APPLICATION OF ABSORBANCY RATIO TO THE ANALYSIS OF CERTAIN PHARMACEUTICAL PREPARATIONS

By

Khalil Abdul-Aziz Quatawneh

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science in Pharmacy
(Pharmaceutical Chemistry) in
the School of Pharmacy of the
American University of Beirut
Beirut, Lebanon
1968

APPLICATION OF ABSORBANCY RATIO TO THE ANALYSIS OF CERTAIN PHARMACEUTICAL PREPARATIONS

By

Khalil Abdul-Aziz Quatawneh

Approved:

Professor Edward N. Vorperian, Pharm. Chem.

Advisor

Professor Usama A. Khalidy, Biochemistry

Member of Committee

Professor Suhayl E. Saheb, Pharm. Chem.

Member of Committee

Date of Thesis Presentation: Aug. 15, 1968.

ACKNOWLEDGEMENT

The author wishes to extend his sincere appreciation and thanks to the Jordanian Government Ministry of Health in giving the chance for a higher education.

Thanks and appreciations are also due to the World Health Organization for the financial support of education and Maintenance during the two years period of advanced study at the School of Pharmacy of the American University of Beirut.

The author wishes to extend further his thanks to the following firms for their kind cooperation and assistance in supplying pure chemicals needed for the experimental work: British Drug House Ltd., Charles E. Frosst & Co., Ciba Pharmaceutical Co., Lakeside Laboratories, Inc., Roussel Corp., Sandoz Pharmaceuticals, Inc., Searle, G.D. & Co., Scierlabs, and Specia.

ABSTRACT

The different methods to be chosen for the simultaneous spectrophotometric analysis of binary systems are discussed. The various equations pertinent to such methods are derived. Detailed discussion of the absorbancy ratio technique regarding its theory, advantages, applicability and limitations has been elaborated.

The experimental part deals with the application of this method to the assay of the following pharmaceutical binary combinations:

1. diphenylhydantoin sodium: phenobarbital
2. hexestrol: phenobarbital
3. naphazoline nitrate: phenylephrine HCl
4. chlorpheniramine maleate: phenylephrine HCl
5. progesterone: estradiol benzoate
6. norethynodrel: mestranol
7. megestrol acetate: ethinylestradiol
8. promethazine: acetylsalicylic acid

The method is found to be efficient, accurate and reproducible. It afforded the quantitative determination of the components in the first five combinations. However, for the last three combinations, it would have served as well had it not been to the unfavorable ratio of the components occurring in the samples.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Historically, the absorbance or absorbancy ratio technique was probably first originated by Hüfner. He was perhaps the first to have shown that the ratio of the two absorbance values at two carefully selected wavelengths is always constant for a given solution containing an absorbing substance. During his investigation on hemoglobin determinations, Hüfner observed invariably a constancy in the ratio of the absorbance values measured at 540 and 560 mu of all hemoglobin solutions under consideration. .This absorbancy ratio was later named after him as the 'Hüfner's quotient' (1).

This observation remained dormant in the literature until well after the second world war, since then, many papers appeared in several scientific journals dealing with the absorbancy ratio techniques as well as to pertinent applications involved for the analysis of complex mixtures. Schroeder, Wilcox, Trueblood, and Dekker (2) tabulated the absorbancy ratio values for a relatively large number of substances of chemical importance. In 1954, Hirt, King, and Schmitt (3) described a graphical approach of the absorbance ratio method for a rapid analysis of two component systems. They pointed out that by proper selection of two analytical wavelengths, a

simple straight-line graph could be obtained to show the relationship between the absorbance ratio and the relative concentration of the two components. Furthermore, they stated that the method could be extended to the analysis of three-component systems. In 1957, Moshe Ish-Shalom, Fitzpatrick, and Orchin (4) recognized the importance and significance of this approach of analysis proposed by King and his co-workers and confirmed its implied usefulness and simplicity for assessing the relative concentrations of components in binary mixtures, without any reference to their actual concentrations. They particularly pointed out the fact that for the application of such a method of analysis one needs not know for purposes of calibration or analysis the total concentration of the components.

Nowadays, the pharmaceutical analyst is confronted with a flood of pharmaceutical preparations containing two or more active ingredients. Consequently the analytical problems facing him are getting more and more involved and difficult to resolve. His primary task would, therefore, be to devise newer techniques and approaches of analysis which are essentially simpler to follow, requiring the shortest possible time, without sacrificing reproducbility, accuracy or dependability of results.

Though the absorbancy ratio method, whenever applicable, could have served long ago as a powerful tool in handling complex analytical problems, and yet it took almost over a century for people working in analytical fields to realize its importance and significance. However, during the last decade, some workers revived its potentiality in handling problems pertinent to the analysis of binary and ternary mixtures. Pernarowski and his co-workers (1,5,6,7), applied the method successfully to the analysis of several pharmaceutical preparations. In 1966, Henning Sattler (8) applied this technique as a possible alternate to the various procedures conventionally used in the quality control of complex drug formulations. Therefore, it is suggestable that when one is encountered with complicated analytical problems, it is often rewarding to consider the possibility of applying absorbancy ratio technique as one of the methods of choice in resolving such cases. Because the adaptation of such a method may eliminate the tedius and time consuming procedures involved in effecting separations prior to the analysis of the sample. Hence, the main advantage of the absorbancy ratio lies in its simplicity time wise and procedure wise. All that is required is to prepare a standard working curve, by

plotting absorbance ratio values versus relative composition, from which the relative concentrations of the components in the given sample could be directly assessed. However, for the determination of the absolute concentrations of the components in the sample, advantage is taken of using isoabsorptive points which approach will be substantiated in full details in subsequent pertinent sections.

As we shall see later, the advantages of the absorbancy ratio technique may be restressed as follows:-

1. Speed and ease in the setting up and use of the analytical method (3).

2. No need for preliminary separation of the components of a given mixture prior its analysis.

3. Its potentiality for assessing relative as well as absolute concentrations of binary mixtures of components having suitable spectra with isoabsorptive wavelengths, even if the initial total weight is not known. This is very useful particularly when the mixture cannot be weighed for some reason such as when it contains some other non-absorbing compounds as part of the mixture or as impurities.

The use of this method is not limited to a particular region of the electromagnetic spectrum. But it is usually

applied in the ultra-violet and intra-red regions. It is, however, more valuable in the ultra-violet than in the intra-red at least in the analysis of pharmaceutical products. Drugs usually contain other substances together with the active ingredients such as excepients, solvents, preservatives, adjuvants, etc. These added materials often absorb in the infra-red region and, therefore, complicate the problem of analysis making it impossible to apply this method without prior separation of the active components in their purest form. In addition, the percentage error is higher when the analysis is carried out in the infra-red than in the ultra-violet region. The accuracy of the results may be affected by the region due to instrumental and experimental factors rather than to theoretical ones (5).

Before ending this introductory note one has to admit that there is much left unsaid. However, pertinent points will be brought up to light in the subsequent sections as reasoning to support the discussions may afford.

THEORETICAL ASPECTS

Absorptiometry

A. Beer's Law

The most fundamental law underlying the practice of spectrophotometry is known as Beer-Lambert or Beer-Bouguer law, sometimes known more simply as "Beer's law". In fact, it is a combination of two laws, the Bouguer (1729) or Lambert (1760) law and Beer's (1852) law (9). The first states that when a monochromatic plane-parallel beam of light enters a homogeneous absorbing medium at right angles to the plane, the rate of decrease in the intensity of the radiant energy with the length of light path through this absorbing medium is proportional to the radiant power of the beam. In other words, each unit length of the homogeneous absorbing material through which light passes absorbs the same fraction of light incident on this unit. The light will be diminished in geometric (not arithmetic), or exponential progression. The second law states that the radiant power of a beam of parallel monochromatic radiation decreases in a similar manner as the concentration of the light absorbing constituent increases.

The combined law may be expressed mathematically, when a single substance is the absorbing one, as

$$\text{Log } Po/P = A = abc \qquad (1)$$

Where Po = incident radiant power

P = transmitted radiant power

A = absorbance

a = absorptivity (a constant depending on the wavelength of radiation and the nature of absorbing material)

b = internal cell length in cm.

c = concentration of the absorbing substance in gms/liter

It may be expressed also as

$$\text{Log } Po/P = A = EbC \qquad (2)$$

Where E is molar absorptivity and C is the contration of the absorbing substance in moles/liter.

B. Deviation from Beer's Law

In quantitative photometric work, probably the most generally useful indicator of error is Beer's law. Deviation from this law is usually tested by plotting absorbance versus concentration. When a straight line passing through the origin is obtained, the substance is said to obey Beer's law. The evidence for failure will be the production of a non-linear curve. Deviation may be

classified as either positive if the plot curves up towards the absorbance axis or negative if it bends towards the concentration axis.

Deviation may be due to one or more of the following reasons:

1. Dissociation or association of the absorbing solute or its interaction with the solvent in a way that will alter its concentration.

2. The use of too wide bandwidth of radiation. In fact, in deriving Beer's law, the use of a monochromatic beam of radiation is implied. The width of radiation band may have a great effect on the results specially where there are very narrow absorption bands. If the absorbance is measured at a sharp maximum using a slit width which permits the passage of wavelengths on either side of the maximum, the measured absorbance will be smaller than the true value. If, however, the measurement is made at a maximum or in the vicinity of a shoulder, the measured absorbance will be greater than the true value.

3. Change in temperature often shifts ionic equilibria, and, in addition, an increase in temperature exerts a bathochromic shift on ions in solution.

4. Scattered radiation, stray light entering the detector housing, and other instrumental errors.

C. Concentration Limit on Validity

The restriction that absorption centers not interact with themselves or other species causes Beer's law to be a limiting law applicable mainly in dilute solutions. At high concentration the charge distribution either in the absorbing or excited species or both may be altered. It follows that the energy needed for excitation and hence the position, shape, and height of the absorption region may be altered.

The index of refraction of the solution (n) will change as the concentration changes. The impact of refractive index on molar absorptivity can be shown to be:

$$E. \frac{n}{(n^2 + 2^2)}$$ rather than E itself which is usually considered to be constant with concentration. An appreciable change in the refractive index will, therefore, lead to deviation from Beer's law when E rather than

$$E. \frac{n}{(n^2 * 2^2)}$$ is used (10).

Spectrophotometric Analysis of Binary Mixtures

A. Spectral Characteristics of the Components

The simplest way for spectrophotometric determination of a two component system depends on the spectral characteristics of its components. The Spectra of the two components in a binary mixture, taken for two equi-

concentration (by weight) solutions and using the same solvent, may have either of the following forms:

1. There exists a region where just only one of the two components will absorb. Examples of this type are illustrated from our preliminary screening of several drugs used in pharmaceutical formulations, e.g.

Ephedrine sulfate and tripplennamine HCl (Fig. 1)

Khellin and 7. (2-3 dihydroxypropyl) theophylline (Fig.2)

Aminopyrine and diallyl barbituric acid (Fig. 3)

Tetracycline HCl and chloramphenicol (Fig. 4)

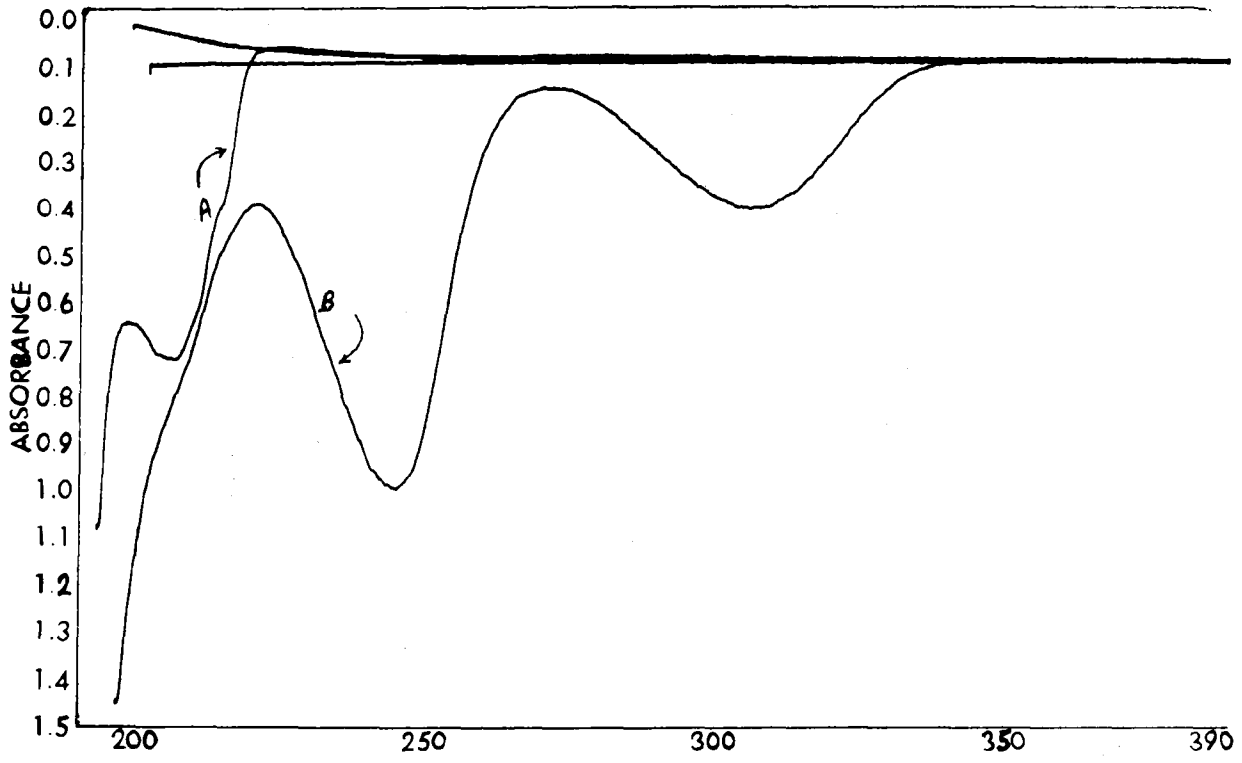

Figure 1. UV spectra of: (a) 20 ugm./ml. of ephedrine sulfate in water.(A).
(b) 20 ugm./ml. of tripplennamine HCl in water (B).

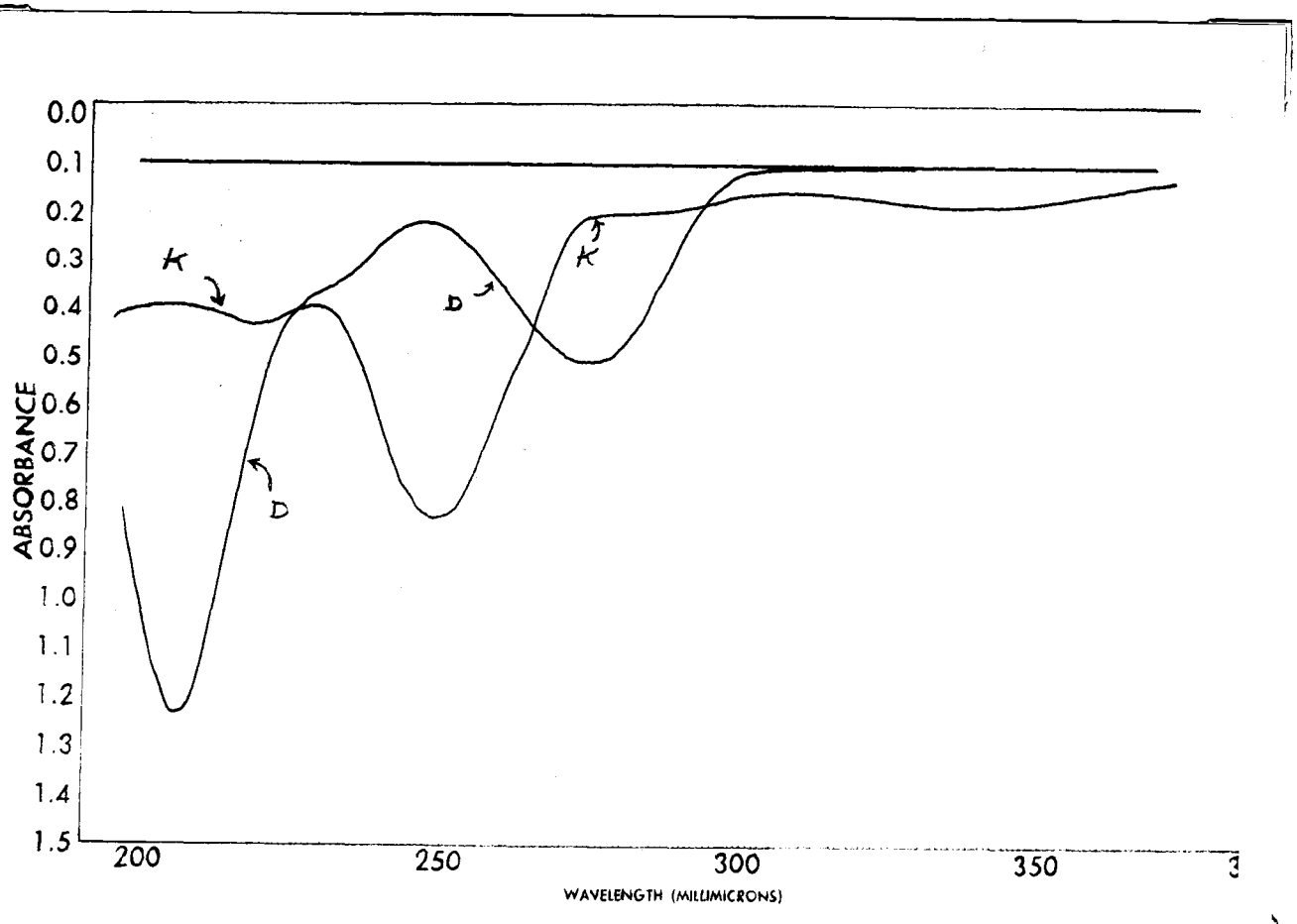

Figure 2. UV spectra of: (a) 11.28 ugm./ml. of khellin in water (K).

