Application of Absorbancy Ratios to the Analysis of Pharmaceuticals IV

Analysis of Ternary Mixtures

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Four equations that may be used in the analysis of ternary mixtures are derived. All of these equations are based on the relationship between absorbancy ratio values and the relative concentration of one of the components in the ternary mixture. To indicate the applicability of these equations, three ternary mixtures were investigated, one in the ultraviolet region and two in the infrared region of the electromagnetic spectrum. Methods of plotting data are discussed and the utility of such plots under specific analytical situations are pointed out.

PREVIOUS PAPERS in this series dealt with the analysis of binary and certain types of ternary mixtures by the absorbancy ratio method of analysis (1–3). Comprehensive derivations and a survey of the literature may be found in the original work of the author (4). For the purposes of this paper, Q value implies an abbreviation for absorbancy ratio value. Similarly, the absorbancy ratio value obtained by, for example, dividing the absorbancy observed at 250 mμ by that observed at 275 mμ, the solute and the solution being the same for both measurements, is symbolized as $Q:250:275$. A Q curve illustrates the relationship between absorbancy ratio values and the relative concentration of binary or ternary mixtures. Other terminology and symbology shall be that suggested by the National Bureau of Standards (5). The symbol for cell length (b) shall not be used in the derivations below since the value is assumed to be equal to unity.

THEORY

When the three components of a ternary mixture have individual spectral characteristics such that each interferes during analysis with the other two at all wavelengths, then the equations derived below may be used for the simultaneous determination of the substances. As in most of the derivations in this series, it is assumed that two of the three components in the mixture have a characteristic isoabsorptive point. Figure 1 illustrates the type of spectral characteristics suited to the equations below.

The total absorbancy at λ_5 (A_5) is equal to the sum of the absorbancies due to X, Y, and Z. The following equation is, therefore, valid

$$A_5 = a_7\, Cx + a_5\, Cy + a_1\, Cz \quad \text{(Eq. 1)}$$

Cx, Cy, and Cz are the concentrations of X, Y, and Z in the mixture. Preceding each of these symbols are the absorbancy index values for the three components at this wavelength. For exactly the same reasons

$$A_4 = a_6\, Cx + a_6\, Cy + a_2\, Cz \quad \text{(Eq. 2)}$$

Continuing the derivation in the same way as indicated in the first paper in this series (1), Eq. 1 is divided by Eq. 2. The concentration terms are converted to fractional quantities by dividing each term of the equation by $Cx + Cy + Cz$. This results in the following equation

$$\frac{A_5}{A_4} = \frac{a_7\, Fx + a_5\, Fy + a_1\, Fz}{a_6\, (Fx + Fy) + a_2\, Fz} \quad \text{(Eq. 3)}$$

However

$$Fy = 1 - Fz - Fx \quad \text{(Eq. 4)}$$

The right-hand member of Eq. 4 is now substituted for Fy in Eq. 3 and the terms are collected. Each term is now divided by a_6.

$$\frac{A_5}{A_4} = \frac{\dfrac{a_7\, Fx}{a_6} + \dfrac{a_5\,(1 - Fz - Fx)}{a_6} + \dfrac{a_1\, Fz}{a_6}}{\dfrac{a_6\,(1 - Fz)}{a_6} + \dfrac{a_2\, Fz}{a_6}} \quad \text{(Eq. 5)}$$

The spectral characteristics in Fig. 1 indicate that a_7/a_6 is the absorbancy ratio value for pure X (Qx) at these two wavelengths. Similarly, a_5/a_6 is the absorbancy ratio value for pure Y (Qy). The absorbancy ratio value for the mixture is equal to A_5/A_4 and may be designated as Qo. These symbols are substituted into Eq. 5 and the equation is rearranged

$$Qo = \frac{Fx(Qx - Qy) + Fz\,(a_1/a_6 - Qy) + Qy}{1 + Fz\,(a_2/a_6 - 1)} \quad \text{(Eq. 6)}$$

Equation 6 contains two unknowns and, therefore, cannot be used to determine the components in the mixture. Another equation is required to complete the analysis. This equation may be derived from the spectral characteristics of the three components at λ_3 and λ_4 in exactly the same manner as shown above. Therefore

$$Qo_1 = \frac{Fx\,((Qx_1 - Qy_1) + Fz\,(a_3/a_6 - Qy_1) + Qy_1}{1 + Fz\,(a_2/a_6 - 1)} \quad \text{(Eq. 7)}$$

