Analysis of Alkaloid Mixtures by Charge-Transfer Complexation

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Abstract □ Binary mixtures of weak and strong UV-absorbing alkaloids were analyzed by a charge-transfer spectrophotometric method, utilizing iodine in ethylene dichloride as the acceptor. In the uncomplexed form, the strong absorbing alkaloid (papaverine, quinine, ergotamine, or reserpine) was measured at a wavelength where there was no interference from weak absorbers (at 335, 332, 315, or 300 nm, respectively). The weak absorbing alkaloid (ephedrine, codeine, atropine, or homatropine methylbromide) was determined by computing its contribution to the total charge-transfer band at 295 nm where absorbance was linearly additive for mixtures. The greater increase in the original ϵ-values of the weak absorbers upon complexation with iodine relative to the corresponding increase in the ϵ-values of the strong absorbers led to good recoveries even at the low dose ratios of the weakly absorbing, and often more potent, alkaloids.

Keyphrases □ Alkaloids—weak and strong UV absorbing in binary mixtures, charge-transfer spectrophotometric analysis □ Spectrophotometry, charge transfer—analysis, weak and strong UV-absorbing alkaloids in binary mixtures □ UV-absorbing alkaloids, strong and weak—binary mixtures, charge-transfer spectrophotometric analysis

Alkaloids occupy a prominent position among extensively employed medicaments. They are frequently prescribed in admixture with each other. Among the convenient methods of analysis of such combinations is the direct UV assay (1, 2). However, the absorption bands of the various alkaloids differ greatly in intensity (1, 3), so the simultaneous analysis of binary systems containing weak and strong UV-absorbing alkaloids is challenging. The problem is made more difficult when the dose ratio of the two types in the mixture is not in favor of the weak absorbing alkaloid, which often happens to be the more potent [*e.g.*, ergotamine–hyoscyamine (4:1), papaverine–codeine (6:1), and papaverine–homatropine methylbromide (20:1)]. For these reasons, the assay of these combinations usually includes a separation procedure[1] (1, 2).

Although the separation of alkaloid mixtures into individual compounds is feasible, it is not always possible to obtain clearcut separations, particularly with alkaloids having close pKa values and similar solubility characteristics (1). Consequently, a method for the simultaneous determination of alkaloids in such combinations without prior separation may be of value.

Recent publications (4, 5) reported that a considerable increase in the band intensity of many alkaloids could be attained *via* charge-transfer complexation with σ-acceptors such as iodine in chlorinated solvents. Such an increase in the original ϵ-values was much greater for weak UV absorbers such as the tropine alkaloids, ephedrine, codeine, and sparteine (up to 100 times the value of the uncomplexed alkaloid) than for strong absorbers such as papaverine, quinine, reserpine, and strychnine (1.5–6 times the original value) (4). Subsequent examination of binary mixtures of the two types revealed a leveling of the maxima of the two components even at the low dose ratio of the weak absorber.

This finding constituted the basis of the present work, which was concerned with the simultaneous analyses of binary mixtures of ephedrine, tropine alkaloids, or codeine with the stronger absorbing papaverine, quinine, ergotamine, or reserpine in formulations. The method was also applied to the analogous combination of sparteine with benzyl alcohol.

EXPERIMENTAL[2]

Alkaloids—Pharmaceutical grade ephedrine base, ephedrine hydrochloride, papaverine hydrochloride, codeine phosphate, atropine sulfate, hyoscyamine hydrobromide, homatropine methylbromide, quinine sulfate, ergotamine tartrate, reserpine, and sparteine sulfate were utilized as working standards.

Alkaloid Dosage Forms—The following commercial preparations were analyzed: tablets of ephedrine hydrochloride with papaverine hydrochloride[3], tablets of ephedrine with reserpine[4], injection of homatropine methylbromide with papaverine hydrochloride[5], pulvules of codeine sulfate with papaverine hydrochloride[6], tablets of total belladonna alkaloids with ergotamine tartrate[7], and injection of sparteine sulfate[8].

Reagents—*Buffer*—Prepare 0.2 *M* pH 9.5 buffer, standardized against the glass electrode with a calomel reference electrode, by dissolving 34.8 g of dibasic potassium phosphate in 900 ml of water. Adjust to pH 9.5 and dilute to 1 liter with water.