(b) 11.28 ugm./ml. of dyphylline in water (D).

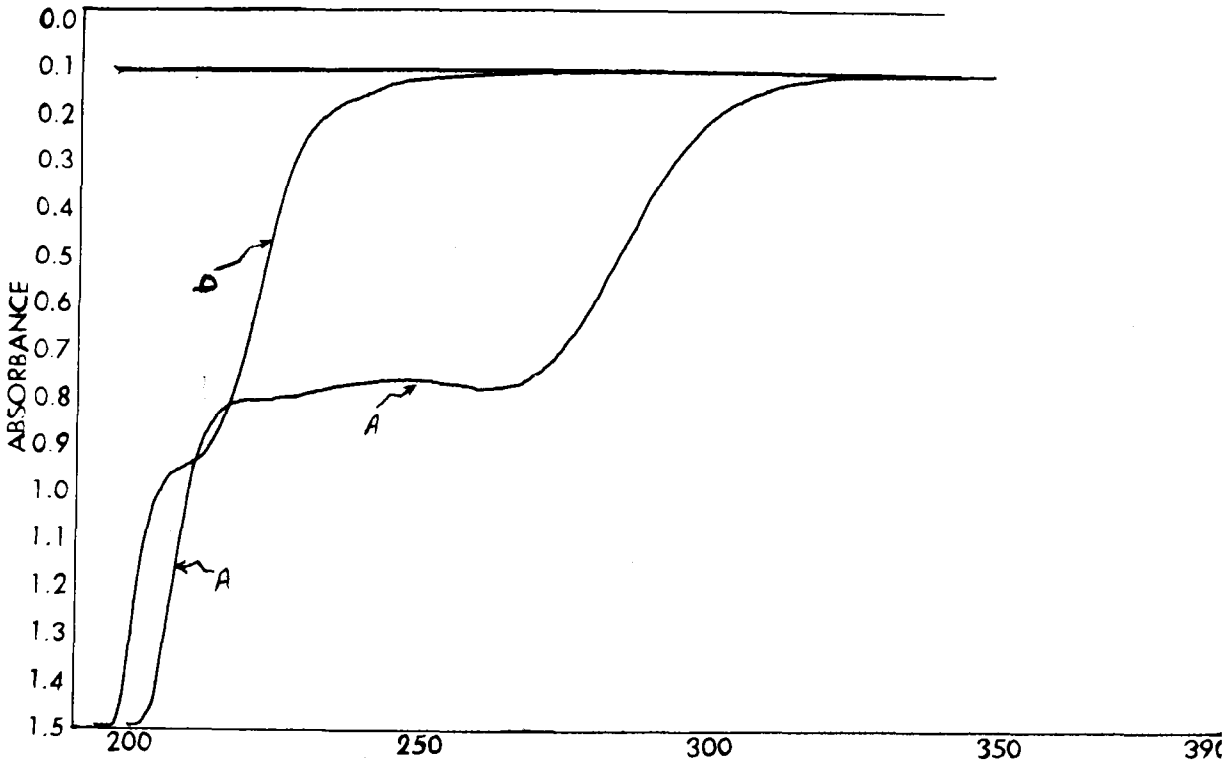

Figure 3. UV spectra of: (a) 19.6 ugm./ml. of aminopyrine in water (A).
(b) 19.6 ugm./ml. of dial in water (D).

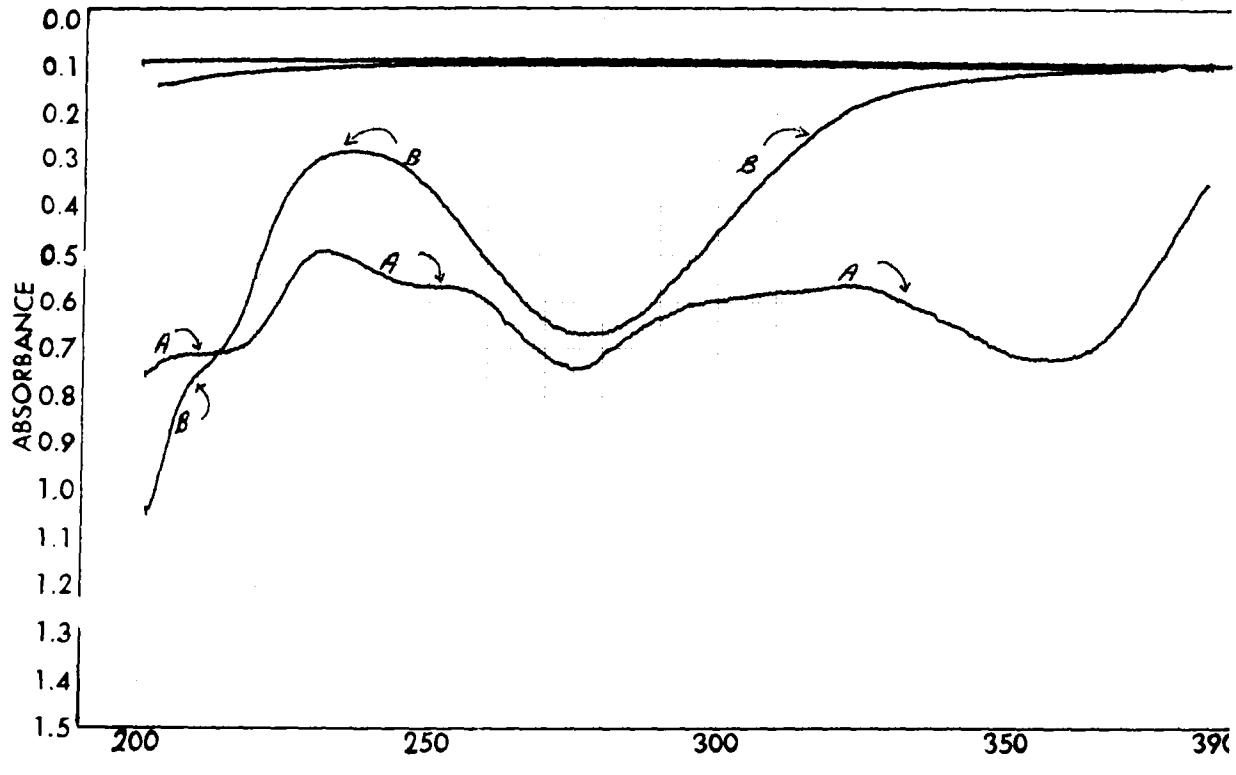

Figure 4. UV spectra of: (a) 19.6 ugm./ml. of tetracycline-HCl in water (A).

(b) 19.6 ugm./ml. of chloramphenicol in water (B).

2. The two substances absorb all through the whole region concerned and their spectra reveal no isoabsorptive point (a wavelength at which the two substances have the same absorptivity value). This is exemplified by methoin and phenobarbitone spectra (Fig. 5).

3. Each of the two components absorbs through the whole region where the analysis is to be carried out, but their spectra have one or more isoabsorptive wavelengths. The presence of such an isoabsorptive point constitutes the very heart of the absorbancy ratio method of analysis. Various illustrations of this case will be found in the experimental part of this thesis.

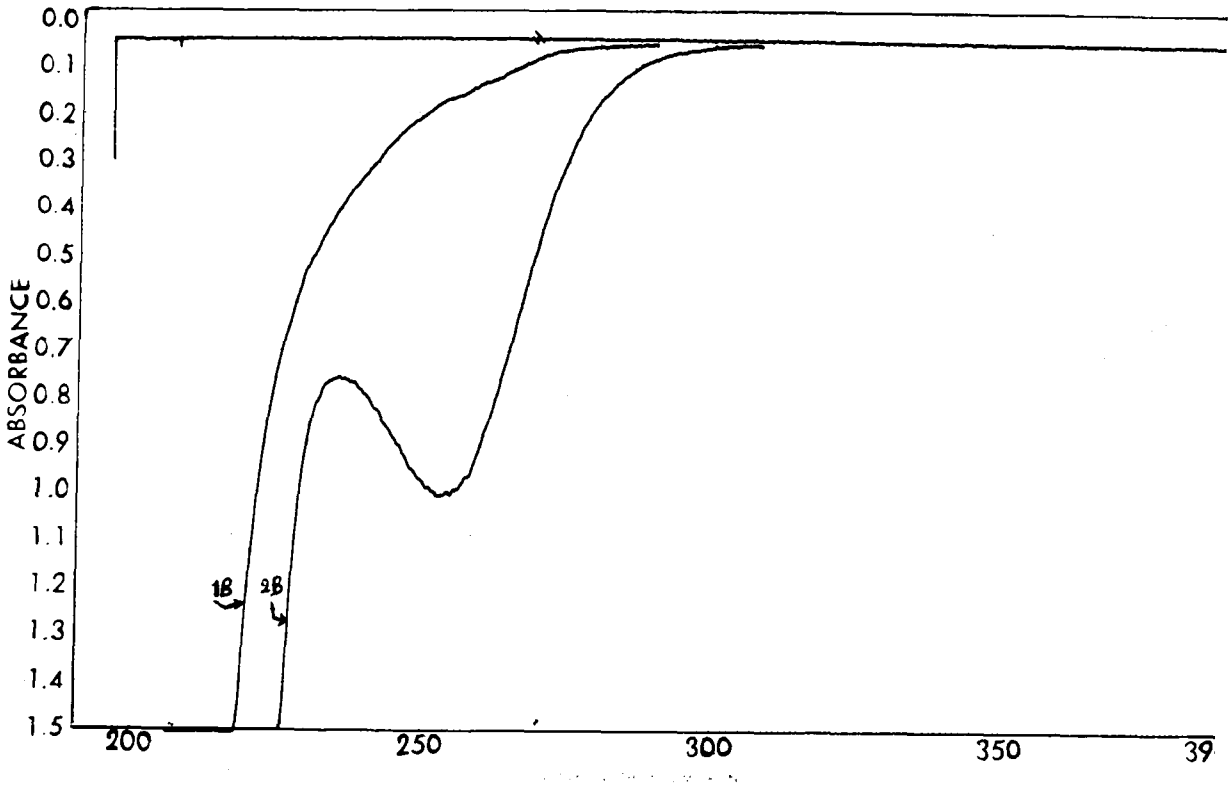

Figure 5. UV spectra of: (a) 29.79 ugm./ml. of methoin in N/10 aqueous NaOH (1B).
(b) 29.79 ugm./ml. of phenobarbitone in N/1o aqueous NaOH.

B. Analytical Approach

The spectra of any two substances in a binary mixture that absorb radiation of a certain region in the electromagnetic spectrum fall within the previous categories. The type of spectrophotometric analysis to be followed for a given mixture will depend upon the spectral characteristics of its components. The analytical approaches may be categorized as follows:

1. For the first type (e.g. Figs. 1, 2, 3, and 4), a suitable wavelength in the region, in which just one component absorbs, is selected to carry out the analysis of that particular component. Analysis is then run as if we had only this single component in solution. It is performed simply by comparing the extent of absorption of radiant energy at the selected wavelength by a solution of the test material and a series of standard solutions. A simple one component calculation based on Beer's law gives the concentration of that single absorbing component in the mixture.

To complete the analysis for the second component of the mixture, another suitable wavelength, is chosen. If at this wavelength the first component does not absorb, analysis is carried out as above. However, if both substances absorb radiation, the absorbance value of the second component will be

$$A_{2nd} = A_{total} - A_{1st} \qquad (3)$$

But the absorbance of the first component "A_{1st}" can easily be calculated since its concentration has already been assessed in the previous step mentioned above. Intricate calculation may be avoided by simply using an equi-concentration (by weight) of the first component as occurring in the mixture as the blank whereby an automatic subtraction of A_{1st} is effected.

2. In the second type (e.g. Fig. 5), when no region could be detected in which just one component absorbs and when the spectra of the two components have no isoabsorptive wavelength, it is still possible to determine their concentrations by making measurements at two selected wavelengths. A pair of simultaneous equations may be used to determine the respective concentrations of the two components under consideration. The validity of such simultaneous equations is based on the assumption that the substances concerned contribute additively to the total absorbance at each of the two chosen wavelengths. If this assumption is taken for granted, then at a given wavelength, the total absorbance for a certain mixture can be expressed as:

$$A_{total} = A_1 + A_2 + A_3 \ldots\ldots\ldots + A_i \qquad (4)$$

where i stands for the number of absorbing components in

that mixture. But it is known that A for each component is equal to

$$A = abc$$

Substituting each A by its value, we get

$$A_{total} = A_1bc_1 + a_2bc_2 + a_3bc_3 \ldots\ldots + a_ibc_i \qquad (5)$$

$$A_{total} = b(a_1c_1 + a_2c_2 + a_3c_3 \ldots\ldots + a_ic_i) \qquad (6)$$

To calculate the concentration of each component, absorbance measurements must be made at as many wavelengths as there are components in the system. Generally the wavelengths are so chosen that at each a different component has a high absorbance value while the sum of the absorbances of all the others is small. The absorptivity constants of all constituents must be determined at all chosen analytical wavelengths. This requires measurements of absorbance values for the pure components at the specified wavelengths so that the respective absorptivities could be calculated. Hence, if absorptivity values are known for the pure components, one may analyze an i component system by making absorbance measurements at i different wavelengths. A series of i equations is then obtained and these must be solved simultaneously. The solution of such i simultaneous equations is a formidable task for more than four equations even for an electric calculator (11). For a binary mixture, however, calculations are rather easy when applied to the following equations:

$$A_1 = a_{x1}\, bc_x + a_{y1}\, bc_y \qquad (7)$$

$$A_2 = a_{x2}\, bc_x + a_{y2}\, bc_y \qquad (8)$$

Where A_1 and A_2 are the absorbance values of the mixture at the two wavelengths 1 and 2 respectively, a_{x1} and a_{x2} are the absorptivities of component x at the two wavelengths, and a_{y1} and a_{y2} are that of component y at the same two wavelengths. The b term is eliminated from the two equations by using 1 cm cell for all the measurements. The values A_1 and A_2 are determined experimentally for the sample solution. The four absorptivities a_{x1}, a_{x2}, a_{y1} and a_{y2} can be determined experimentally under the same conditions using standard solutions of the two pure components. Though these values may sometimes be found in the literature, but it is advisable to be determined by the analyst under the same set of experimental conditions.