WAVELENGTH
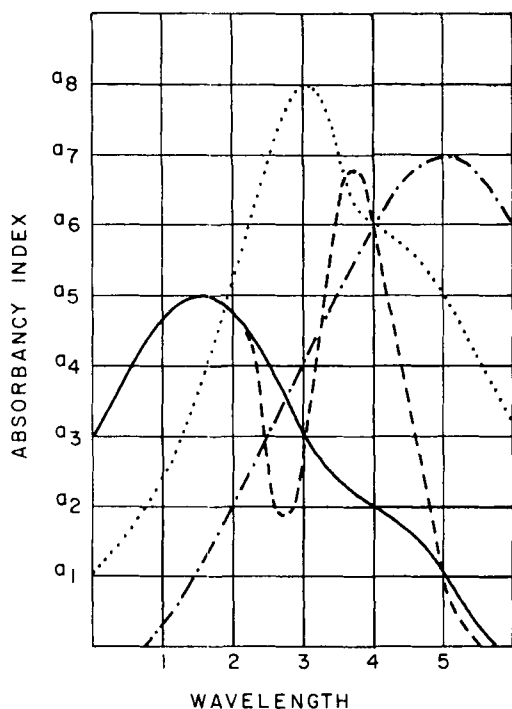
Fig. 1.—Hypothetical spectrophotometric curves of X ($-\cdot-\cdot-\cdot$), Y ($\ldots\ldots$), and Z (---) illustrating spectral characteristics necessary for ternary Q analysis. The spectrum for Z ($- - -$) illustrating a common isoabsorptive point is also shown.

Equations 6 and 7 may now be solved simultaneously for the fraction of two of the three components in the mixture.

The above equations are simplified considerably if the three components in the mixture have a common isoabsorptive point. The total absorbancies at λ_3 and λ_4 may be defined as above except that the absorbancy index value of component Z at λ_4 is equal to a_5. By carrying out the derivation in exactly the same manner as above, Eqs. 6 and 7 become

$$Qo = Fx (Qx - Qy) + Fz (Qz - Qy) + Qy \quad \text{(Eq. 8)}$$

and

$$Qo_1 = Fx (Qx_1 - Qy_1) + Fz (Qz_1 - Qy_1) + Qy_1 \quad \text{(Eq. 9)}$$

These two equations may now be solved simultaneously for the fractions of X and Z in the mixture.

The disadvantages of this type of analysis are apparent from the above equations. Two simultaneous equations must be solved in order to complete the analysis. This, however, is somewhat simpler than the solution of three equations simultaneously as would normally be required for a three-component mixture. The major disadvantage occurs when there is no common isoabsorptive point in the spectra of the three components. When this occurs, absorbancy index values at three wavelengths for one of the three components are required to apply the above equations.

The first paper in this series (1) showed that a plot of the absorbancy ratio values of binary mix-

tures *vs.* the relative concentration of such mixtures was a straight line if one of the two wavelengths used in the analysis represented an isoabsorptive point. A similar situation holds for ternary mixtures. If the fraction of one of the components in a ternary mixture is held constant, a plot of absorbancy ratio *vs.* the fraction of one of the other two components results in a straight line. An examination of Eq. 6 will indicate the reason for this. With Fz a constant, this equation assumes the form of $y = mx + b$. The slope and intercept of this line will depend on the fraction of Z in the mixture. If a series of solutions are prepared, each series containing a fixed quantity of Z and varying quantities of X and Y, and the absorbancy ratios derived from these plotted *vs.* the relative concentration of either X or Y in the mixture, a series of straight lines will result. These lines will intersect at some point either on the graph or at an extrapolated distance from it.

A similar condition holds for Eq. 8. In this case, however, a series of straight lines will result which have the same slope but different intercepts. This occurs because of the isoabsorptive point at λ_4. The analytical use of this type of plot and of that above will be discussed in more detail below. The most important consideration here is that if independent analysis of Z is possible by other means, the above equations, based on the principles of Q analysis laid down in earlier publications (1–3) may be used to analyze for the other two components in the mixture and hence obviate the necessity of solving simultaneous equations.