Potassium Iodide Solution (*0.2* M)—Dissolve 3.32 g of analytical grade potassium iodide in water and dilute to volume in a 100-ml volumetric flask with water. Prepare fresh every 3 days.

Iodine Solution (10^{-3} M)—Dissolve 25.5 mg of resublimed iodine in spectrograde ethylene dichloride in a 100-ml volumetric flask. The solution is stable for 1 week at 4°.

Standard Solutions—*Alkaloidal Salts*—Dissolve an accurately weighed amount of the appropriate working standard in water and dilute the solution quantitatively and stepwise to obtain a concentration of the salt equivalent to 1.0 mg of base/ml. Pipet 1.0 ml of this solution into a 30-ml separator containing 5 ml of buffer. Extract with two 5-ml portions of ethylene dichloride, passing the separated organic layers through 2 g of anhydrous sodium sulfate supported by glass wool in a small funnel. Collect the filtrate in a 10-ml volumetric flask, wash the filter with a few drops of ethylene dichloride, and dilute to volume with the same solvent.

Ephedrine and Reserpine Bases—Dissolve an accurately weighed amount of the appropriate working standard in ethylene dichloride and dilute to obtain a concentration of 0.1 mg/ml.

[1] Manufacturer's Control Method, EL-Nile Co., Cairo, Egypt, personal communication with Dr. Z. Gad.

[2] Spectra were determined on a Spektromom-203 spectrophotometer, Mom, Budapest, Hungary.

[3] Asmasone (El-Nile Co., Cairo, Egypt) contains (per tablet): ephedrine hydrochloride, 10 mg; papaverine hydrochloride, 30 mg; phenobarbital sodium, 10 mg; and dexamethasone, 0.1 mg.

[4] Renir (Massengill, Bristol, Tenn.) contains (per tablet): reserpine, 0.25 mg; and ephedrine, 8 mg.

[5] Surparyl (Memphis Labs., Egypt) contains (per 2 ml): papaverine hydrochloride, 50 mg; and homatropine methylbromide, 2.5 mg.

[6] Copavin (Lilly, Indianapolis, Ind.) contains (per pulvule): codeine sulfate, 15 mg; and papaverine hydrochloride, 15 mg.

[7] Bellergal (Sandoz, Basel, Switzerland) contains (per tablet): total alkaloids of belladonna leaf as malates, 0.1 mg; ergotamine tartrate, 0.3 mg; and phenobarbital, 20 mg.

[8] Spartocin (Ayerst, New York, N.Y.) contains (per 1-ml ampul): sparteine sulfate, 150 mg; and benzyl alcohol, 0.5%.

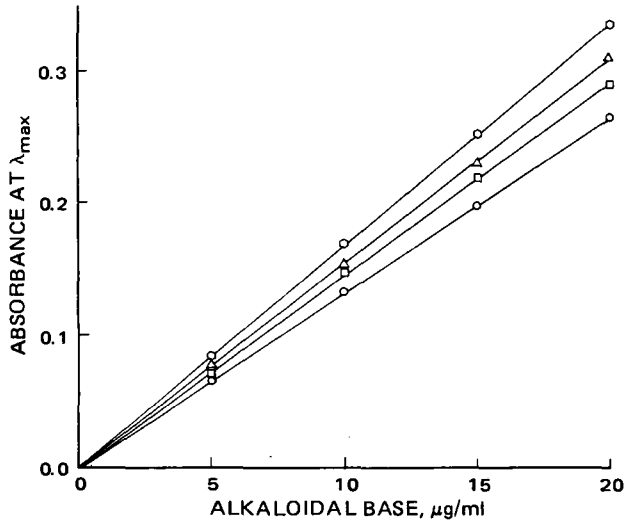

Figure 1—*Calibration curves of alkaloidal bases in ethylene dichloride. Key:* ○, *reserpine at 300 nm;* △, *quinine at 332 nm;* □, *papaverine at 335 nm; and* ○, *ergotamine at 315 nm.*

Homatropine Methylbromide—Proceed as directed under alkaloidal salts, substituting 2 ml of 0.2 *M* potassium iodide solution for the buffer.

Preparation of Samples—*Synthetic Mixtures*—Proceed as directed under alkaloidal salts, using the calculated amount of the proper salts to give the desired ratio of the component alkaloidal bases in the final dilution.