If, however, the relative concentrations rather than the absolute concentrations of the different components in the mixture are required, the previous equations could be further modified as follows:

Assuming that in the analysis the internal cell length, b, is one cm, then:

$$A_1 = a_{x1}\, c_x + a_{y1}\, c_y \qquad (9)$$

$$A_2 = a_{x2}\, c_x + a_{y2}\, c_y \qquad (10)$$

Solving for c_x and c_y one can obtain

$$c_x = \frac{A_1 a_{y2} - A_2 a_{y1}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \qquad (11)$$

$$c_y = \frac{A_2 a_{x2} - A_1 a_{x2}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \qquad (12)$$

Now if we let

$$F_x = \frac{c_x}{c_x + c_y} \qquad (13)$$

$$F_y = \frac{c_y}{c_x + c_y} \qquad (14)$$

Hence $F_x + F_y = 1$ (15)

Substituting c_x and c_y in equation 14 by their values obtained from equations 11 and 12 respectively and then dividing the numerator and denomenator by A_2 to get:

$$F_y = \frac{a_{x1} - \frac{A_1}{A_2} \cdot a_{x2}}{\frac{A_1}{A_2}(a_{y2} - a_{x2}) - (a_{y1} - a_{x1})} \qquad (16)$$

Having obtained the value of F_y, F_x can be easily determined by the use of equation 15. However, one may, as well, determine F_x first, by substituting, in equation 13 the values of c_x and c_y followed by the assessment of F_y by equation 15.

Another convenient equation for finding the relative concentration may be derived in the following manner:

$$\frac{A_1}{A_2} = \frac{a_{x1}c_x + a_{y1}c_y}{a_{x2}c_x + a_{y2}c_y} \qquad (17)$$

Dividing the numerator and denomenator by $(c_x + c_y)$ and then substituting F_x for $\left(\frac{c_x}{c_x + c_y}\right)$ and F_y for $\left(\frac{c_y}{c_x + c_y}\right)$ we get

$$\frac{A_1}{A_2} = \frac{a_{x1}F_x + a_{y1}F_y}{a_{x2}F_x + a_{y2}F_y} \qquad (18)$$

Substituting F_y by its value, $1 - F_x$ (from equation 15), to get:

$$\frac{A_1}{A_2} = \frac{a_{x1}F_x + a_{y1}(1 - F_x)}{a_{x2}F_x + a_{y2}(1 - F_x)} \qquad (19)$$

$$= \frac{F_x(a_{x1} - a_{y1}) + a_{y1}}{F_x(a_{x2} - a_{y2}) + a_{y2}} \qquad (20)$$

Solving for F_x, we get

$$F_x = \frac{A_2a_{y1} - A_1a_{y2}}{A_1(a_{x2}-a_{y2}) - A_2(a_{x1}-a_{y1})} \qquad (21)$$

The equations 18 - 21, in fact, illustrate the application of the absorbancy ratio method to the analysis of a mixture

whose components spectra are revealing no isoabsorptive point. It is seen that these equations are not more simple than the previous conventional equations 11 - 16. The use of the absorbancy ratio method, on the other hand, is much more valuable in the analysis of mixtures of type three where an isoabsorptive point does occur. This will be discussed later in more details.

Using the forementioned equations, 16 and 21, one may determine the relative concentrations without necessarily knowing the initial total concentration of the components in the mixture. If, however, the original total weight or concentration of the constituents is known beforehand, another simplified approach could be used to ascertain the relative as well as the actual concentrations. In this case, only one absorbance measurement at a suitable wavelength is needed (4). To elucidate the above statement mathematically, let $(c_x + c_y)$ be equal the original total concentration of the two components in a binary mixture. If assumed that the cell length, b, is one cm, then at a certain analytical wavelength:

$$A = a_x c_x + a_y c_y \qquad (22)$$

Dividing by $(c_x + c_y)$ we get

$$\frac{A}{c_x + c_y} = \frac{a_x c_x}{c_x + c_y} + \frac{a_y c_y}{c_x + c_y} \qquad (23)$$

$$\frac{A}{c_x + c_y} = a_x F_x + a_y F_y \qquad (24)$$

$$= a_x F_x + a_y(1-F_x) \qquad (25)$$

$$= a_x F_x + a_y - a_y F_x \qquad (26)$$

$$= F_x(a_x - a_y) + a_y \qquad (27)$$

In equation 27 the term F_x only is the unknown. The absorbance and the absorptivities can be ascertained experimentally. Finding the value of F_x, and knowing the initial total concentration, one could easily calculate for the values of the absolute concentrations of the two components in the mixture.

3. The third approach will be discussed fully in the following chapter.

ABSORBANCY RATIO METHOD

Finally, we come to the so called absorbance or absorbancy ratio method which is sometimes abbreviated simply by Q analysis. This method of analysis applies in cases where the spectra of the components in a binary mixture reveal the presence of one or more isoabsorptive points. When the spectral characteristics of the components are suitable, the application of this technique to analysis is often capable of yielding very satisfactory results. The standard deviation for the average recovery of the individual components in a binary mixture, when assayed by this method, approximates to 1% when analyses are carried out in the ultra-violet region; and to 2 to 3% in the infra-red region (5).

Q analysis may be used for finding the relative as well as the absolute concentrations. In this method also one needs not know either for purpose of calibration or for analysis the total concentration of the components in order to determine the relative concentration. If absolute concentrations are desired, the initial total weight or concentration of the sample must be known or otherwise should be determined.

Since this work is concerned with the application of this method to the analysis of pharmaceutical

preparations, I will try to discuss it in a somewhat detailed and stepwise manner in the following sections.

Constancy of the Absorbance Ratio Value

This method is based on the fact that, under the same set of experimental conditions, the ratio of two absorbance values determined on the same solution at two different wavelengths is always constant. This stems from the fact that absorptivity of the solution at a certain wavelength is constant when measured under identical test conditions. The ratio of the two absorbance values of a particular solution at two selected wavelengths is equal to the ratio of the two absorptivities of this solution at these two wavelengths. In mathematical terms we have:

$$\frac{A_1}{A_2} = \frac{a_1 bc}{a_2 bc} = \frac{a_1}{a_2} \qquad (28)$$

But $\frac{a_1}{a_2}$ is constant. Therefore, it implies that $\frac{A_1}{A_2}$ is also a constant value. This ratio is called the absorbance or absorbancy ratio and is referred to by the symbol Q together with the specifications of the two wavelengths at which the measurements have been carried out.

Derivation of Equations

As mentioned before, the usefulness of the absorbancy ratio method of analysis depends on the presence of an isoabsorptive wavelength. A comparison of equations derived for the case of the absence of such a point to that where an isoabsorptive point does exist will be shown below. It is then very easy for one to recognize the simplicity and usefulness of the later type.

1. When No Isoabsorptive Point is Present

Two suitable wavelengths are chosen for measuring the absorbancies. At the two wavelengths the total absorbance values of the two components "x and y" of a binary mixture are given by:

$$A_1 = a_{x1}bc_x + a_{y1}bc_y \qquad (29)$$

$$A_2 = a_{x2}bc_x + a_{y2}bc_y \qquad (30)$$

The absorbancy ratio "Q_{1-2}" is equal to

$$Q_{1-2} = \frac{A_1}{A_2} = \frac{b(a_{x1}c_x + a_{y1}c_y)}{b(a_{x2}c_x + a_{y2}c_y} \qquad (31)$$

$$= \frac{a_{x1}c_x + a_{y1}c_y}{a_{x2}c_x + a_{y2}c_y} \qquad (32)$$

Divide the numerator and denomenator by $(c_x + c_y)$ to get

$$Q_{1-2} = \frac{a_{x1}\left(\frac{c_x}{c_x+c_y}\right) + a_{y1}\left(\frac{c_y}{c_x+c_y}\right)}{a_{x2}\left(\frac{c_x}{c_x+c_y}\right) + a_{y2}\left(\frac{c_y}{c_x+c_y}\right)} \qquad (33)$$

$$= \frac{a_{x1}F_x + a_{y1}F_y}{a_{x2}F_x + a_{y2}F_y} \qquad (34)$$

Substitute F_y by its value, $1 - F_x$, to have

$$Q_{1-2} = \frac{a_{x1}F_x + a_{y1}(1-F_x)}{a_{x2}F_x + a_{y2}(1-F_x)} \qquad (35)$$

$$= \frac{F_x(a_{x1} - a_{y1}) + a_{y1}}{F_x(a_{x2} - a_{y2}) + a_{y2}} \qquad (36)$$

To use this equation, one has to find the values of four absorptivities as in the case of the previously mentioned simultaneous equation method (i.e. equation 16). At first sight equation 36 does not warrant any advantages over equation 16, however, the first may be used advantageously by graphics to assess the relative concentration without recourse of determining the four absorptivities.

Equation 36 shown above indicates that a plot of Q_{1-2} versus F_x is a curve, the extremities of which are given by the expressions $\frac{a_{x1}}{a_{x2}}$ (the absorbancy ratio for component x) and $\frac{a_{y1}}{a_{y2}}$ (the absorbancy ratio for component

y). To construct such a curve, Q_{1-2} observed for various relative concentrations of mixtures of x and y are to be used. This type of analysis must necessarily be graphical. The relative concentrations of the two components in an unknown sample are determined by the use of this absorbance ratio graph with no additional steps. All one has to do is to find the Q_{1-2} for the unknown sample solution. It is easy then to assess the relative concentrations of the unknown sample constituents directly from the working curve.

To find the absolute concentrations, however, the initial weight of the sample in its pure form (with no absorbing compounds other than the main components) should be known, otherwise a further step has to be undertaken. This further step requires the knowledge of four absorptivities and hence the absorbance ratio method will lose its usefulness.

2. <u>When an Isoabsorptive Point (s) is present</u>

In this case, equations are derived with the same sequence of thought as indicated in the previous case except for the fact that here the isoabsorptive wavelength must be incorporated as one of the two reference points. We refer to the isoabsorptive wavelength by the symbol i and the other reference wavelength by 2. At these two

reference points Q will be equal to

$$Q_{2-i} = \frac{a_{x2}c_x + a_{y2}c_y}{a_i(c_x+c_y)} \qquad (37)$$

In this equation, a_i stands for the absorptivity of either of the two components or for any conceivable mixture of both at the isoabsorptive wavelength, i.e. a solution of any of the two components or of the two mixed together will have the same absorptivity at that specified isoabsorptive wavelength. Again dividing the numerator and denomenator by $(c_x + c_y)$ and using the terms F_x and F_y for their respective values we have:

$$Q_{2-i} = \frac{a_{x2}F_x + a_{y2}F_y}{a_i(F_x + F_y)} \qquad (38)$$

$$= \frac{a_{x2}F_x + a_{y2}(1-F_x)}{a_i(1)} \qquad (39)$$

$$Q_{2-i} = \frac{F_x(a_{x2} - a_{y2}) + a_{y2}}{a_i} \qquad (40)$$

$$= F_x\left(\frac{a_{x2}}{a_i} - \frac{a_{y2}}{a_i}\right) + \frac{a_{y2}}{a_i} \qquad (41)$$

But $\frac{a_{x2}}{a_i}$ is equal to Q_x, the absorbance ratio for pure x;

and $\frac{a_{y2}}{a_i}$ is equal to Q_y, the absorbance ratio for pure y.

Substituting each for its corresponding value in the above equation 41 we get:

$$Q_{2-i} = F_x(Q_x - Q_y) + Q_y \qquad (42)$$

This is the equation of a straight line with a slope value equal to $(Q_x - Q_y)$ and an intercept value of Q_y. Its ends are given by $\frac{a_{x2}}{a_i}$ and $\frac{a_{y2}}{a_i}$, the absorbancy ratio for the pure components x and y respectively. Such a curve is usually obtained by using standard solutions of variable relative concentrations and is referred to as the standard working curve, hereafter referred to simply as the working curve. To find the relative concentration of a two component system, this curve may be used with even more reliable results than the curve plotted from equation 36 above. Here I would like to restress the point that the sample solution should be prepared in the same way as the standard solutions and be subjected to the same experimental treatment so that much more reliable and valid results could be attained.

By investigating equation 42, one can easily realize that the Q_{2-i}, value is independent of the absolute concentrations of the components, but is rather dependent on their relative concentrations. This general statement also holds true for the Q value of any

single component in that it is independent of its actual concentration. In other words, the Q value will be the same at any dilution of the solution provided that the concentration lies within the limit of accurate range of absorbance readings.

To find the relative concentration, the above equation 42, may be used directly or by plotting the working curve. In this case knowledge of absorptivity values is not necessary and in addition knowledge of the initial total weight of the components in the mixture is also not essential.

Unlike the previous case, where there was no isoabsorptive point, here it is possible to assess absolute concentrations even though the initial total weight of the sample components is not known. This is clarified by the following equations:

The absorbance of a two component system at the isoabsorptive point is given by:

$$A_i = a_i \, b \, c_{total} \qquad (43)$$

where c_{total} is the total concentration of the two components in the sample.

Using a one cm cell, it becomes:

$$A_i = a_i \, c_{total} \qquad (44)$$

$$c_{total} = \frac{A_i}{a_i} \qquad (45)$$

In words, the total concentration of the components in the mixture is equal to its absorbance at the isoabsorptive wavelength divided by its absorptivity at that wavelength. It is good at this point to be reminded of the fact that the absorptivity for the mixture is the same as for any of the pure components at this point.

From equation 42 we have

$$F_x = \frac{Q_{2-i} - Q_y}{Q_x - Q_y} \qquad (46)$$

F_x is the fraction of component x in the mixture of total weight c_{total}. Therefore, the absolute concentration of component x in the mixture is

$$c_x = F_x \, c_{total} \qquad (47)$$

$$= \left(\frac{Q_{2-i} - Q_y}{Q_x - Q_y}\right) \cdot \frac{A_i}{a_i} \qquad (48)$$

The value of the absolute concentration of component y is then easily assessed since the total weight, c_{total}, is known. It is also possible to calculate for c_y from the equation

$$c_y = \frac{(Q_{2-i} - Q_x)}{Q_y - Q_x} \cdot \frac{A_i}{a_i} \qquad (49)$$

To avoid the use of a_i (the absorptivity at the isoabsorptive wavelength) another equation could be

derived as follows. The absorbance value for the mixture solution at the isoabsorptive point is given by

$$A_i = a_i \, b \, c_{total} \qquad (50)$$

Similarly the two absorbance values for the two pure components at that wavelength, i, are expressed by:

$$A_{x\text{-}std.i} = a_i \, b \, c_{x\text{-}std.} \qquad (51)$$

$$A_{y\text{-}std.i} = a_i \, b \, c_{y\text{-}std.} \qquad (52)$$

$x_{std.}$ and $y_{std.}$ means that the components x and y are used in their pure forms as standards.

Dividing equation 50 by either of the two equations 51 and 52 we get

$$\frac{A_i}{A_{x\text{-}std.i}} = \frac{c_{total}}{c_{x\text{-}std.}} \qquad (53)$$

$$\frac{A_i}{A_{y\text{-}std.i}} = \frac{c_{total}}{c_{y\text{-}std.}} \qquad (54)$$

$$c_{total} = c_{x\text{-}std.}\frac{A_i}{A_{x\text{-}std.i}} = c_{y\text{-}std.}\frac{A_i}{A_{y\text{-}std.i}} \qquad (55)$$

Therefore, using as a standard a solution of any of the two components in its purest form, the total concentration of the unknown sample mixture could be determined. It is clear that equation 55 is valid for absorbance measurements taken at the isoabsorptive wave-

length. This is one of the major advantages associated with the existence of such a point.

As it was mentioned before, the relative concentration may be assessed by the direct application of equation 46 or through the absorbance ratio curve. Having assessed the absolute value of the original total concentration or weight of the unknown sample through equation 55 and its relative composition through equation 46, it is then easy to calculate for the absolute concentration values of its components. For component x, for example, its absolute concentration would be:

$$c_x = \frac{(Q_{2-i} - Q_y)}{Q_x - Q_y} \cdot \frac{A_i \, c_{x-std.}}{A_{x-std.i}} \qquad (56)$$

Since $\frac{A_{x-std.i}}{c_{x-std.}}$ equals a_i (the absorptivity of component x at the isoabsorptive wavelength), it follows that

$$\frac{A_i \, c_{x-std.}}{A_{x-std.i}} = \frac{A_i}{a_i} \qquad (57)$$

Equation 56 is, therefore, the same as equation 48 with the term a_i substituted by its derived value $(\frac{A_{x-std.i}}{c_{x-std.}})$.

Extension of the Method to the Analysis of Ternary Mixtures

During their theoretical discussions and elaboration of procedures, King and Schmitt concluded that "it is possible to extend the two component absorbance - ratio method to three components, at the expense of some rapidity and convenience, but still with some advantage over the conventional solving of three simultaneous equations" (3). The absorptiometric principles involved in its application to the analysis of ternary mixtures do not differ from those for binary mixtures. Derivation of equations is based on Beer's law and the principles of absorbancy ratio analysis discussed in the previous sections. The major disadvantages of this type of analysis is primarily due to the almost complete lack of common isoabsorptive points in the spectra of the three components (7).

