Modern Methods of Pharmaceutical Analysis

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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 methods 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, or 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 true 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 analyses 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.

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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.

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Chapter 1

SEPARATION OF DRUGS FROM EXCIPIENTS

I. INTRODUCTION

Many practical problems in pharmaceutical analysis require the determination of a substance which is dispersed in a sample matrix. Successful solution of the analytical problem is usually accomplished in two stages: (1) separation of the substance from the matrix and (2) quantitation of the isolated compound. Devising a suitable separation procedure is almost always the most difficult part of the development of a new analytical method for a formulated drug.

The most common techniques for separating drugs from other compounds of their formulations are extraction and column chromatography. These techniques will be discussed in some detail in this chapter. Distillation and precipitation are much less useful in organic analysis than in inorganic analysis and will not be discussed.

II. LIQUID-SOLID EXTRACTION

A. Principles of Liquid-Solid Extraction

Extraction of a substance from a solid matrix is frequently encountered in the analysis of solid dosage forms, impurities in a drug substance, and raw materials such as leaves, roots, animal tissues, etc. which contain a medicinal substance. The substance may be present as a simple physical mixture with the matrix components, it may be adsorbed onto or absorbed into those components, or it may be held in a more complex fashion, as in the case of a natural product contained within the cellular structure of the tissue that produces it.

Extraction from a physical mixture is relatively simple. The solvent is chosen to provide good solubility of the substance of interest and minimal solubility of other components of the mixture. The rate of dissolution of the analyte may be an important factor in selection of the solvent in some cases. The volume of solvent required to effect complete extraction from a simple mixture will be a little greater than the minimum volume required to dissolve the analyte, the excess solvent being used to flush residual solution of the analyte from the surface of the solid. However, in many practical applications a fraction of the analyte adsorbs onto insoluble components of the sample matrix, and extraction becomes more difficult.

Extraction of adsorbed material is more difficult because the extraction process becomes less efficient as the ratio of unextracted material to solvent decreases. The major qualitative features of these extractions can be illustrated for a solute/adsorbent system with the following properties:

1. The heat of adsorption is relatively large (>> 10 Kcal/mol), and is independent of the amount of solute already on the surface.
2. The surface has a limited capacity for adsorption.

Under these conditions the adsorption process can be described by the Langmuir isotherm,[1]

$$\theta = \frac{M}{q + M} \qquad (1)$$

where θ = fraction of the adsorbent covered with analyte, M = molarity of the solution in contact with the adsorbent, and q = molarity of the solution which gives 50% coverage of the adsorbent.

Upon equilibrating the analyte/adsorbent mixture with solvent, the mass balance relation may be written

$$\theta M_o + MV = A \qquad (2)$$

where M_o = maximum number of moles than can be adsorbed by the solid, V = volume of solvent, and A = total moles of analyte present.

Combining Equations 1 and 2 and solving for the fraction, f, of the analyte extracted gives

$$f = \frac{A - \theta M_o}{A} = \frac{-(V_q + M_o - 1) + \sqrt{(V_q + M_o - 1)^2 + 4AV_q}}{2A} \qquad (3)$$

The way f changes when a fixed amount of solid is extracted with increasing volumes of solvent is shown in Curve C of Figure 1. It is clear that increases in the volume of solvent used do not result in proportional increases in the fraction extracted, and that it is difficult to achieve a reasonably quantitative recovery with a single extraction. A series of extractions are necessary to achieve this, and if the adsorption is relatively weak (K is large), the fraction recovered increases rapidly with number of extractions. An example is given in Table 1. The advantage of a series of extractions over a single extraction using the same total volume of solvent is a very general result which is observed for most adsorption mechanisms and for liquid-liquid extraction systems as well.

Quantitative recovery of a strongly adsorbed substance is difficult to achieve even with multiple extractions, and proper selection of conditions for the extraction is the key to success in these cases. The solvent should be chosen according to the following criteria:

1. The solvent must be compatible with the operations to be performed following the extraction.
2. The solvent must be chemically compatible with the analyte.
3. Of otherwise equivalent solvents, the one in which the analyte is most soluble should be used.
4. The solvent should be adsorbed by the matrix in the same manner as the analyte, as this will tend to displace the analyte from adsorption sites.

If necessary, another compound may be added to the solvent to help displace the analyte. For example, a small amount of a low-molecular-weight carboxylic acid might be added to assist in extraction of an organic acid, or a low-molecular-weight amine in the extraction of an alkaloid or other basic substance. Performing the extraction at elevated temperature will also improve the recovery of adsorbed materials. Higher temperatures also favor rapid equilibration which can be very important when dissolution of the adsorbed substance is slow. The kinetics of adsorption processes have been discussed in numerous places.[2,3]

Extraction of substances adsorbed into a matrix or held in a complex manner in tissues, etc. is much more difficult to discuss in general terms. It is generally true that a series of extractions with lesser volumes of solvent will be more effective than a single