Tablets of Ephedrine Hydrochloride with Papaverine Hydrochloride—Place one powdered tablet, or its equivalent from a composite of 20 powdered tablets, in 2 ml of 0.1 *N* HCl in a 30-ml separator. Extract with 5 ml of freshly distilled ethyl acetate. Discard the organic layer and neutralize the aqueous phase with 0.1 *N* NaOH. Add 5 ml of buffer and extract with two 10-ml fractions of ethylene dichloride, passing the separated organic layers through 2 g of anhydrous sodium sulfate suitably supported in a small funnel. Wash the filter with about 3 ml of ethylene dichloride and collect the filtrate and washing in a 25-ml volumetric flask. Dilute to volume with the same solvent.

Tablets of Ephedrine with Reserpine—Place two powdered tablets, or the equivalent from a composite of 20 tablets, in a 30-ml beaker. Extract with two 4-ml portions of warm (40–50°) ethylene dichloride. Filter the fractions through a small piece of cotton wool, wash the powder in the beaker and the filter with a small amount of warm solvent, and collect the filtrate and washing in a 10-ml volumetric flask. Cool to room temperature and dilute to volume with ethylene dichloride.

Injection of Homatropine Methylbromide with Papaverine Hydrochloride—Pipet 1.0 ml, or the measured contents of a single-dose container of the injection, into a 30-ml separator containing 5 ml of buffer and 2 ml of 0.2 *M* potassium iodide solution. Continue as directed for the tablets of ephedrine hydrochloride with papaverine hydrochloride, beginning with: "extract with two 10-ml fractions of ethylene dichloride . . . ."

Pulvules of Codeine Sulfate with Papaverine Hydrochloride—Place the weighed contents of one capsule in 5 ml of buffer in a 30-ml separator. Continue as directed for the tablets of ephedrine hydrochloride with papaverine hydrochloride, beginning with: "extract with two 10-ml fractions of ethylene dichloride . . . ."

Tablets of Total Belladonna Alkaloids with Ergotamine Tartrate—Proceed as directed for the pulvules of codeine sulfate with papaverine hydrochloride, substituting 10 powdered tablets, or their equivalent from a composite of 20 tablets, for the capsule contents.

Sparteine Sulfate Injection—Proceed as directed for the standard solutions of alkaloidal salts, substituting 1.0 ml or the measured contents of a single-dose container of the injection, for the diluted solution of the alkaloidal salt.

General Procedure—Pipet a volume of the prepared sample equivalent to 0.1–0.2 mg of the strong absorbing alkaloid (papaverine, quinine, ergotamine, or reserpine) into a 10-ml volumetric flask. Dilute to volume with ethylene dichloride and determine the absorbance at the proper wavelength (335, 332, 315, or 300 nm, respectively).

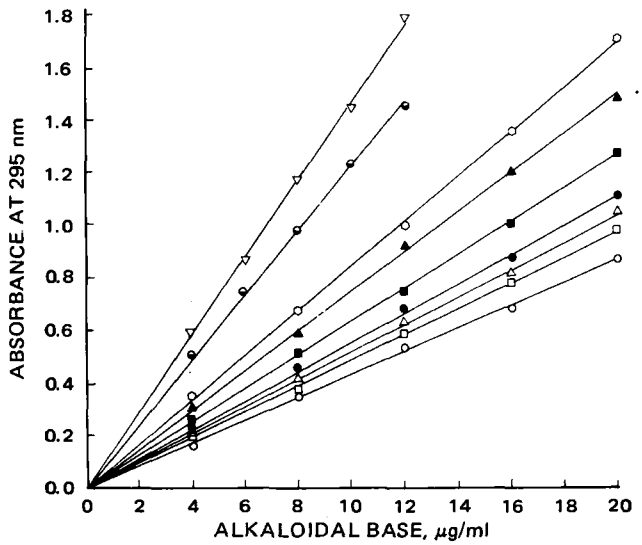

Figure 2—*Calibration curves of the charge-transfer band at 295 nm for ephedrine* (▽), *sparteine* (◒), *reserpine* (○), *homatropine methyliodide* (▲), *quinine* (■), *atropine* (●), *papaverine* (△), *ergotamine* (□), *and codeine* (○).