Since in this thesis, all the experiments are carried out on binary mixtures, it will not be attempted here to discuss or derive the equations used in the analysis of ternary mixtures. Detailed elaboration of this type of analysis can be found in the following two major references (3, 7).

EXPERIMENTAL

General Procedure for Screening Prospective Binary Mixtures

The spectral characteristics of the individual components of the binary mixture are first determined. This may require the usage of different solvents, so that the best to be chosen will be that which will reveal the most suitable spectra for analysis. Variation of pH of the solution is sometimes very useful in modifying the spectral characteristics of some compounds in such a way whereby the absorbancy ratio analysis becomes feasible.

When the spectra of the two components in a binary mixture are found to be suitable, the isoabsorptive point(s) is then to be located. The following two procedures may be used for locating such a point.

1. The spectrum of a solution of one substance is recorded relative to a solution of the other substance (i.e. the second solution serves as the blank). The wavelength at which an absorbancy reading of zero is observed represents an isoabsorptive point provided that the concentration of the two substances are identical.

2. The spectra of the two substances are recorded relative to their common blank. When the two compounds

have identical concentrations, the point(s) of intersection of their spectra represents an isoabsorptive point.

The two wavelengths to be used in the analysis are then chosen. These will be usually the wavelength at which one of the two substances exhibits maximum absorption and the isoabsorptive wavelength. The absorbancy ratio values at these two selected wavelengths are determined for a number of standard mixtures of well known different relative compositions. The working curve is then plotted as "Q" versus the percentage of one of the two components in the mixture.

Apparatus

Perkin-Elmer model 202 ultraviolet - visible spectrophotometer was used in this investigation. The analytical balance used was Mettler's balance type H16. All the volumetric glassware necessary to the investigation were of Kimax grade A type.

Purity of Chemicals

Most of the chemicals used were received from K and K Laboratories. They were labeled "for investigational and manufacturing use only - not for drug use". Some chemicals, however, were obtained from other firms and this

will be notified later upon their mentioning in the text.

Pharmaceutical Combinations Screened

The spectral characteristics, of a quite large number of medicinal compounds, published (12, 13) were studied. The possible binary combinations anticipated to fit the absorbancy ratio analysis were selected for further investigation. Over twenty of such combinations were studied, but the following were found to be favorable for analysis by the proposed method.

A. Diphenylhydantoin Sodium: Phenobarbital

1. Spectral Characteristics

To obtain an isoabsorptive point equi-concentrations (by weight) of diphenylhydantoin, rather than its sodium salt, and phenobarbital are to be used. Equi-concentrations (by weight) of diphenylhydantoin sodium and phenobarbital will exhibit no isoabsorptive point regardless of pH modifications. When water or alcohol were used diphenylhydantoin and phenobarbital spectra revealed no isoabsorptive point. However, 0.4 normal aqueous NaOH was found to be a good solvent which when used, the spectra of these two compounds exhibited two isoabsorptive points at 232 and 236.5 mu. Nevertheless, 0.4N aqueous NaOH is not

an ideal solvent. The instability of phenobarbital should be kept in mind when using such an alkaline medium. The spectral measurements should be made within one hour of the preparation of the solution; under which conditions, the decomposition of phenobarbital is minimized if not completely avoided.

The wavelengths chosen for the analysis were 255 mu (the point at which phenobarbital absorbs ultra-violet radiant energy strongly) and 236.5 mu (the isoabsorptive point). The spectra of two equal concentrations of diphenylhydantoin and phenobarbital are shown in (Fig. 6).

2. Procedure

In two separate 500 ml. volumetric flasks dissolve about 25 mg. accurately weighed of each of phenobarbital and diphenylhydantoin using 0.01N aqueous NaOH. From these two standard solutions prepare the following dilutions in 8 separate 25 ml. volumetric flasks (Table I).

Table I

Standard Mixtures of Phenobarbital and Diphenylhydantoin Used for Plotting the Q-Curve

Solution	1	2	3	4	5	6	7	8
ml. of Phenobarbital Solution	0.5	2	4	6	8	10	4	0
ml. of Diphenylhydantoin Solution	4	4	4	4	4	4	0	4

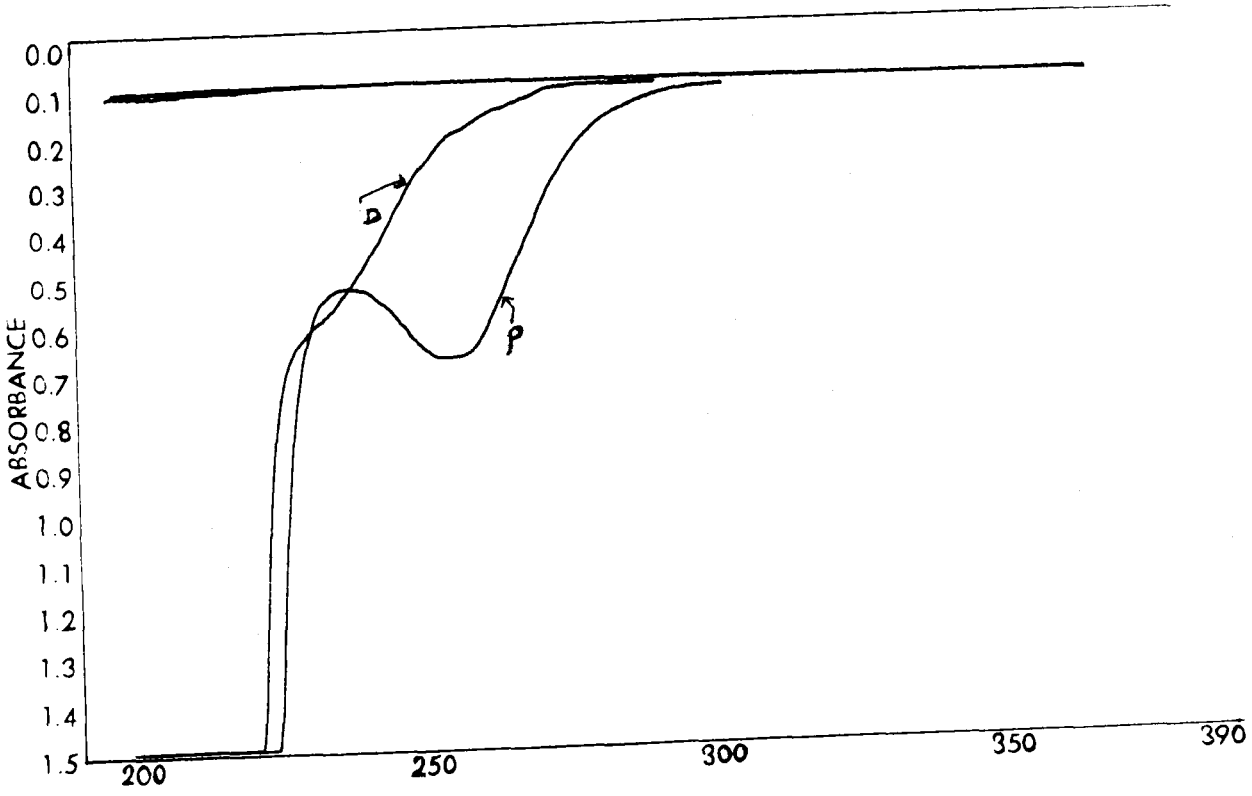

Figure 6. UV spectra of: (a) 20 ugm./ml. of phenobarbital in 0.4N aqueous NaOH (P).
(b) 20 ugm./ml. of diphenylhydantoin in 0.4N aqueous NaOH (D).

Pipet 10 ml. of N/1 aqueous NaOH into each flask and then adjust the volume with 0.01N aqueous NaOH rather than distilled water. This is to compensate for any excess alkali in the mixtures containing larger aliquots of phenobarbital solution.

The unknown sample solution is prepared in the following manner. Weigh accurately, as completely as possible, the contents of about twenty capsules, and ascertain the weight of powder per capsule. Mix the powder well and transfer an accurately weighed portion, representing about 25 mg. of diphenylhydantoin, to a 500 ml. volumetric flask. Dissolve and make up to volume with 0.01 N aqueous NaOH. Filter the solution through Whatman No.1 filter paper discarding the first 25 ml. of the filtrate and collecting the rest in a dry clean container. From this filtrate, pipet an aliquot of 5 ml. into 25 ml. volumetric flask. Pipet 10 ml. N/1 aqueous NaOH and adjust to volume with 0.01N aqueous NaOH. It is very important to note here that the addition of the 10 ml. aliquot of N/1 aqueous NaOH to each solution is made just before taking the spectrum of that solution. The spectra of all solutions are taken against their common blank (Fig. 7). From these spectra the absorbancy values for each solution (both the standards and the

unknown) are measured at the two selected wavelengths (255 and 236.5 mu). A plot of $Q_{255-236.5\ mu.}$ versus the percentage of phenobarbital in the standard mixtures gives a straight line as shown in (Fig. 8).

One commercial sample (the only one available in the Lebanese market) containing 100 mg. diphenylhydantoin sodium and 50 mg. phenobarbital per capsule, was analyzed by this method. The results of analysis are shown in (Table II).

Five synthetic mixtures of known composition were prepared and analyzed. The results of this analysis are shown in (Table III).

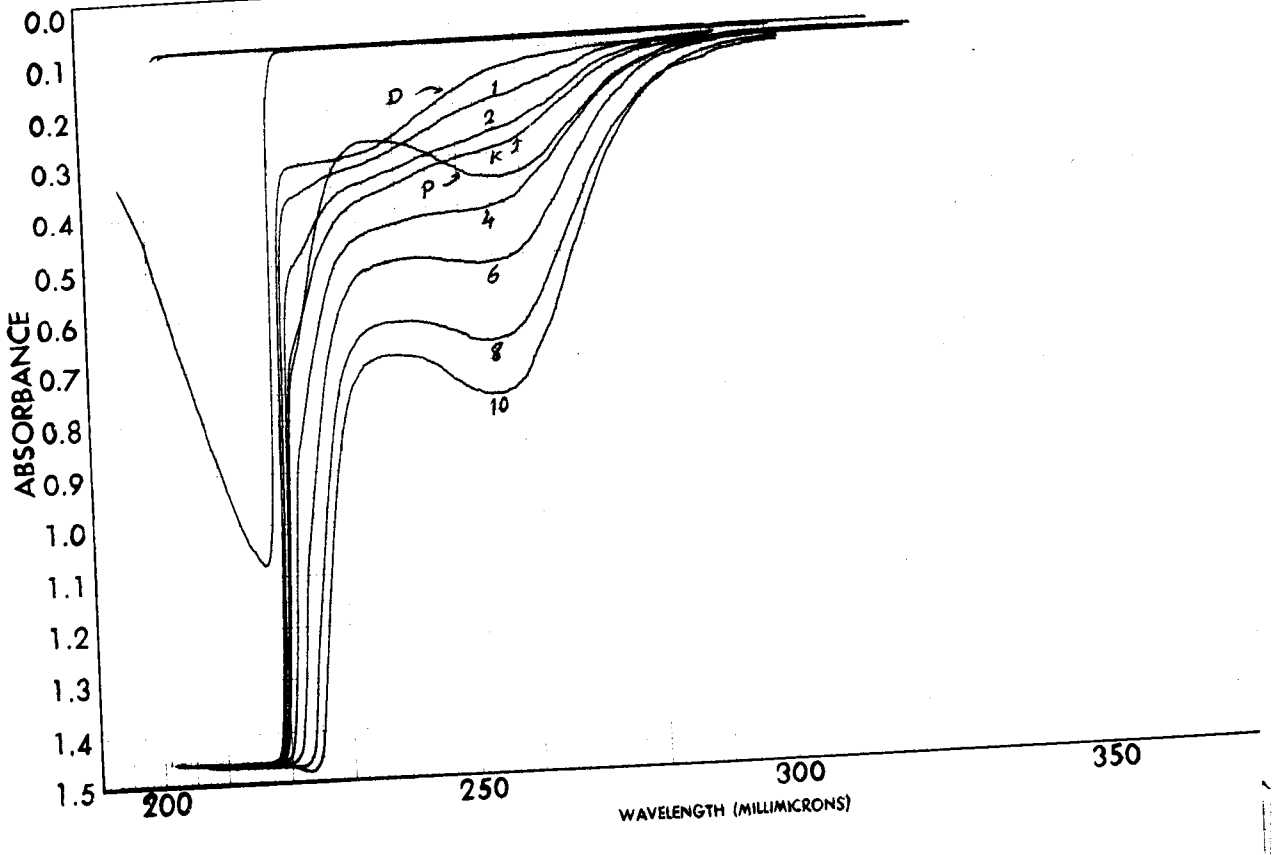

Figure 7. UV spectra, using 0.4N aqueous NaOH as the solvent, of: (a) phenobarbital (P)
(b) diphenylhydantoin (D)
(c) standard mixtures of both (1,2,4,6,8 and 10)
(d) the unknown sample (K)

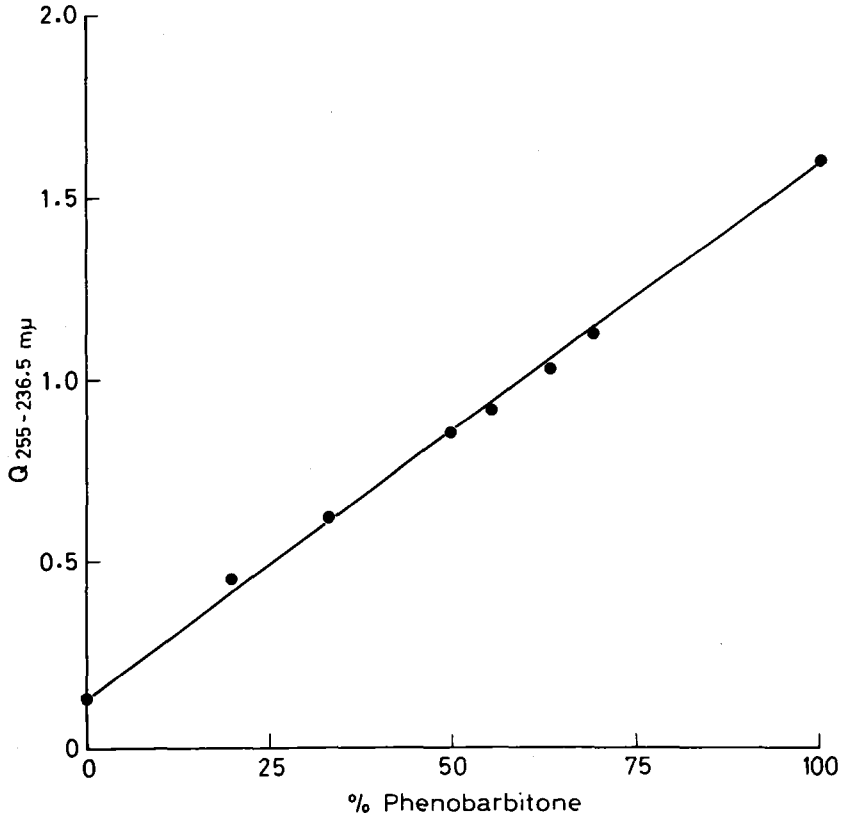

Figure 8. Q curve for phenobarbital and diphenylhydantoin.

Table II

Results of the Analysis of One Commercial Preparation Containing 100 mg. Diphenylhydantoin Sodium and 50 mg. Phenobarbital

Runs	Total Weight; mg./capsule		% Phenobarbital*		Phenobarbital; mg./capsule		Diphenylhydantoin Sodium; mg./capsule	
	Labeled	Found	Labeled	Found	Labeled	Found	Labeled	Found
1	150	157.3	35.3	36	50	53.5	100	103.8
2	150	157	35.3	36.5	50	54	100	103
3	150	154.5	34.3	37.0	50	54	100	100.5
4	150	156	35.3	36.0	50	53	100	103
5	150	157.3	35.3	36.0	50	53.5	100	103.8

* The percentage is taken on the basis of diphenylhydantoin rather than its sodium salt.