EXPERIMENTAL

The apparatus and general techniques used in the analysis of the ternary systems described below have been reported in previous publications (1, 2). Two ternary systems were analyzed in order to show the validity of the above equations.

Analysis of Mixtures Containing Phenyl Salicylate, Salicylic Acid, and Gentisic Acid.—These three substances are not appreciably soluble in water. Stock solutions, therefore, were prepared with the aid of alcohol. Since the latter two substances under investigation are acidic, the spectral characteristics can be expected to vary with the pH of the solution being studied. All solutions, therefore, were prepared in such a way that each contained 1.00% concentrated hydrochloric acid in the final dilution. The spectrophotometric curves for these three substances are shown in Fig. 2. Salicylic acid, phenyl salicylate, and gentisic acid absorb ultraviolet radiant energy most strongly at 303, 307, and 330 mμ, respectively. Minima occur at 272, 260, and 270 mμ for phenyl salicylate, salicylic acid, and gentisic acid, respectively. An isoabsorptive point for these three substances occurs at 314 mμ.

Three wavelengths, 303 mμ (the peak for salicylic acid), 314 mμ (the isoabsorptive point), and 330 mμ (the peak for gentisic acid) were chosen for the analysis. Q:303:314 and Q:330:314 values for these three substances were determined and are reported in Table I.

It was pointed out above that a series of straight lines result when the absorbancy ratio is plotted *vs.* the per cent of one of the components in the mixture
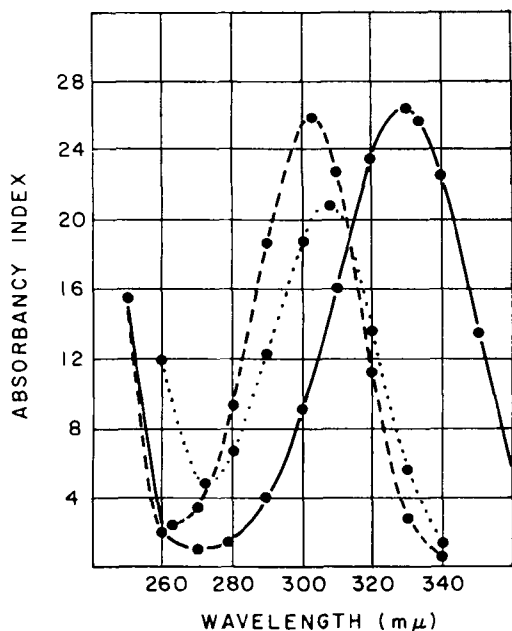
Fig. 2.—Spectrophotometric curves for phenyl salicylate (. . . . .), salicylic acid (– – – –), and gentisic acid (———) in 0.1 N hydrochloric acid.

Fig. 3.—Q curves for gentisic acid, salicylic acid, and phenyl salicylate (salol).

if one of the three components in such a mixture is held constant. Four series of solutions were so prepared that the first contained 0% phenyl salicylate, the second 20% phenyl salicylate, the third 40% phenyl salicylate, and the fourth 60% phenyl salicylate. The amounts of salicylic acid and gentisic acid within a series were allowed to vary. For any particular series, therefore, the amount of phenyl salicylate is a constant. The variables in the series are the quantities of gentisic acid and salicylic acid. Q:330:314 and Q:303:314 values were determined for all solutions and the results plotted in the usual way. These results are shown graphically in Fig. 3 and the equations for these lines are indicated by the data tabulated in Table II. Within experimental limitations, the slopes of these lines are equal. Hence, the lines are parallel. The intercepts of the lines differ, indicating the validity of Eqs. 8 and 9. These equations may now be rewritten in their numerical form. All slope values (that is, the difference between two Q values as shown in Eqs. 8 and 9) were determined by the method of least squares from data accumulated on mixtures. There is, therefore, a slight difference between these values and those obtained from the data in Table I. As stated in previous papers (1, 2), the method of least squares gives a more accurate estimate of the slope and hence such values are carried into future calculations