Pipet a second aliquot of the prepared sample equivalent to 0.05–0.1 mg of total alkaloids into a 10-ml volumetric flask. Add 1.0 ml of iodine reagent solution and dilute to volume with ethylene dichloride. Place the solution in a thermostated water bath at 25 ± 1° for 30 min and then transfer to a 1-cm quartz cell. Determine the absorbance at 295 nm *versus* a blank prepared from 1.0 ml of iodine reagent solution diluted to 10.0 ml with ethylene dichloride and treated similarly.

Calculate the amount of each alkaloid in the preparation by reference to the proper calibration curve and:

$$\text{concentration of strong absorbing alkaloid } (\mu g/ml) = \frac{A_u}{\alpha} \quad \text{(Eq. 1)}$$

$$\text{concentration of weak absorbing alkaloid } (\mu g/ml) = \frac{[A_{295} - (fA_u)]}{\beta} \quad \text{(Eq. 2)}$$

where:

A_u = absorbance of the solution containing the alkaloids in the uncomplexed form at the proper λ_{max} (335, 332, 315, or 300 nm for papaverine, quinine, ergotamine, or reserpine combinations, respectively)

α = slope of the calibration curve (Fig. 1) for the corresponding alkaloidal base in ethylene dichloride as determined by the method of least squares (0.0146, 0.0155, 0.0132, or 0.0168 for papaverine, quinine, ergotamine, or reserpine, respectively)

A_{295} = absorbance of the solution containing iodine-complexed alkaloids corrected to the same dilution as A_u

f = factor derived from the ratio of the slopes of the calibration curves of the strong absorbing alkaloid charge-transfer band at 295 nm (Fig. 2) and the same alkaloidal base at its λ_{max} in ethylene dichloride (Fig. 1) (3.56, 4.13, 3.70, or 5.06 for papaverine, quinine, ergotamine, or reserpine, respectively)

β = slope of the calibration curve for the weakly absorbing alkaloid present in the mixture (charge-transfer band at 295 nm, Fig. 2) as determined by the method of least squares (0.145, 0.123, 0.055, or 0.044 for ephedrine, sparteine, atropine, or codeine, respectively)

Sparteine Sulfate Injection—Pipet two equal aliquots of the prepared sample equivalent to 0.02–0.1 mg of sparteine into separate 10-ml volumetric flasks marked T and R. Add 1.0 ml of iodine reagent solution to Flask T and dilute each flask to volume with ethylene dichloride. Allow the flasks to stand in a thermostated water bath at 25 ± 1° for 30 min. Then determine the absorbance of the solution in Flask T *versus* the solution in Flask R at 295 nm.

Table I—Charge-Transfer Bands in Ethylene Dichloride

Alkaloid	295[a] nm	375[a] nm
Reserpine	46,200	32,000
Sparteine	28,800	14,700
Ergotamine	27,300	13,600
Quinine	24,500	—
Ephedrine	23,400	12,100
Papaverine	17,600	—
Atropine	15,900	6,200
Codeine	13,200	6,700

[a] Based on molecular weight of free base; average of three determinations.

Calculate the amount of sparteine in the preparation from:

$$\text{concentration of sparteine base } (\mu g/ml) = \frac{A}{0.123} \quad \text{(Eq. 3)}$$

where A = absorbance determined at 295 nm, and 0.123 = slope of the calibration curve of sparteine base at 295 nm (Fig. 2).

The conversion factor to convert sparteine base concentration into sparteine sulfate is 1.418.

RESULTS AND DISCUSSION

Charge-Transfer Spectra in Ethylene Dichloride—With iodine in ethylene dichloride, all alkaloids examined showed a major charge-transfer band at 295 nm with molar absorptivities of up to 46,200 (Table I). Most alkaloids also exhibited a second band of lower intensity at 375 nm. Typical charge-transfer spectra of the two types, exemplified by codeine and papaverine, are shown in Figs. 3 and 4, which also illustrate the spectra of uncomplexed alkaloids.

The consistency of the 295-nm band, in spite of the structural variations among the different alkaloids, is probably due to the common origin of the transition in n-electron transfer from the nitrogen of the alkaloid to the acceptor iodine. This is probably the case even for alkaloids having an aromatic heterocyclic nitrogen in addition to an alicyclic one, such as quinine and ergotamine, and for alkaloids containing only aromatic heterocyclic nitrogen, such as papaverine. The large association constants previously reported (4) and the high intensity of the charge-transfer bands (4, 5) (Table I) are common to complexes of n-donors with iodine (6, 7) and are much larger than those of typical aromatic π-donor complexes of iodine (6).