Table III

Results of Analysis of Synthetic Mixtures Containing Known Amounts of Phenobarbital and Diphenylhydantoin

Total Weight; mg./500ml.		% Phenobarbital		Phenobarbital; mg./500 ml.		Diphenylhydantoin-Na; mg./500 ml.	
Taken	Recovered	Taken	Recovered	Taken	Recovered	Taken	Recovered
125.0	124.13	16.6	16.5	20.75	20.48	104.25	103.65
125.0	122.5	20.0	21.0	25.0	25.72	100.0	96.78
125.0	125.68	50.0	50.0	62.5	62.84	62.5	62.84
125.0	127.5	66.6	66.0	83.25	84.15	41.75	43.35
125.0	119.6	75.0	75.5	93.75	90.3	31.25	29.3

B. Hexestrol: Phenobarbital

1. Spectral Characteristics

The spectral characteristics of hexestrol as well as phenobarbital depend on the pH of the medium. The pH of the solution has a significant effect on the ultra-violet absorption of these two compounds. However, it is extremely simple to prepare solutions of exactly reproducible pH by mixing accurately measured volumes of reagents of known concentrations. In N/10 NaOH in 50% ethanol, hexestrol and phenobarbital revealed the spectra shown in (Fig. 9). Two iso-absorptive points are exhibited at the two wavelengths 257 and 276.5 mu. The later is found most appropriate to be used as the isoabsorptive reference point. The second reference point is taken at the wavelength 296.5 mu. (a point where hexestrol reveals maximum absorption).

2. Procedure

In 50% ethanol, prepare hexestrol solution to contain about 6 mg., accurately weighed, of hexestrol per 100 ml. (solution A). Similarly prepare phenobarbital solution, using the same solvent, to contain exactly the same concentration as the hexestrol solution mentioned above (solution B). In 11 separate 25 ml. volumetric flasks prepare the following dilutions as indicated in (Table IV)

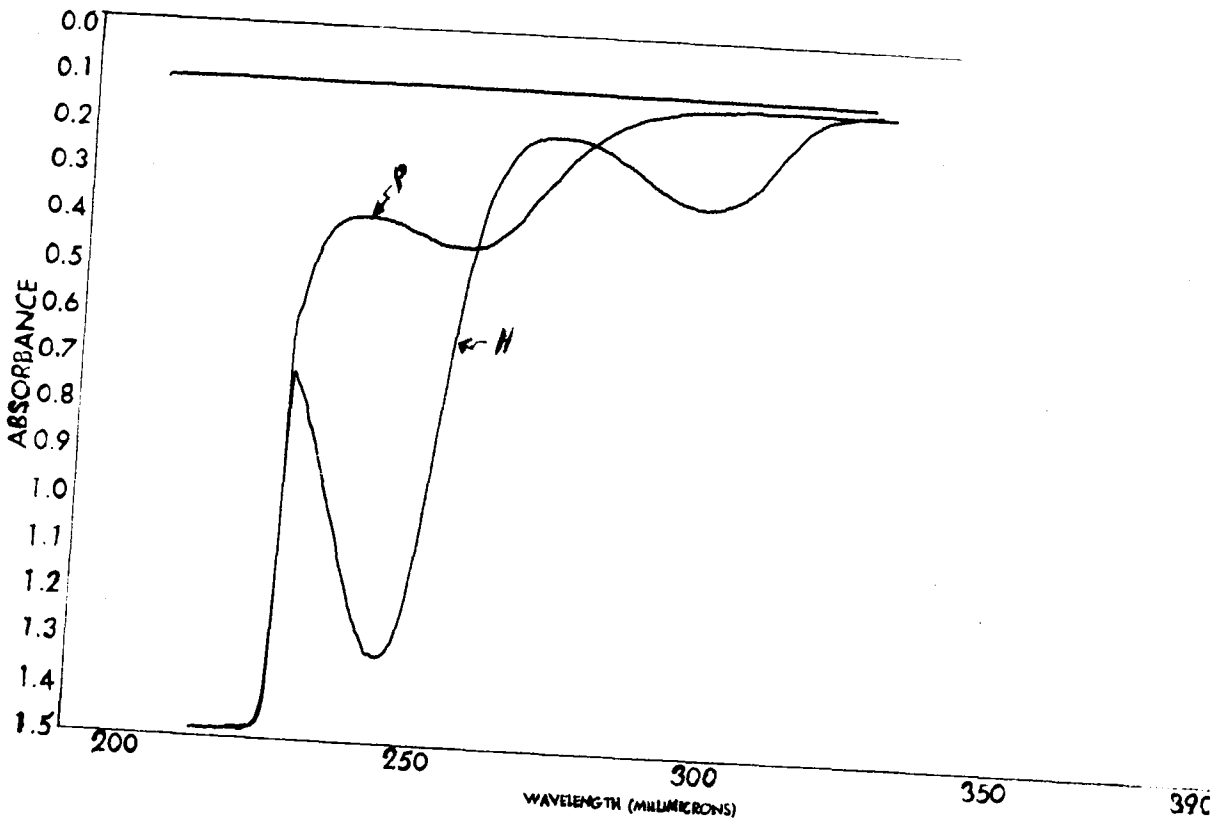

Figure 9. UV spectra of: (a) 12.36 ugm./ml. of hexestrol in N/10 NaOH in 50% ethanol (H) (b) 12.36 ugm./ml. of phenobarbital in N/10 NaOH in 50% ethanol (P).

Table IV

Standard Mixtures of Phenobarbital and Hexestrol Used for Plotting the Q-Curve

Solution	1	2	3	4	5	6	7	8	9	10	11
ml. of Solution A	0.5	2	4	5	7	8	9	10	12	5	0
ml. of Solution B	5	5	5	5	5	5	5	5	5	0	5

Pipet 2.5 ml. of N/1 aqueous NaOH into each flask and then make up to volume with 50% ethanol.

The unknown sample solution is prepared in a similar manner. Ten tablets, containing 20 mg. phenobarbital and 3 mg. hexestrol per tablet, are weighed and powdered. The average weight per tablet is then ascertained. A portion of the well mixed powder, representing about 30 mg. of phenobarbital and 4.5 mg. hexestrol, is accurately weighed, transferred to a 500 ml. volumetric flask, dissolved, and made up to volume with 50% ethanol. The solution thus prepared is filtered through Whatman No.1 filter paper discarding the first 25 ml of the filtrate and collecting the rest in a dry clean container. From this filtrate, an aliquot of 5 ml. is pipetted into 25 ml. volumetric flask. 2.5 ml. N/1 aqueous NaOH is then pipetted, and the volume is adjusted with 50% ethanol. The UV spectra of all the solutions are taken against a blank of N/10 NaOH in 50% ethanol as shown in (Fig. 10).

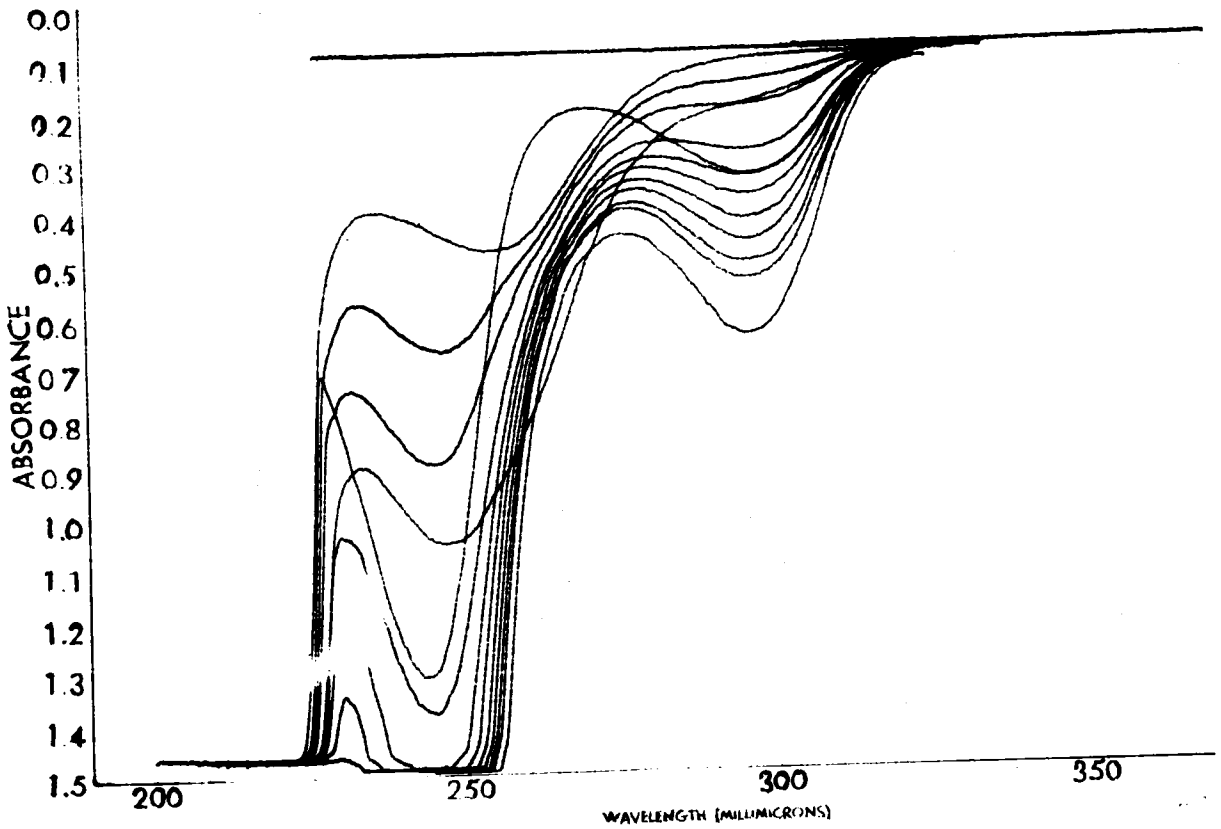

Figure 10. UV spectra of standard mixtures of hexestrol and phenobarbital as well as the unknown sample using N/1o NaOH in 50% ethanol as the solvent.

The $Q_{296.5-276.5\ mu}$ values calculated from absorbancy readings are plotted versus the various fractions of hexestrol in the stated mixtures. This plot results in a straight line as shown in (Fig. 11). The relative composition and the absolute concentration of the unknown sample is then determined. One commercial sample (which was available in the Lebanese market) containing 20 mg. of phenobarbital and 3 mg. of hexestrol was analyzed by this method. The results are shown in (Table V).

Table V

Results of the Analysis of One Commercial Preparation Containing 20 mg. Phenobarbital and 3 mg. Hexestrol

Runs	Total weight; mg./tablet		% Hexestrol		Hexestrol; mg./tablet		Phenobarbital; mg./tablet	
	Labeled	Found	Labeled	Found	Labeled	Found	Labeled	Found
1	23	26.3	13.05	13.3	3	3.5	20	22.8
2	23	26.5	13.05	13.0	3	3.45	20	23.05
3	23	25.6	13.05	13.5	3	3.46	20	22.14
4	23	26.3	13.05	13.5	3	3.55	20	22.75

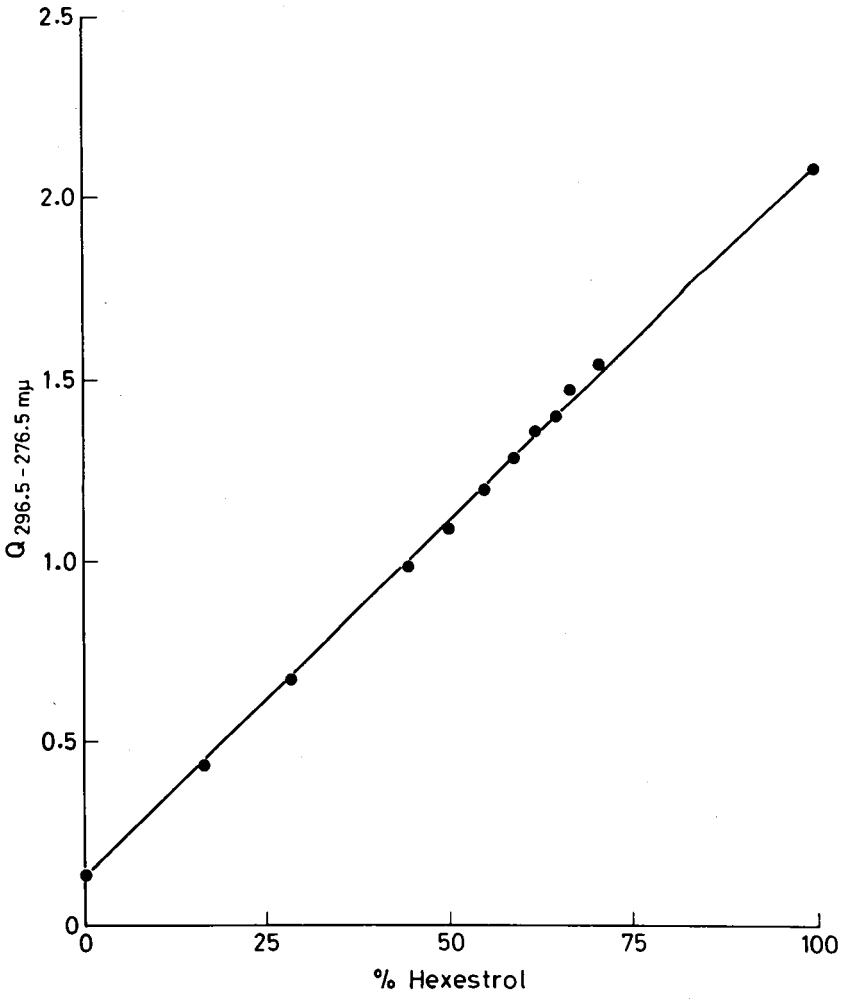

Figure 11. Q curve for hexestrol and phenobarbital.

The following four synthetic mixtures were prepared to contain known amounts of hexestrol and phenobarbital and analyzed for both relative and absolute concentrations. The results are shown in (Table VI).

Table VI

Results of Analysis of Synthetic Mixtures Containing Known Amounts of Hexestrol and Phenobarbital

Total Weight; mg.		% Hexestrol		Hexestrol; mg.		Phenobarbital; mg.	
Taken	Recovered	Taken	Recovered	Taken	Recovered	Taken	Recovered
60.0	61.8	25.0	25.0	15.0	15.45	45.0	46.35
60.0	63.2	50.0	50.0	30.0	31.6	30.0	31.6
60.0	62.35	66.66	65.0	40.0	40.53	20.0	21.82
60.0	57.37	75	75.5	45.0	43.31	15.0	14.06

C. Chlorpheniramine Maleate: Phenylephrine HCl

1. Spectral Characteristics

Chlorpheniramine maleate and phenylephrine HCl aqueous solutions exhibited an isoabsorptive point at 270.5 mu. This point together with the wavelength 261.5 mu (where chlorpheniramine maleate has a maximum absorption) were selected as the two reference points for its analysis. The UV spectra of two equi-

concentration (by weight) aqueous solutions of these two compounds are shown in (Fig. 12).

2. Procedure

Weigh accurately about 10 mg. of chlorpheniramine maleate and transfer it to a 100 ml. volumetric flask; dissolve and make up to volume with water (Solution A). Into another 100 ml. volumetric flask, transfer exactly an equal weight of phenylephrine HCl; dissolve and make up to volume with water (Solution B). The following dilutions are then prepared using 11 separate 25 ml. volumetric flasks, adjusting their volumes with water (Table VII).

Table VII

Standard Mixtures of Chlorpheniramine Maleate and Phenylephrine HCl Used for Plotting the Q-Curve

Solution	1	2	3	4	5	6	7	8	9	10	11
ml. of Solution A	0.5	1	2	3	5	6	8	10	12	5	0
ml. of Solution B	5	5	5	5	5	5	5	5	5	0	5

The UV spectra of these solutions are taken against a blank of distilled water (Fig. 13). From these spectra, the $Q_{261.5-270.5\ mu}$ values are then calculated and plotted versus the percentages of chlorpheniramine

maleate to give a straight line curve (Fig. 14).

The unavailability of commercial products, listed in pharmaceutical compendia, neither on the local market nor in Jordan; and the shortage of time, permitting us to order these products from abroad, obliged us to be satisfied by preparing synthetic mixtures to replace the actual commercial products.

Five such synthetic mixtures were prepared to contain known amounts of chlorpheniramine maleate and phenylephrine HCl and analyzed by the proposed method. The results of analysis are shown in (Table VIII).