$$Q{:}330{:}314 = 1.224\ Fx + 0.141\ Fz + 0.143$$

and

$$Q{:}303{:}314 = -0.809\ Fx - 0.318\ Fz + 1.383$$

Fx and Fz represent the fractional quantities of gentisic acid and phenyl salicylate, respectively. Experimentally, absorbancies are measured at the three wavelengths indicated above, the absorbancy
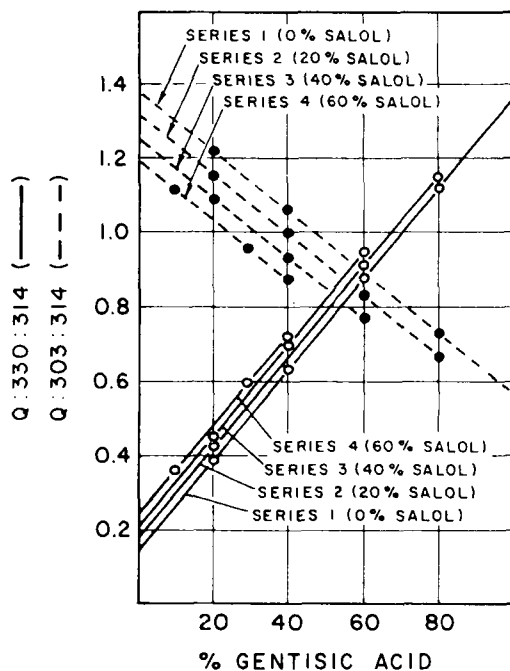
TABLE I.—Q VALUES FOR PHENYL SALICYLATE, SALICYLIC ACID, AND GENTISIC ACID[a]

Compound	Q:330:314	Q:303:314
Phenyl salicylate	0.282 ± 0.004	1.071 ± 0.002
Salicylic acid	0.143 ± 0.001	1.383 ± 0.006
Gentisic acid	1.367 ± 0.004	0.571 ± 0.004

[a] Each Q value is the mean of five determined values the standard deviation is reported after each value.

TABLE II.—EQUATIONS OF Q CURVES SHOWN IN FIG. 3

Series	% Phenyl Salicylate in Mixtures	Line Q:330:314 vs. % Gentisic Acid Slope	Intercept	Line Q:303:314 vs. % Gentisic Acid Slope	Intercept
1	0	0.0122	0.148	−0.00809	1.380
2	20	0.0122	0.174	−0.00822	1.324
3	40	0.0124	0.203	−0.00807	1.255
4	60	0.0125	0.224	−0.00823	1.199

ratio values calculated, and the above two equations solved simultaneously to determine the quantity of gentisic acid and phenyl salicylate in the mixture. The amount of salicylic acid is obtained by subtracting the sum of the fractional quantities of these two components from 1.

Five solutions containing these three substances were prepared and analyzed. The results obtained are shown in Table III.

Simultaneous Analysis of Quinine, Brucine, and Strychnine.—The simultaneous analysis of brucine and strychnine by the absorbancy ratio technique was described in a previous publication (2). If the alkaloid quinine is added to such a binary mixture, the resulting ternary mixture can be analyzed by

TABLE III.—RESULTS OF THE ANALYSIS OF MIXTURES CONTAINING GENTISIC ACID, SALICYLIC ACID, AND PHENYL SALICYLATE

Solution	Gentisic Acid Present, %	Found, %	Salicylic Acid Present, %	Found, %	Phenyl Salicylate Present, %	Found, %
1	20.0	20.5	60.0	60.4	20.0	19.1
2	10.0	9.9	30.0	31.2	60.0	58.9
3	40.0	40.2	60.0	59.8	0.0	0.0
4	20.0	20.2	40.0	39.4	40.0	40.4
5	0.0	0.1	40.0	41.7	60.0	58.2

TABLE IV.—EQUATIONS OF Q CURVES SHOWN IN FIG. 4

Series	% Quinine in Mixture	Line $Q:7.78:6.83$ vs. % Brucine Slope	Intercept
1	0	1.096	0.612
2	25	1.269	0.625
3	50	1.491	0.626
4	70	1.564	0.623

applying the principles inherent in Eq. 6. This, however, presupposes that the cinchona alkaloid can be determined at some wavelength at which brucine and strychnine transmit all or nearly all of the radiant energy passing through the solution.