This consistency of the major band is used to advantage in the present assay of alkaloid mixtures, since its intensity is taken as a measure of the total alkaloids present.

Linearity of Beer's Plots of 295-nm Band—Absorbance values

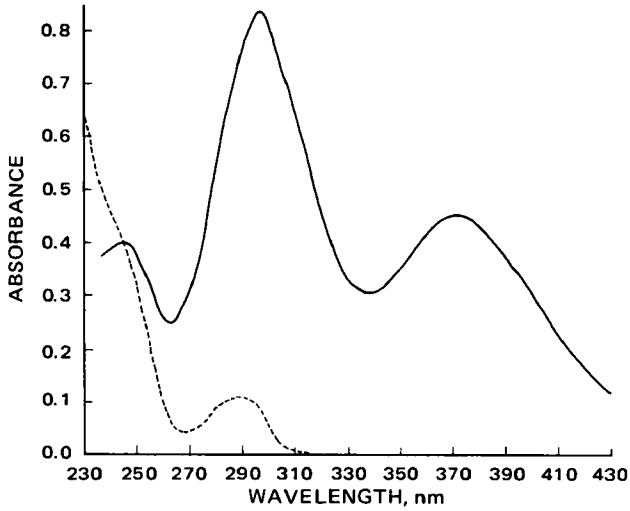

Figure 3—*Spectra of iodine-complexed and uncomplexed codeine in ethylene dichloride. Key: —, codeine (20 μg/ml) with iodine (25.5 μg/ml); and - - - -, codeine (20 μg/ml).*

of the charge-transfer band at 295 nm for each component alkaloid in a mixture were linearly additive, a feature that is imperative in the assay of mixtures (1), under certain conditions. First, the absorbance readings are to be taken after 30 min of equilibration in a constant-temperature water bath. Fixation of time and temperature is essential to minimize the changes in absorbance with time due to the conversion of outer charge-transfer complexes to inner complexes common to n-donor complexes of iodine (8).

Second, the ratio of the molar concentration of the acceptor iodine, [A], to the total alkaloid donor, [D], in the final dilution should not be less than 1.5:1. In the upper concentration ranges, this acceptor–donor ratio is within the stipulated range that is free from Beer's law deviations (4, 6, 8). Although anomalies are usually expected in lower concentration ranges where, in this case, [A] $\gg$ [D] (6, 8–11), no significant deviation from linearity was observed. This finding may be explained as follows.

Anomalies due to self-association (10) or the presence of termolecular complexes (11, 12) were apparently minimized due to the low concentrations employed in the assay (6) (2–20 μg of alkaloid/ml, corresponding to 6×10^{-6}–6×10^{-5} M for an alkaloid of an average molecular weight of 300, and iodine concentration of 10^{-4} M). These relatively low concentrations were attainable because of the large ϵ-values of the charge-transfer bands. Anomalies due to charge-transfer contact pairs with solvent (13, 14) were probably eliminated due to the low tendency of ethylene dichloride to form this type of

Table II—Analysis of Papaverine–Codeine Mixtures

			Recovery			
	Composition, mg %[a]		Papaverine		Codeine	
Mixture	Papaverine	Codeine	%[b]	± SD, %	%[b]	± SD, %
1	0	100	—	—	99.81	0.81
2	5	50	98.75	2.21	101.00	1.56
3	25	25	100.23	0.95	99.65	0.97
4	40	20	99.51	1.29	99.29	0.71
5	50	10	101.35	1.14	98.74	1.60
6	80	5	100.62	0.86	102.55	3.27
7	100	4	99.48	0.55	96.35	2.81
8	80	2	99.60	0.41	95.52	4.32
9	100	0	100.13	0.75	—	—
Mean recovery, %			99.934		99.114	
SE, %			0.326		0.811	
Pooled SD[c], ± S_p, %			0.815		2.137	

[a] Calculated as free bases. [b] Average of five determinations. [c] Calculated from the general formula:

$$S_p = \sqrt{\frac{\Sigma(x_i - \bar{x}_1)^2 + \Sigma(x_i - \bar{x}_2)^2 + \cdots + \Sigma(x_i - \bar{x}_n)^2}{f_1 + f_2 + \cdots + f_n}}$$

where $f_1 + f_2 + \cdots + f_n$ are the available degrees of freedom (16).

