efficiency

The stationary phase (solvent) efficiency results from the solute-solvent interaction,

Table 2
VARIANCE CONTRIBUTIONS DUE TO BAND SPREADING OUTSIDE THE CHROMATOGRAPHIC COLUMN

| Excessive bandwith contribution | Means to reduce variance |
|---|---|
| Sample injection volume is too great | Inject a smaller volume |
| Sample injection too slow | Improve injection technique |
| Too much "dead" volume between point of injection and column | Reduce space by adding column packing or modifying injection system |
| Detector volume | Keep volume of detector small to minimize diffusion |
| Detector-column connecting line | Minimize length or eliminate completely so gas flow is not slowed down; keep volume of line small to minimize diffusion |

and it determines the relative position of solute bands on the chromatogram. A striking advantage of gas chromatography is that appropriate selection of the liquid phase will separate substances which have the same vapor pressure. The combined effects of several types of interaction forces determine the solubilities of the solutes in the stationary phase (hydrogen bonding, complex formation, etc.) and consequently the observed separation. The combined effects of these forces are expressed by the partition coefficient, k, where

$$k = \frac{\text{amount of solute per unit volume of liquid phase}}{\text{amount of solute per unit volume of gas phase}} \qquad (8)$$

The greater the difference in partition coefficients, the larger the separation of two compounds and the shorter the column length needed to effect the separation.

The efficiency of a stationary phase for a particular separation is measured by $\alpha$, the relative retention, which is the ratio of the partition coefficients for the substances being separated:

$$\alpha = \frac{k_2}{k_1} \qquad (9)$$

Calculation of the solvent efficiency is shown in Figure 4. Note that adjusted retention times are used to calculate relative retention.

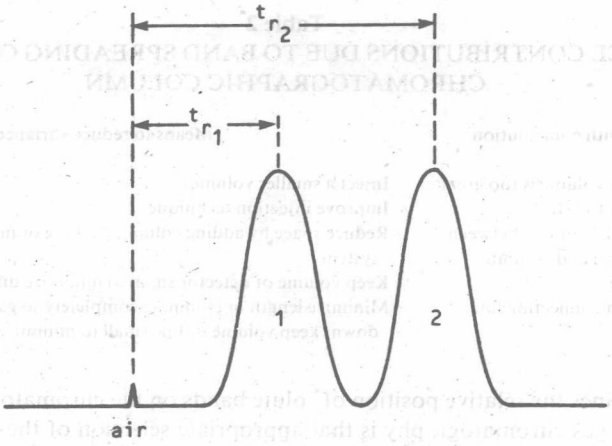
Although k and therefore $\alpha$ are temperature dependent, $\alpha$ is essentially constant over a limited temperature range. With an increase in temperature, the solute spends less time in the stationary phase and more time in the carrier gas, so elution time and separation will both decrease.

To properly evaluate the separation of two consecutive peaks, one must consider resolution, R, which is demonstrated in Figure 5. It is a measure of both column and solvent efficiencies and relates peak width and maxima separation. R must be 1.5 or greater to achieve complete (baseline) separation of two peaks.

For two peaks which are spaced closely enough that they have nearly equal widths, Purnell[13] proposed an equation to relate the relative retention and the resolution to the number of theoretical plates for a separation:

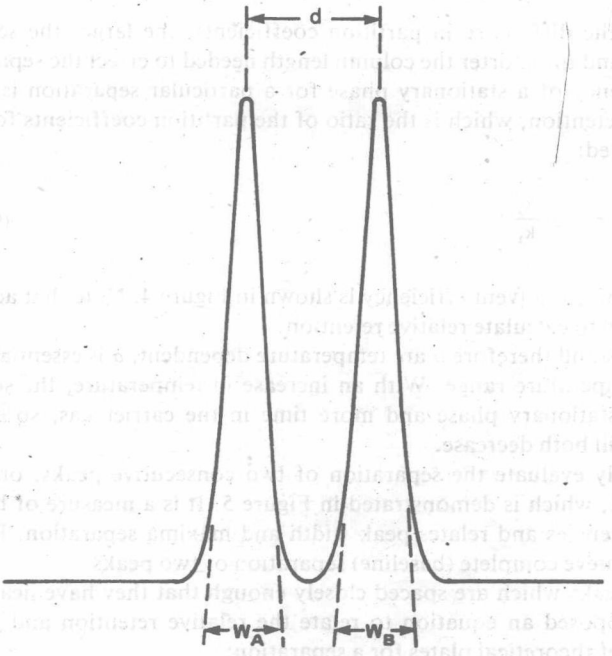
$$N_{req} = 16\left(\frac{\alpha}{\alpha - 1}\right)\left(\frac{k_2 + 1}{k_2}\right)^2 R \qquad (10)$$

where $k_2$ is the partition coefficient of the longer retained component. This equation is used to determine the number of plates required for a specified separation. Figure 6




$$\alpha = \frac{t_{r_2}}{t_{r_1}} = \frac{k_2}{k_1}$$

**FIGURE 4.** Calculation of the stationary phase efficiency.




$$R = \frac{2d}{\omega_A + \omega_B}$$

**FIGURE 5.** Calculation of the resolution, R, of two consecutive peaks.