Table VIII

Results of the Analysis of Five Synthetic Mixtures Containing Known Amounts of Chlorpheniramine Maleate and Phenylephrine HCl

Total Weight; mg./100ml.		% Chlorpheniramine Maleate		Chlorpheniramine Maleate mg./100 ml.		Phenylephrine HCl mg./100ml.	
Taken	Recovered	Taken	Recovered	Taken	Recovered	Taken	Recovered
10.0	9.67	9.1	9.0	0.91	0.87	9.09	8.8
10.0	9.71	28.55	28.6	2.85	2.78	7.14	6.93
10.0	10.12	50.0	49.5	5.0	5.01	5.0	5.11
10.0	9.62	58.33	58.0	5.83	5.58	4.17	4.04
10.0	10.33	66.66	66.75	6.67	6.89	3.334	3.43

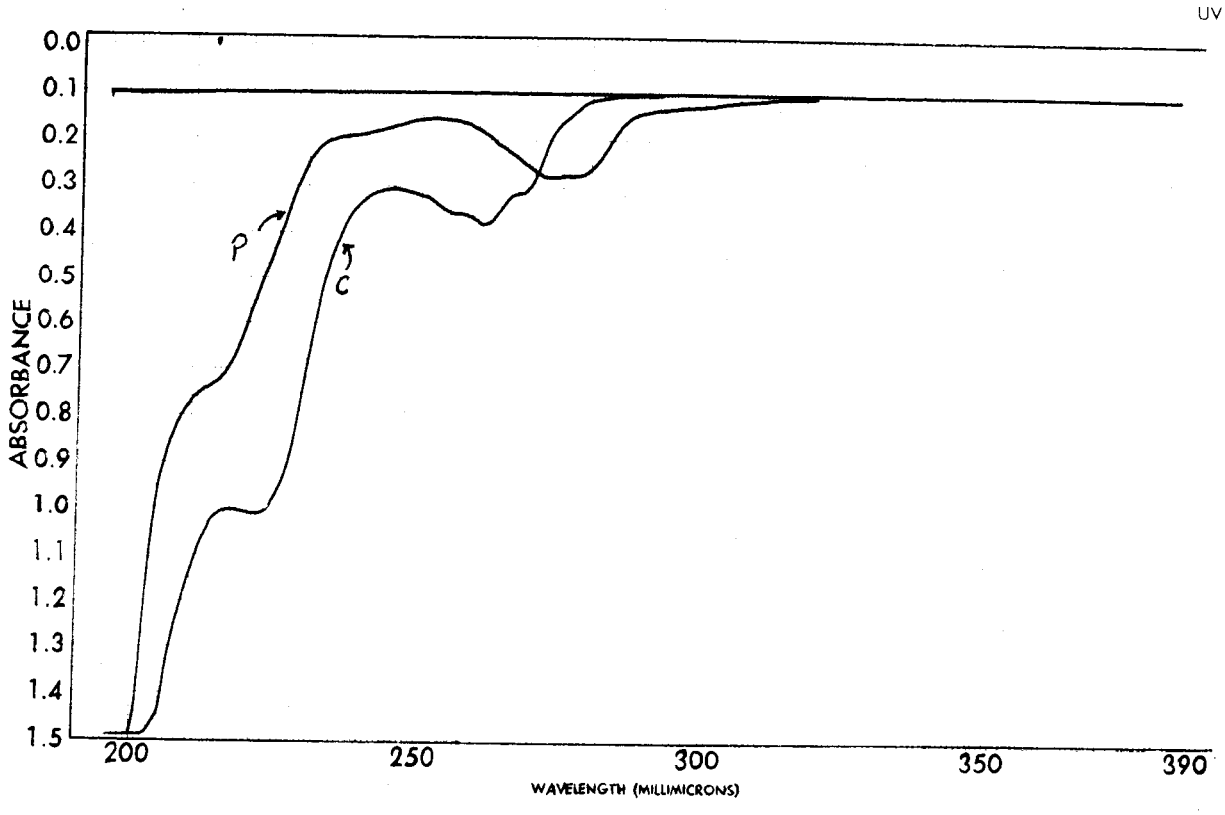

Figure 12. UV spectra of: (a) 20.5 ugm./ml. of chlorpheniramine maleate in water (C) (b) 20.5 ugm./ml. phenylephrine HCl in water (P).

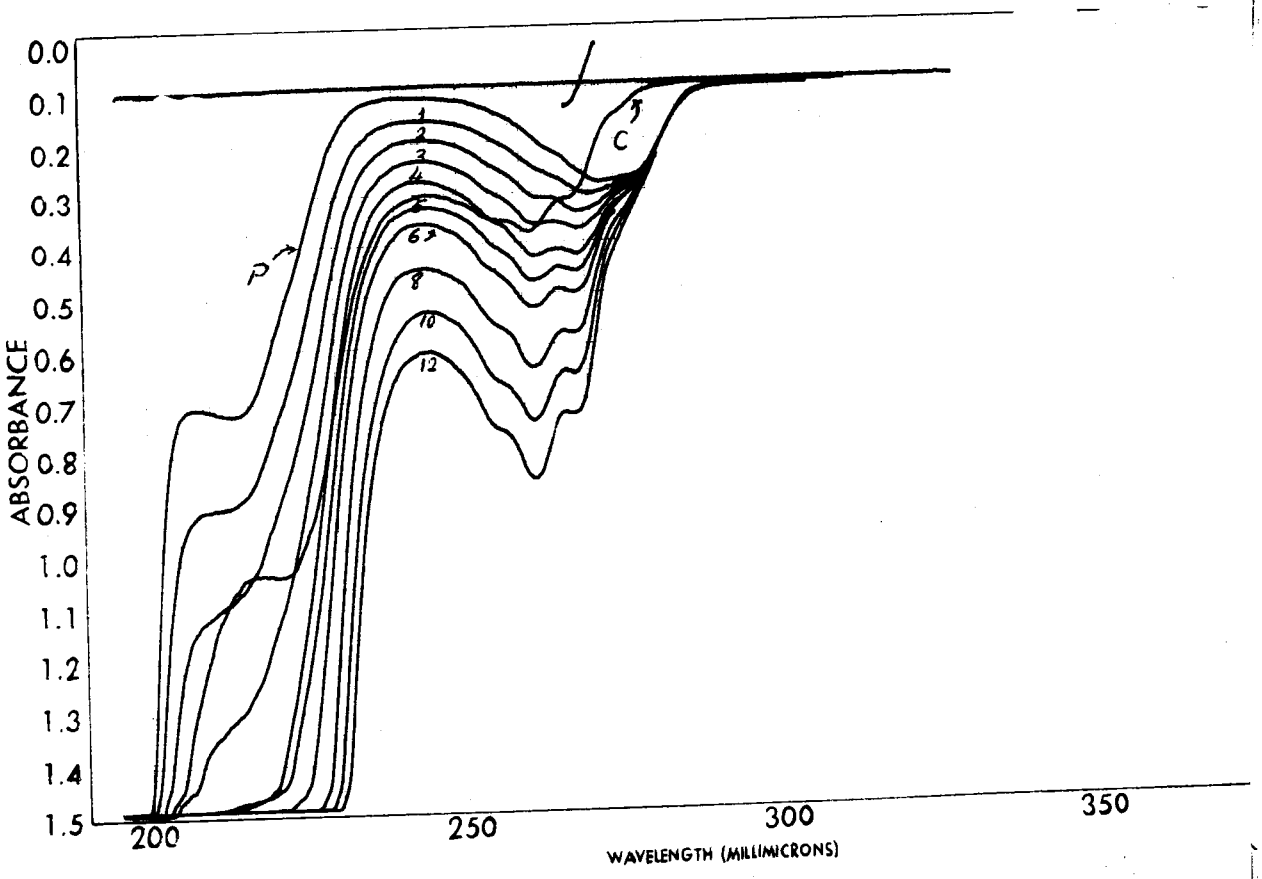

Figure 13. UV spectra of aqueous solutions of:

(a) chlorpheniramine maleate (C)

(b) phenylephrine HCl (P)

(c) standard mixtures of both (1,2,3,4,5,6, 8,10 and 12).

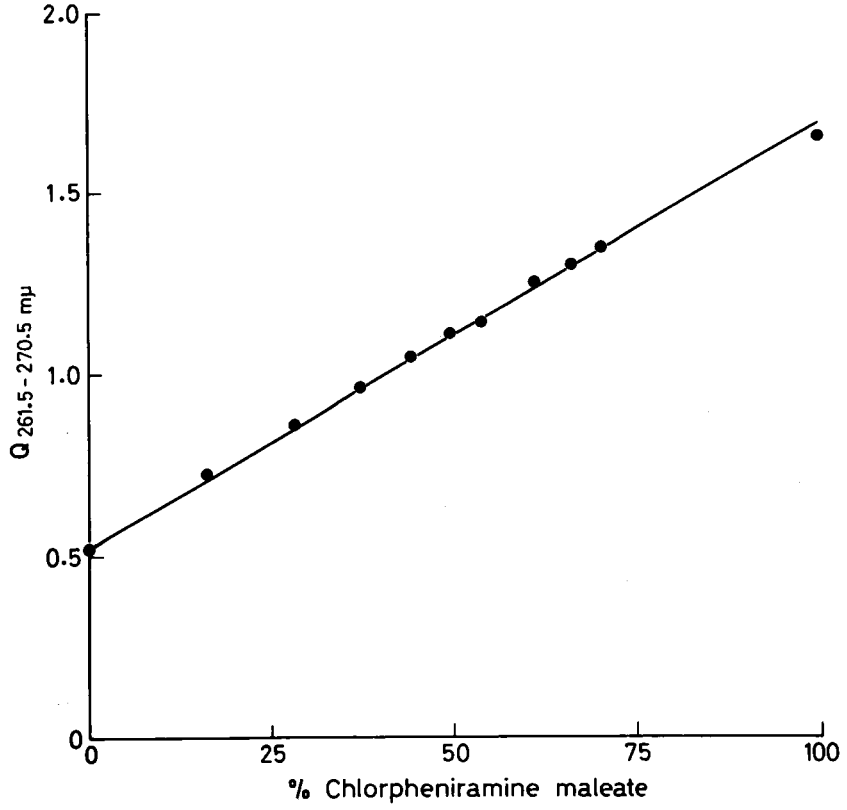

Figure 14. Q curve for chlorpheniramine maleate and phenylephrine HCl.

D. Phenylephrine HCl: Naphazoline Nitrate*

1. Spectral Characteristics

Phenylephrine HCl and naphazoline nitrate exhibited no isoabsorptive point in water or N/10 aqueous HCl. When N/10 aqueous NaOH was used, however, two isoabsorptive points were revealed at 252 and 294 mu. The first was used as the isoabsorptive reference point together with the wavelength 281 mu (the peak for naphazoline nitrate) as the second reference point. The UV spectra taken for equal concentrations (by weight) of these two substances in N/10 aqueous NaOH are shown in (Fig. 15).

2. Procedure

Prepare naphazoline nitrate solution in distilled water to contain about 6 mg., accurately weighed, per 100 ml. (Solution A). Similarly, prepare phenylephrine HCl solution in distilled water to contain exactly the same concentration as above (Solution B). In 10 separate 25 ml. volumetric flasks, prepare the following dilutions (Table IX).

---

* Received from Scer-Labs.

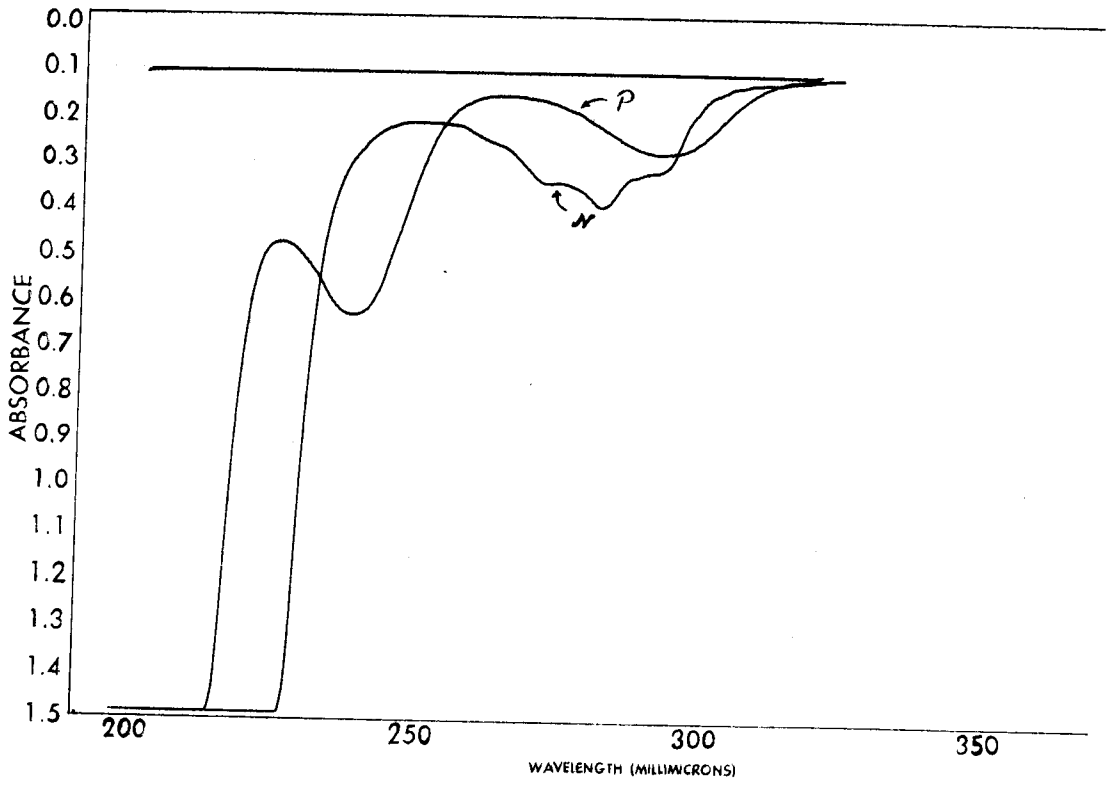

Figure 15. UV spectra of: (a) 12.2 ugm./ml. of naphazoline nitrate in N/10 aqueous NaOH (N) (b) 12.2 ugm./ml. of phenylephrine HCl in N/10 aqueous NaOH (P).

Table IX

Standard Mixtures of Naphazoline Nitrate and Phenylephrine HCl Used for Plotting the Q-Curve

Solution	1.	2	3	4	5	6	7	8	9	10
ml. of Solution A	0.5	1	2	3	4	6	8	10	4	0
ml. of Solution B	4	4	4	4	4	4	4	4	0	4

Into each of the above volumetric flasks, pipet 2.5 ml. N/1 aqueous NaOH and make up to volume with distilled water. The UV spectra of these solutions against a blank of N/10 aqueous NaOH are shown in (Fig. 16). A plot of $Q_{281-252\ mu}$ versus the percentage of naphazoline nitrate gives a straight line (Fig. 17).

Five synthetic mixtures containing known amounts of these two compounds were prepared and analyzed for both their relative and absolute concentrations. The results are tabulated as follows (Table X).

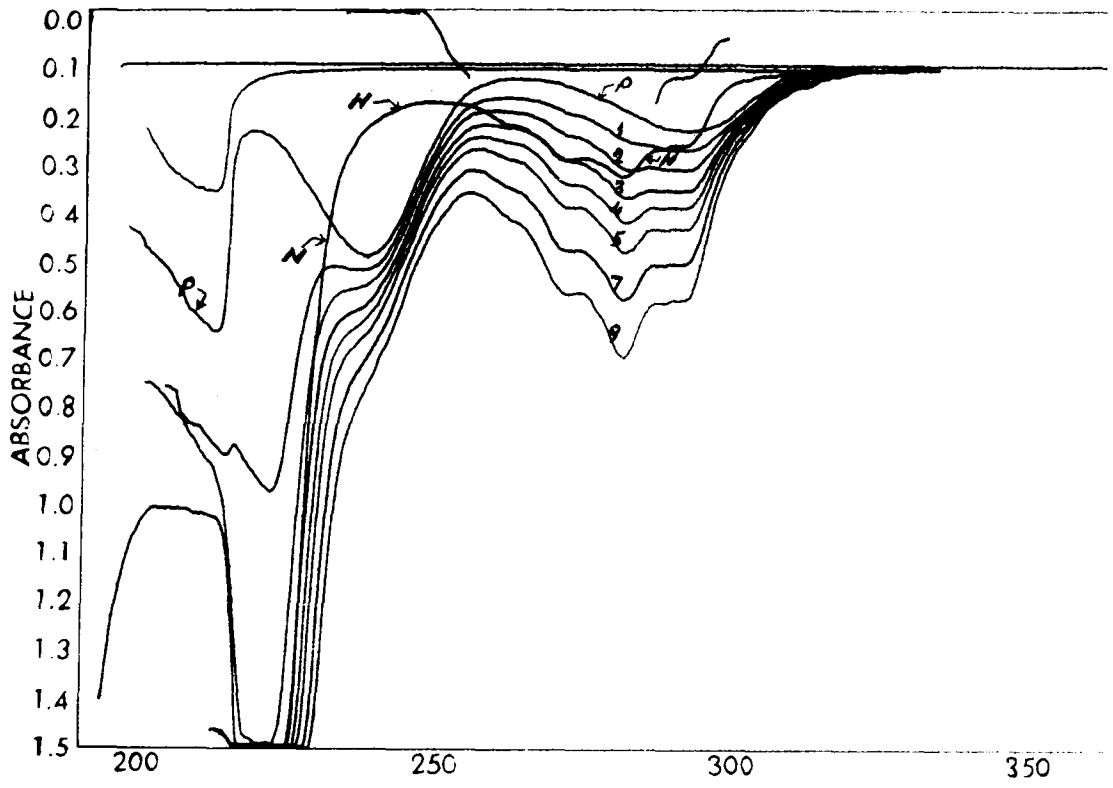

Figure 16. UV spectra, using N/10 aqueous NaOH as the solvent, of: (a) naphazoline nitrate (N) (b) phenylephrine HCl (P) (c) standard mixtures of both (1,2,3,4,5, 7 and 10).