Table III—Analysis of Quinine–Atropine Mixtures

Mixture	Composition, mg %[a]		Recovery			
			Quinine		Atropine	
	Quinine	Atropine	%[a]	±SD, %	%[a]	±SD, %
1	0	75	—	—	100.35	0.88
2	10	50	101.25	1.68	99.58	0.63
3	30	30	100.08	0.79	99.61	0.43
4	50	10	99.64	0.97	100.72	0.58
5	80	5	100.55	1.04	99.00	1.02
6	50	2	98.73	1.54	103.7	2.61
7	80	2	100.61	0.82	96.70	4.05
8	50	1	99.48	1.26	93.88	5.34
9	75	0	100.38	0.86	—	—
Mean recovery, %			100.09		99.192	
SE, %			0.310		0.921	
Pooled SD^a, ± S_p, %			0.819		2.259	

[a] See footnotes to Table II.

complexes with alkaloids (5), especially when the species in excess was the acceptor, *i.e.* of the same electron type as the solvent.

These considerations were observed in the construction of calibration curves for the various alkaloids based on the 295-nm charge-transfer band (Fig. 2). Under these conditions, absorbances were linearly additive for mixtures over a reasonable range of the relative concentrations of the two components.

Wavelength Selection—In the assay of binary mixtures of weak and strong UV-absorbing alkaloids, the free bases, liberated by pH 9.5 buffer, were extracted into ethylene dichloride. The absorbance of one aliquot of the extract was read at a preselected wavelength to determine the strong absorbing component present in the uncomplexed form. Iodine was added to a second aliquot, and the absorbance of the total charge-transfer band was read at 295 nm.

The selection of the proper wavelength for measurement of uncomplexed strong absorbing alkaloid was based on the following observations. The alkaloids examined (papaverine, quinine, ergotamine, and reserpine) have very strong bands in ethylene dichloride at or below 270 nm (at 240, 236, 242, and 270 nm, respectively) and weaker, but still fairly strong, bands around or above 300 nm (at 335, 332, 315, and 300 nm, respectively). Maximum sensitivity may be attained by taking the measurements at the intense short wavelength bands. However, overlap with end absorption by many weak absorbers occurred at short wavelengths, leading to interference (Figs. 3 and 4). Accordingly, the long wavelength bands were used for the selective determination of the strong UV-absorbing alkaloids. At these wavelengths, none of the weakly absorbing alkaloids tested had any significant absorption (1, 2, 15), as was verified experimentally.

The amount of each alkaloid in the binary mixture may be calculated using Eqs. 1 and 2.

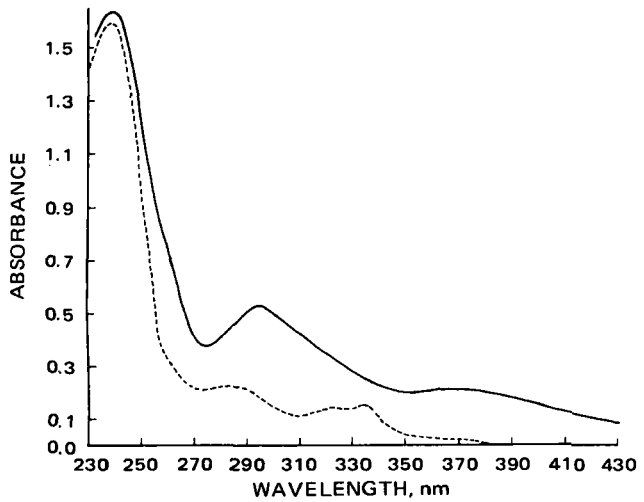

Figure 4—*Spectra of iodine-complexed and uncomplexed papaverine in ethylene dichloride. Key: —, papaverine (10 μg/ml) with iodine (25.5 μg/ml); and - - - -, papaverine (10 μg/ml).*

Application to Synthetic Mixtures—The validity of Eqs. 1 and 2 was tested by analyzing synthetic binary mixtures containing known amounts of alkaloids. Four mixtures were selected as typical examples of pharmaceutical preparations, namely, papaverine hydrochloride with codeine phosphate, quinine sulfate with atropine sulfate, ergotamine tartrate with hyoscyamine hydrobromide, and reserpine with ephedrine.