Infrared spectra of solutions of quinine (0.5 to 2.0 Gm. quinine per 100 ml. of chloroform) were compared with spectra of brucine and strychnine and, on this basis, it was apparent that quinine can be analyzed in the presence of these two substances by measuring absorbancies at 6.13 μ. Solutions of quinine in chloroform were first considered. A linear relationship was observed between the absorbancy at 6.13 μ and the concentration of quinine (C_q) in the solution expressed in terms of Gm. per 100 ml. The equation of this line was found to be: $A_{6.13\,\mu} = 0.283\ Cq + 0.025$. It should be pointed out that th s equation is valid only for the conditions of operation cited earlier (2). The intercept of the above equation indicates that there is a slight deviation from Beer's law at this wavelength. To avoid gross errors in the analysis, this equation was used to calculate Cq for all determinations at this wavelength.

Equation 6 presupposes a knowledge of absorbancy index values for quinine at the two wavelengths at which brucine and strychnine are analyzed (6.83 and 7.78 μ). In a manner similar to that described above, the absorbancies of solutions of quinine at these two wavelengths were plotted vs. the concentration (Cq) in Gm. per 100 ml. of chloroform. The equations of the two straight lines are: $A_{6.83\,\mu} = 0.173\ Cq$ and $A_{7.78\,\mu} = 0.095\ C_q + 0.004$. Both equations indicate that Beer's law is obeyed at these wavelengths.

The inherent qualities of Eq. 6 were discussed above. To check the validity of these observations in the infrared region of the spectrum, four series of solutions were prepared in a manner analogous to that described under the section on the analysis of phenyl salicylate, salicylic and gentisic acids. $Q:7.78:6.83$ values were determined for each of the solutions within a series and the results plotted in the usual way. These results are shown graphically in Fig. 4 and in tabular form in Table IV. This table shows the slopes to be different and the intercepts the same. This, however, is a rather unique case. It was pointed out above that there should
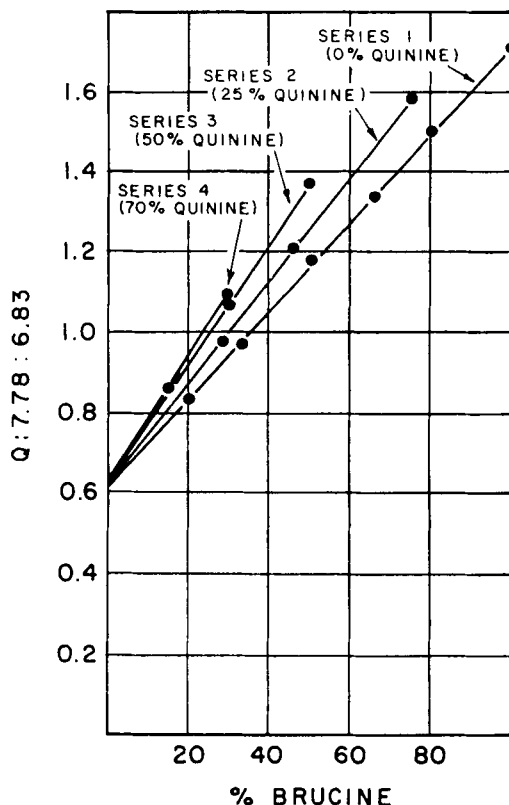
Fig. 4.—Q curves for brucine, strychnine, and quinine.

be a point of intersection in lines such as these. In this case, this point occurs at or near the intercept. In other ternary mixtures, however, this would not be the case. The Q value at the point of intersection would not necessarily coincide with the intercept value.

On the basis of the data reported here or in a previous publication (2), Eq. 6 may be rewritten as follows

$$Q:7.78:6.83 = \frac{1.096\ Fb + Fq\ (0.095/0.371 - 0.612) + 0.612}{1 + Fq\ (0.173/0.371 - 1)}$$

Fb is the fraction of brucine in the ternary mixture and Fq the fraction of quinine in the same mixture. Experimentally, the numerical value of Fq is obtained by determining the absorbancy of the mixture at 6.13 μ, applying the necessary correction factors cited above, and dividing the value obtained for Cq by weight of the sample taken in the analysis.