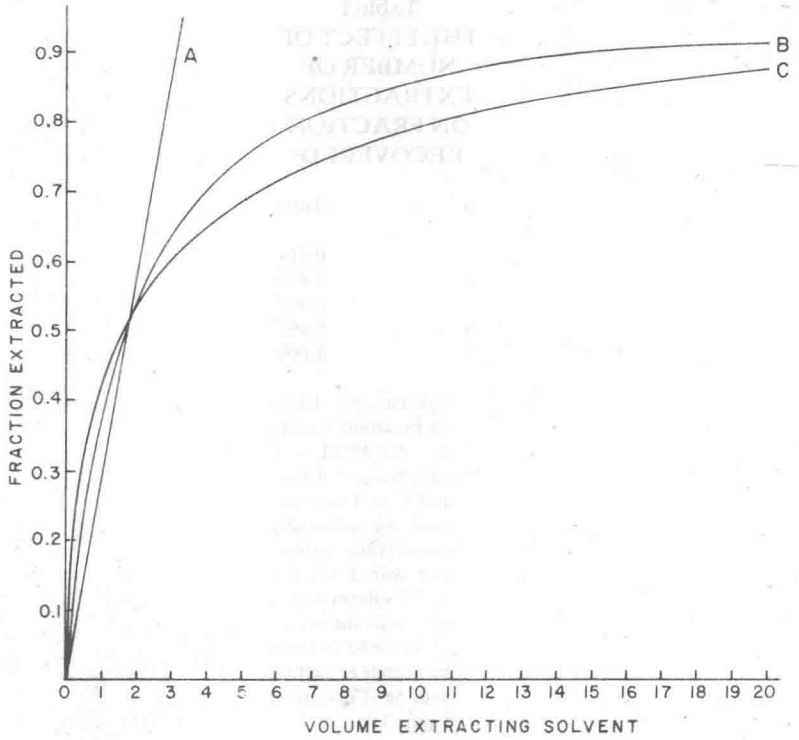

FIGURE 1. Comparison of extractions in three physical systems. The curves are calculated assuming 1 mol of solute is initially present and parameters adjusted so that 50% extraction is achieved with 1.67 *l* of the extraction solvent. Curve A— Liquid-solid extraction of a simple physical mixture. Solubility is assumed to be 1 mol/1.67 *l*. Curve B— Liquid-liquid extraction. The partition coefficient is assumed to be 0.6 and the initial solution concentration 1 *M* (see Equation 6). Curve C— Liquid-solid extraction with adsorption on the solid described by the Langmuir isotherm. M_o = 1 mol and q = 0.3 *M* (see Equation 3).

extraction with the same total volume of solvent, but the rate of dissolution of the analyte is often very slow and becomes the limiting factor in designing the extraction. Very little is known about the mechanisms of extraction of natural products from tissues, but it appears in many cases that the rate of extraction is as dependent on the rate of diffusion of solvent into the matrix as it is on diffusion of the analyte out of it. The rate of extraction can be maximized by prior comminution of the sample and by operating at elevated temperatures. In some cases, chemical destruction of the matrix can also be used to increase the extraction rate. Because a considerable amount of time is often required for extractions of this type, continuous rather than batch processes are usually used.

B. Procedures in Liquid-Solid Extraction

Both continuous and batch process for laboratory scale extractions have been reviewed by Craig and Craig.[4] The simplest batch procedure is agitation of the solids directly with the solvent and separation of the solid and liquid phases by filtration, centrifugation, or decantation. Fresh solvent is added and the process repeated as often as necessary to achieve quantitative recovery of the analyte. The same process can be carried out in a Soxhlet extractor, as pictured in Figure 2. The solvent in the receiving

Table 1
THE EFFECT OF NUMBER OF EXTRACTIONS ON FRACTION RECOVERED[a]

n	fTotal
1	0.418
2	0.876
3	0.970
4	0.998
5	1.000

[a] Calculations based on Equation 3 using q = 0.3 *M*, M_o = 1 mol, $A_{initial}$ = 1 mol, and V = 1 *l*. Compare the essentially quantitative extraction with 5 × 1.0 *l* = 5 *l* solvent in this case with the recovery of 0.686 achieved by a single extraction with 5*l* (Curve C in Figure 1).

flask of the Soxhlet extractor is distilled into the extraction tube where it equilibrates with the solids. When the level of solvent in the tube reaches the top of the overflow arm, the solvent siphons back into the receiving flask. This process repeats itself automatically as long as desired. The advantages of this procedure include automatic repetition and more concentrated analyte solution than obtained by the manual procedure, since the solvent is recycled. Drawbacks include the requirements for a reasonably volatile solvent and stability of the analyte at the boiling point of the solution in the receiving flask, as well as a lower efficiency than the continuous extraction procedures.

The most common continuous process is that in which solvent from a reservoir percolates through a bed of the solid. This is quite efficient because the lowest concentration of analyte will be found towards the head of the column, where the solids are being washed with essentially pure solvent. Optimum performance of a column depends upon good solvent/solid contact which requires a finely divided solid and slow enough flow so that channeling is avoided. To help prevent channeling, a bed of finely divided insoluble material or a glass frit is placed at the bottom of the column. A column can also be operated by recycling the solvent using the apparatus shown in Figure 2; the advantages and disadvantages are the same as with the Soxhlet apparatus, except that the efficiency is improved.

III. LIQUID-LIQUID EXTRACTIONS

A. Principles of Liquid-Liquid Extractions

The behavior of two solvents which are only partially miscible is necessarily nonideal with respect to one another. Nonetheless, a third substance dissolved in the two-phase