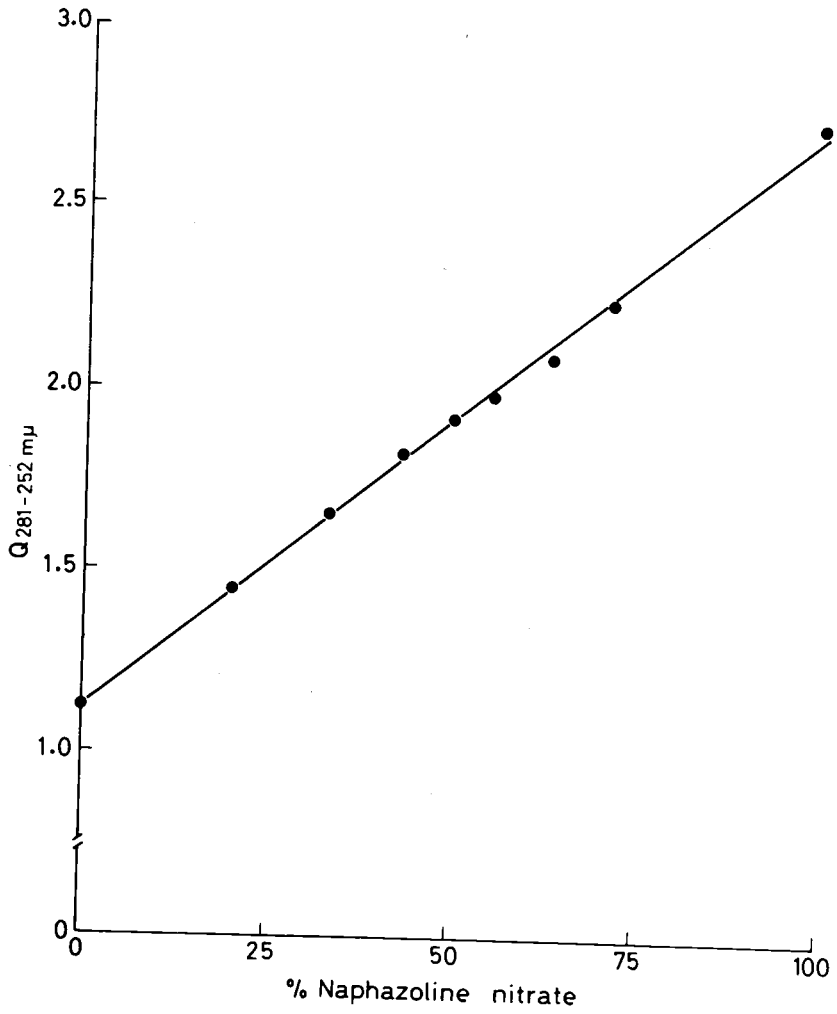

Figure 17. Q curve for naphazoline nitrate and phenylephrine HCl.

Table X

Results of the Analysis of Five Synthetic Mixtures Containing Known Amounts of Naphazoline Nitrate and Phenylephrine HCl.

Total Weight; mg./100 ml.		% Naphazoline Nitrate		Naphazoline Nitrate; mg./100 ml.		Phenylephrine HCl; mg./100 ml.	
Taken	Recovered	Taken	Recovered	Taken	Recovered	Taken	Recovered
6.0	5.92	11.1	11.5	0.67	0.68	5.33	5.24
6.0	6.07	20.0	20.0	1.2	1.21	4.8	4.86
6.0	6.22	42.85	42.0	2.57	2.611	3.43	3.60
6.0	6.18	50.0	50.0	3.0	3.09	3.0	3.09
6.0	5.83	71.4	70.5	4.28	4.112	1.72	1.72

E. Progesterone*: Estradiol Benzoate

1. Spectral Characteristics

The UV spectra of two solutions of progesterone and estradiol benzoate in 95% ethanol having equal concentrations (by weight) are shown in (Fig. 18). Two isoabsorptive points are exhibited at 234 and 263 mu. The two wavelengths selected for carrying out the Q analysis are 220 and 234 mu.

---

*Received from Roussel.

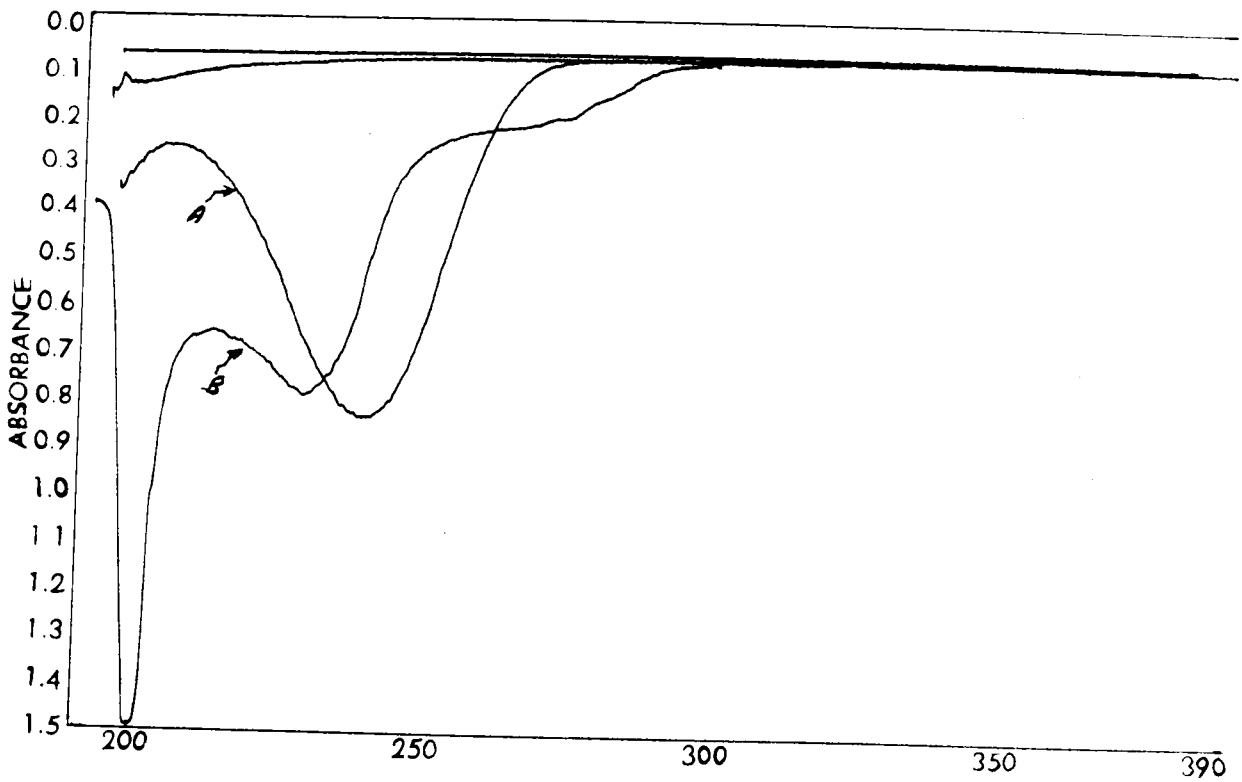

Figure 18. UV spectra of: (a) 15 ugm./ml. of estradiol benzoate in 95% ethanol (B)
(b) 15 ugm./ml. of progesterone in 95% ethanol (A).

2. Procedure

Prepare estradiol benzoate in 95% ethanol to contain about 6 mg., accurately weighed, per 100 ml. (Solution A). Similarly, prepare progesterone solution to contain exactly an equal concentration as the above and using 95% ethanol as the solvent (Solution B). A series of dilutions are then prepared using 9 separate 25 ml. volumetric flasks adjusting the volume with 95% ethanol (Table XI).

Table XI

Standard Mixtures of Estradiol Benzoate and Progesterone Used for Plotting the Q-Curve.

Solution	1	2	3	4	5	6	7	8	9
ml. of Solution A	0.5	1	2	3	4	5	6	3	0
ml. of Solution B	3	3	3	3	3	3	3	0	3

The UV spectra of these solutions against a blank of 95% ethanol are shown in (Fig. 19). A plot of $Q_{220-234\ mu}$ versus the percentage of estradiol benzoate is a straight line (Fig. 20).

Because all the pharmaceutical preparations found on the local market were oily solutions, it was not possible to carry out this method of analysis without

prior separation of the two active ingredients. If such a step is to be carried out, separation of these two substances, each at a time, may be effected and hence, the application of Q analysis will not be needed. However, for non-oily solutions, this method may be applied to give somewhat satisfactory results without any priliminary separation. To test the accuracy and precision in this case, the following five synthetic mixtures were prepared and analyzed. The results of this analysis are given in (Table XII).

Table XII

Results of Analysis of Synthetic Mixtures Containing Known Amounts of Progesterone and Estradiol Benzoate

Total Weight; mg.		% Estradiol-Benzoate;		Estradiol Benzoate; mg.		Progesterone mg.	
Taken	Recovered	Taken	Recovered	Taken	Recovered	Taken	Recovered
6.0	5.97	14.3	14.5	0.86	0.87	5.14	5.1
6.0	5.86	40.0	40.0	2.4	2.34	3.6	3.51
6.0	6.19	50.0	49.5	3.0	3.06	3.0	3.13
6.0	5.93	58.1	59.5	3.49	3.53	2.51	2.4
6.0	6.08	62.5	63.0	3.75	3.86	2.25	2.22

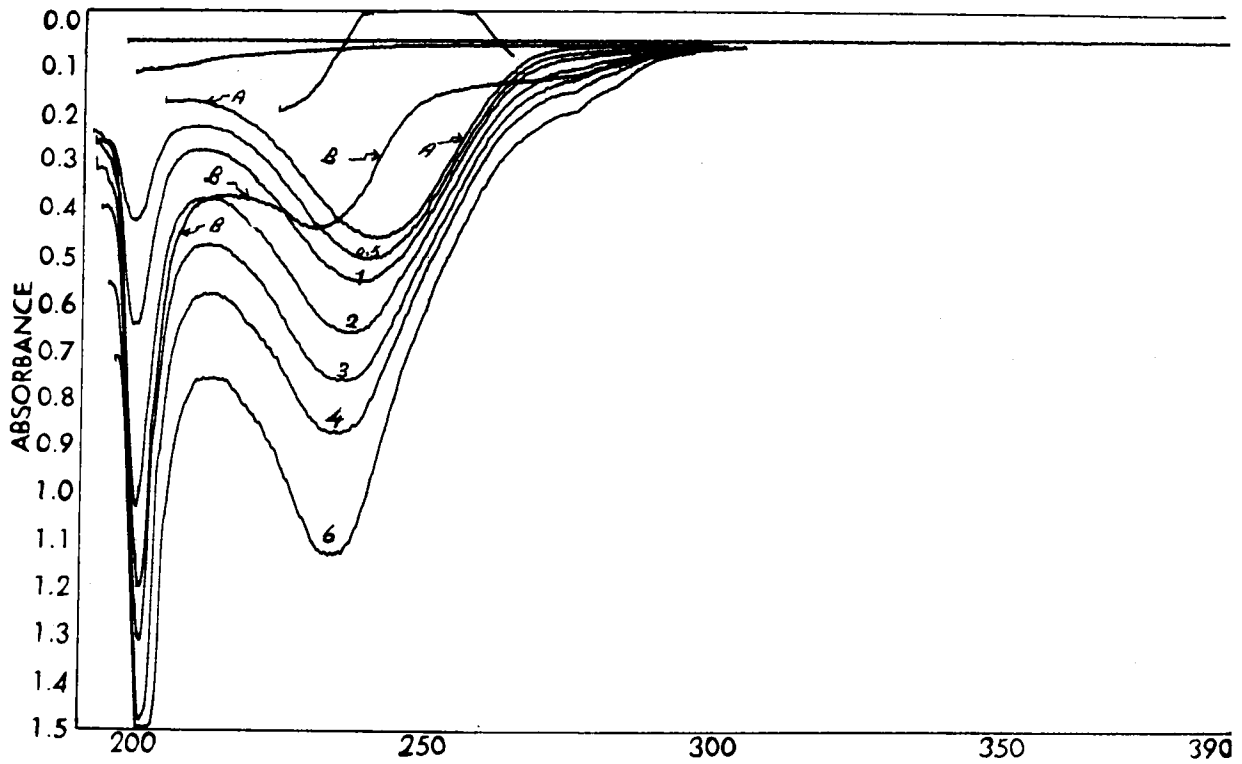

Figure 19. UV spectra, using 95% ethanol as the solvent, of: (a) estradiol benzoate (B)
(b) progesterone (A)
(c) standard mixtures of both (0.5,1,2,3,4 and 6).

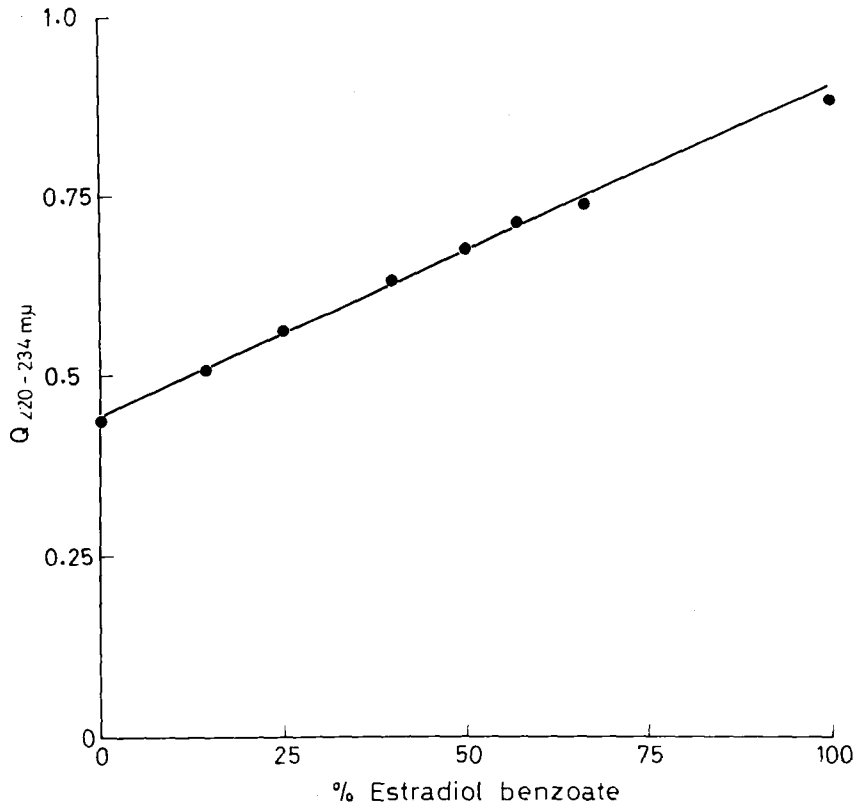

Figure 20. Q curve for estradiol benzoate and progesterone.