TABLE V.—RESULTS OF THE ANALYSIS OF MIXTURES CONTAINING QUININE, BRUCINE, AND STRYCHNINE

Solution	Quinine		Brucine		Strychnine	
	Present, %	Found, %	Present, %	Found, %	Present, %	Found, %
1	50.0	47.0	30.0	31.9	20.0	21.1
2	25.0	24.2	28.7	29.4	46.3	46.4
3	0.0	0.0	80.1	80.8	19.9	19.2
4	40.1	41.3	9.6	10.1	50.3	48.6
5	50.0	51.6	50.0	51.6	0.0	0.0

This value is now substituted into the above equation, the Q:7.78:6.83 value calculated from the absorbancies observed at 7.78 and 6.83 μ, and the fraction of brucine in the mixture calculated by solving this equation. The fraction of strychnine in the mixture can now be determined by subtracting the sum of Fb and Fq from 1.

A number of solutions containing quinine, brucine, and strychnine were prepared and analyzed. The results obtained for five of these solutions are shown in Table V.

DISCUSSION

The absorptiometric principles involved in the analysis of ternary mixtures do not differ from those for binary mixtures. While the equations derived appear to be more complicated, they are still based on Beer's law and the principles of absorbancy ratio analysis outlined in previous publications (1, 2). The comments in these two publications are as applicable here as they were to the analysis of binary systems. The pertinent factors cited therein included slope of the Q curve, accuracy of the absorbancy measurement, relative concentrations of the active ingredients, nature of the pharmaceutical, and spectral characteristics of the components of the mixture.

Several points arise, however, from the experimental data accumulated on ternary mixtures. It was pointed out above that a straight line results if the Q values of ternary mixtures in which one of the components is held constant are plotted *vs.* the relative concentration of one of the components in the mixture. This is shown graphically in Figs. 3 and 4. The question arises as to the utility of such plots. Three possible situations can result from such plots. The first involves the case where all three components have an isoabsorptive point in common, the second where only two of the three components in the mixture have a common isoabsorptive point, and the third where no suitable isoabsorptive point occurs.

If the absorbancy ratio values of ternary mixtures and the relative concentrations are compared in the usual way, and only two of the three components share an isoabsorptive point, then a point arises in the series of curves plotted at which all mixtures have the same absorbancy ratio value. Such a point may represent experimental or hypothetical mixtures. In the case of quinine, brucine, and strychnine, the point at which all lines intersect represented an experimental condition. It should be evident that such a point could occur (on extrapolation) to the left of the Y axis if a different ternary mixture were investigated. The utility of such a point of intersection is not difficult to ascertain. In the vicinity of such a point, an analysis of

the ternary mixture by the principles laid down herein would yield poor analytical results. This occurs simply because any number of mixtures have the same Q value. This then indicates that the presence of such a point on the graph is not desirable if the entire range of the mixture (0 to 100%) is to be investigated. It is important to consider, therefore, the relative concentrations of the three components in the mixture. If these are such that Q values obtained on analysis are sufficiently removed from this point of intersection, then the analysis will yield satisfactory results. For example, a 1-1-1 mixture might be easily analyzed, but it would be impossible to analyze accurately a 3-2-1 mixture. By plotting results in the manner shown in Fig. 4, the feasibility of the analysis can be quickly established.

A somewhat different situation occurs in the case of phenyl salicylate, salicylic and gentisic acids. These three substances have a common isoabsorptive point and hence the slope of the Q curves is a fixed quantity. This value is always equal to the slope of the Q curve for the binary mixture composed of two of the three substances in the ternary mixture. The absorbancy index value for the third component at the first wavelength used for the calculation of the Q values is, therefore, the significant constant. If the value of a_1/a_6 approaches that of Qy (see Eq. 6), then the analysis will not be very accurate primarily because the third component is not altering the intercept value of the Q curves to a significant extent. This results in a situation where many mixtures have essentially the same Q value. If a_1/a_6 and Qy differ drastically (but again not so drastically that their determination becomes difficult) then the analysis can be expected to yield results in keeping with good analytical practice.

To summarize, therefore, a series of Q curves for ternary mixtures gives the analyst an indication of the accuracy with which the analysis can be conducted. If a point of intersection is present on the graph, the analyst can expect that analysis of mixtures having compositions defined by the graph in the vicinity of this point will not be accurate. The greater the difference between a_1/a_6 and Qy (on the basis of Eq. 6) the better the results will be.

The reader may well ask what would happen if no suitable isoabsorptive point is present in the spectra of two of the three substances. This obviously complicates the equations derived in this paper but, again, if one of the components is held constant, a plot of absorbancy ratio *vs.* the per cent of one of the other components in the ternary mixture should result in a curve. In the second paper in this series (2), the potassium bromide technique was used in the analysis of two sulfa drugs. This binary mixture can, in fact, be considered as a ternary mixture containing potassium bromide, sulfathiazole,
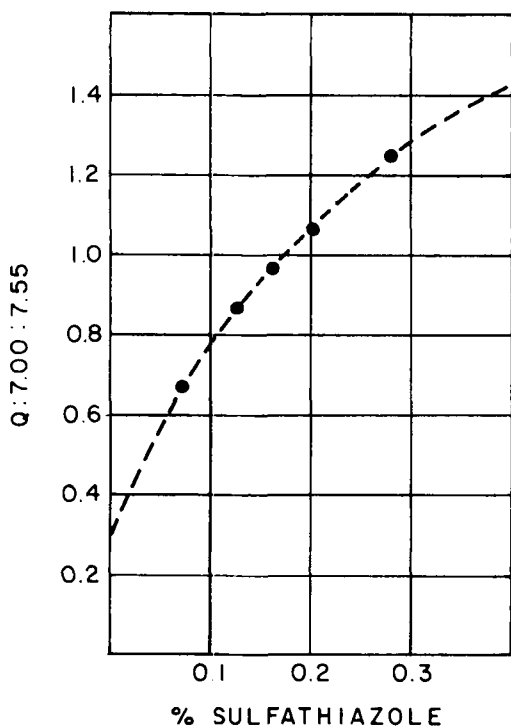
Fig. 5.—*Q* curve for sulfathiazole, sulfapyridine, and potassium bromide.

and sulfapyridine. Potassium bromide has linear but significant absorption (as mentioned in the earlier paper) but is also present in what might be considered as constant quantities. The pellets prepared for this investigation contained very near to 99.6% potassium bromide and 0.4% active ingredient or ingredients. Since it was only the latter value that was representative of the two variables, the 99.6% of potassium bromide was a constant. It was mentioned in the earlier publication that neither of the two wavelengths (7.00 and 7.55 μ) used in the analysis represented an iso-absorptive point. A plot of $Q:7.00:7.55$ *vs.* the % of sulfathiazole in a ternary mixture containing the components under discussion should result in a curve. This is the case and such a curve is shown in Fig. 5. Once such a curve is constructed, it can be used to determine the concentration of sulfathiazole (and sulfapyridine, by proper choice of wavelengths) in unknown mixtures.

The major disadvantage of this type of analysis is the need for a simultaneous solution of two equations. The constants required for these equations are relatively easy to accumulate primarily because most of these are absorbancy ratio values and hence concentration independent. Equation 6 or its equivalent does provide an alternative approach. If the third substance in a mixture can be determined by some other method of analysis in such a way that the other two components do not interfere, then the other two components in the ternary mixture can be resolved by application of the above equation. Such situations often arise in pharmaceutical analysis. If one of the three components in the mixture is acidic, for example, then a volumetric method of analysis might be used to determine its concentration. From a knowledge of the initial weight of the sample, the fraction of this acidic substance in the mixture can be determined. Equation 6 will now yield relative values for the other two components. These, however, can be easily converted to absolute values on the basis of the weight of the sample taken in the analysis.

The accuracy of this technique does not approach that found in the case of binary mixtures. The ternary mixtures investigated could be analyzed to within approximately 2 to 3% of theory. Since this presupposes no separation of the components, the accuracy is probably better than that obtained by more conventional techniques.

CONCLUSION

A number of equations have been derived to show the applicability of the absorbancy ratio technique to the analysis of ternary mixtures. Such mixtures can be resolved to within 2 to 3% of theory provided the spectral characteristics of the individual components meet the criteria listed above. Other limitations of this technique are discussed.

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