F. Miscellaneous Combinations

Among the various pharmaceutical formulations screened, some were found to contain active ingredients having UV spectra which were appropriate for the application of the Q analysis (Figs. 21,22 & 23). The limiting factor in these cases, however, was found to be the proportion of the two active ingredients occurring actually in the pharmaceutical product. A 50-50 mixture would yield far more accurate results than, for example, a 95-5 mixture. In the later case, the precision with which the minor ingredient could be determined is very low. Examples of such pharmaceutical preparations encountered to contain active ingredients with unfavorable proportions are the following:

1. Norethynodrel: Mestranol

These two steroidal compounds usually exist in pharmaceutical preparations in the ratio of 1.5 parts of mestranol to 100 parts of norethynodrel. This makes it very difficult to evaluate the minor component, mestranol, with an acceptable degree of accuracy and precision. Because in this formulation the relative proportion of mestranol to norethynodrel is very small and slight discrepancies in absolute recoveries will result in large percentage differences. To illustrate

this, a difference of 0.01 mg. from a mean value of mestranol content per tablet, 0.075 mg., will result in a 13.3% deviation. Though the absolute difference may seem to be small, yet the percentage deviation is beyond the acceptability for quantitative purposes.

The value of the major component, however, could be assessed with a higher degree of accuracy. In this case, the equation $F_x = \frac{Q_{2-i} - Q_y}{Q_x - Q_y}$ could directly be used to determine its relative composition thus its absolute concentration without recourse to the Q plot.

2. Megestrol Acetate: Ethinyl Estradiol

Megestrol acetate, supplemented with an estrogen, generally ethinyl estradiol, is used as an oral contraceptive. It is formulated as tablets containing 1.25% ethinyl estradiol. The application of the absorbancy ratio analysis to such a pharmaceutical product is governed by the limiting factor mentioned above.

3. Acetylsalicylic Acid: Promethazine

The proportion of acetylsalicylic acid to promethazine as they occur in pharmaceuticals is 100 to 1. Such a low percentage of promethazine limits the applicability of this proposed method of analysis.

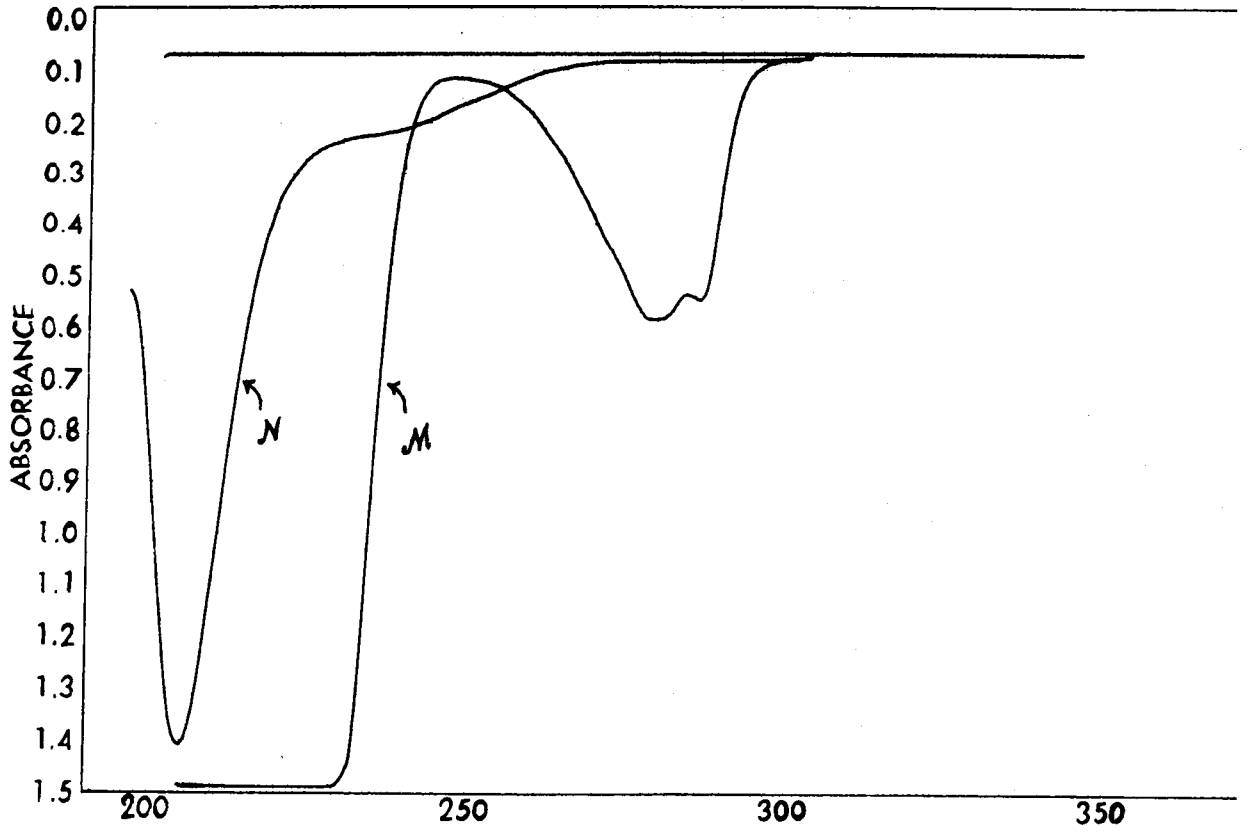

Figure 21. UV spectra of: (a) 82 ugm./ml. of mestranol in 95% ethanol (M)

(b) 82 ugm./ml. of norethynodrel in 95% ethanol (N).

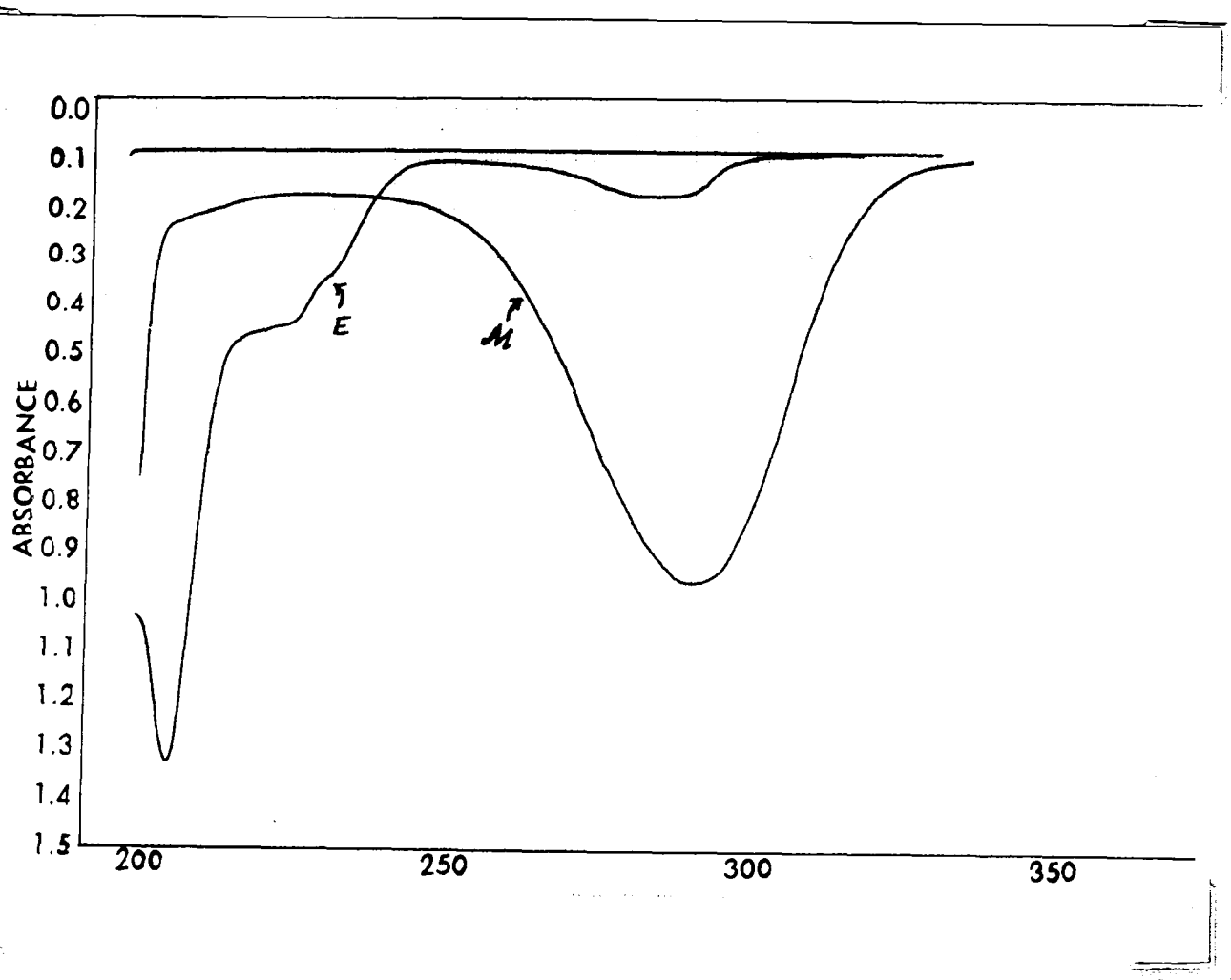

Figure 22. UV spectra of: (a) 14.9 ugm./ml. of megestrol in 95% ethanol (M)
(b) 14.9 ugm./ml. of ethinyl estradiol in 95% ethanol (E).

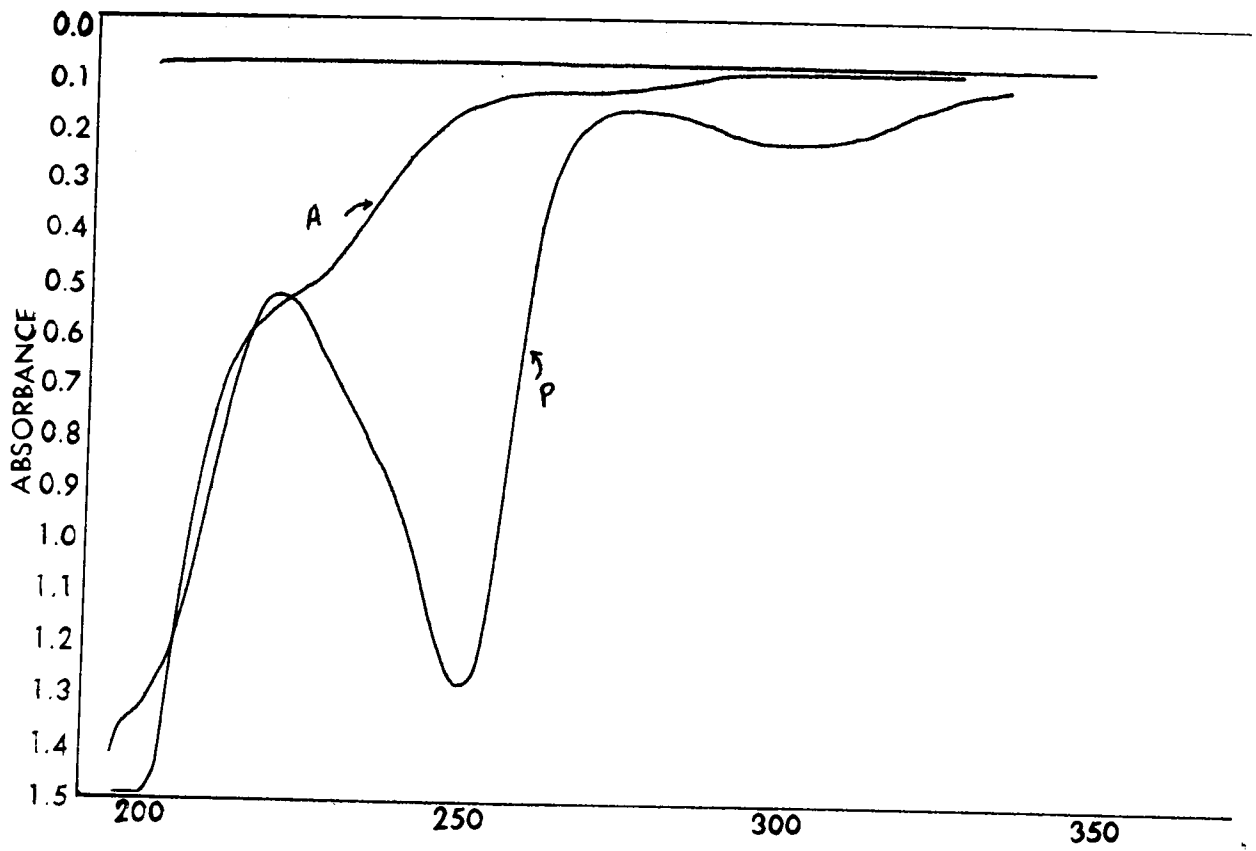

Figure 23. UV spectra of: (a) 14.4 ugm./ml. of acetylsalicylic acid in water (A) (b) 14.4 ugm./ml. of promethazine in water (P).

DISCUSSION

The accuracy of analysis by the absorbancy ratio method is generally influenced by two main factors. The spectral characteristics of the individual components as well as their composite spectrum play a major role in this respect. Equally important is the proportion of the two components in the mixture. For the first case, it is very difficult, except on theoretical basis, to specify ideal conditions primarily because the spectral characteristics of the substances encountered are greatly variable. However, the following generalizations may be safely stated to serve as a guide in cases where absorbancy ratio method of analysis is to be applied. For instance, if the isoabsorptive point occurs near the peaks of the two components, the slope of the Q curve will get nearer to zero, and in turn the analysis will be less accurate. Again if the isoabsorptive point occurs at a wavelength where the composite spectrum of the two substances appears to be very steep, the dependability of absorbance measurements will be greatly influenced and thus affecting the accuracy and precision of the analytical data.

In the analysis of all the previous samples, the commercial products as well as the different synthetic mixtures, two major sources of error were usually encountered. The first appears in the estimation of the absolute value of the total concentration of the two components in the mixture. The other is revealed in the determination of the relative composition of the sample. The two errors add up and aggravate the results for only one of the two components either on the positive or on the negative side. Examples are clearly seen in most of the tabulated experimental results.

A survey of the experimental data shows that this method could be used for the assay of the investigated binary combinations with satisfactory results. Although, in some few cases, the recovered weight may differ from the taken weight by an amount reaching as high as 4%, yet this figure should be judged in relation to the speed and ease of the analysis. This figure decreases as the characteristics of the binary combination concerned, spectral and otherwise, approach the ideal conditions for applying such a method of analysis.

BIBLIOGRAPHY

1. Pernarowski, M., Knevel, A.M., and Christian, J.E., J. Pharm. Sci., 50, 943 (1961).

2. Schroeder, W.A., Wilcox, P.E., Trueblood, K.N., and Dekker, A.O., Anal. Chem., 23, 1740 (1951).

3. Hirt, R.C., King, F.T., and Schmitt, R.G., Anal. Chem., 26, 1270 (1954).

4. Ish-Shalom, M., Fitzpatrick, J.D., and Orchin, M., J. Chem. Educ., 34, 496 (1957).

5. Pernarowski, M., Knevel, A.M., and Christian, J.E., J. Pharm. Sci., 50, 946 (1961).

6. Yokoyama, F., and Pernarowski, M., ibid., 50, 953 (1961).

7. Pernarowski, M., Knevel, A.M., and Christian, J.E., ibid., 51, 688 (1962).

8. Sattler, H., Pharm. Ztg. 39, 1395 (1966).

9. Willard, H.H., Merritt, L.L., Jr., and Dean, J.A., "Instrumental Methods of Analysis," D. Van Nostrand Company, Inc., New York, 1965, p. 76.

10. Strobel, H.A., "Chemical Instrumentation," Addison-Wesley Pub. Co., Inc., U.S.A., 1960, p. 153.

11. Szymanski, H.A., "Theory and Practice of Infrared Spectroscopy," Plenum Press, New York, 1964, p. 310.

12. Sunshine, I., and Gerber, S.R., "Spectrophotometric Analysis of Drugs Including Atlas of Spectra," Charles C. Thomas Publisher, U.S.A., 1963.

13. Hayden, A.L., Sammul, O.R., Selzer, G.B., and Carol, J., "Infrared, Ultraviolet, and Visible Absorption Spectra of Some USP and NF Reference Standards and Their Derivatives," Association of Official Agricultural Chemists, Inc., F.D.A., Washington 25 D.C., Reprinted from J.A.O.A.C., 45, 797 (1962).

14. Glenn, A.L., J. Pharm. Pharmacol., 12, 595 (1960).

15. Wexler, A.S., Appl. Spec., 19, 135 (1965).

16. Brown, T.L., J. Pharm. Sci., 52, 274 (1963).

17. Shah, R.C., Raman, P.V., and Shah, B.M., J. Pharm. Sci., 52, 167 (1963).