The results, illustrated in Tables II–V, show good recoveries and high precision in binary mixtures having the ratio of the two alkaloids in the general range of 1:10–10:1. Fairly good accuracy and precision were obtained in the wider range of 1:20–20:1. However, the method yielded progressively poorer results with more extreme ratios (Tables II–V). It failed on application to quinine–atropine mixtures in the ratio of 250:1 as exists in some commercial dosage forms[9].

The data in Tables II–V suggest the suitability of the charge-transfer spectrophotometric method for the analysis of binary mixtures of strong and weak UV-absorbing alkaloids when present in the ratio of their common doses.

Assay of Formulations—The charge-transfer spectrophotometric method was applied to assay a number of commercial preparations containing binary mixtures of alkaloids of the two types. The potential interference in the assay by other ingredients of some formulations was eliminated by adopting the proper extraction method. Thus, with tablets of ephedrine hydrochloride with papaverine hydrochloride, which also contain dexamethasone and phenobarbital sodium, the steroid was removed, together with most of the barbiturate, by preliminary extraction with ethyl acetate in an acidic medium. The second partition with ethylene dichloride in an alkaline medium effectively isolated the pure alkaloidal bases, retaining any remaining amounts of phenobarbital in the aqueous alkaline phase.

In tablets of total belladonna alkaloids, ergotamine tartrate, and phenobarbital, the single partition at the alkaline pH of the procedure was sufficient to separate the acidic barbiturate from the basic alkaloids.

In the case of sparteine sulfate injection, which contains no other alkaloid, the interference of benzyl alcohol present in the preparation was eliminated by a differential procedure. Placing the ethylene dichloride extract containing the uncomplexed alkaloid in the reference cell nullified any background absorption at 295 nm due to benzyl alcohol (λ_{max} of benzyl alcohol in ethylene dichloride at 256 nm, ϵ 468), which may be extracted with the alkaloidal base. Preliminary experiments on benzyl alcohol solutions in ethylene dichloride revealed no formation of charge-transfer bands with 10^{-4} *M* iodine.

The results of analyses (Table VI) confirm the suitability of the presented method for the analysis of these binary mixtures with an accuracy comparable to that obtained with synthetic mixtures.

Alkaloid Mixtures with Quaternary Salts—The quaternized salts of some tropine alkaloids such as atropine methylnitrate, homatropine methylbromide, and *N*-butylscopolammonium bromide are prescribed more often than the tertiary alkaloids, particularly in combination with papaverine. Their presence in the mixture creates a problem because, in spite of their high water solubility, they are

[9] Cold Caps with Quinine, Spencer-Mead, Valley Stream, N.Y.

Table IV—Analysis of Ergotamine–Hyoscyamine Mixtures

Mixture	Composition, mg %[a]		Recovery			
			Ergotamine		Hyoscyamine	
	Ergotamine	Hyoscyamine	%[a]	±SD, %	%[a]	±SD, %
1	0	75	—	—	99.60	0.16
2	5	50	101.88	1.42	100.91	1.47
3	20	20	99.44	0.48	100.45	1.01
4	40	15	98.65	0.80	100.83	1.39
5	60	10	99.89	0.73	98.90	0.50
6	80	5	101.06	0.65	98.60	1.78
7	100	5	101.54	1.32	96.7	3.77
8	100	0	100.68	0.52	—	—
Mean recovery, %			100.449		99.427	
SE, %			0.443		0.570	
Pooled *SD*[a], ± S_p, %			1.078		1.583	

[a] See footnotes to Table II.

Table V—Analysis of Reserpine–Ephedrine Mixtures

Mixture	Composition, mg %[a]		Recovery			
			Reserpine		Ephedrine	
	Reserpine	Ephedrine	%[a]	±SD, %	%[a]	±SD, %
1	0	75	—	—	100.68	0.74
2	5	60	101.65	1.93	99.75	1.02
3	10	80	98,34	1.07	99.74	0.52
4	10	50	99.49	0.80	100.32	1.39
5	40	40	100.81	0.44	99.61	0.78
6	50	15	99.55	0.74	101.05	1.47
7	80	10	101.09	0.95	100.96	1.79
8	80	0	99.86	0.94	—	—
Mean recovery, %			100.112		100.301	
SE, %			0.384		0.291	
Pooled *SD*[a], ± S_p, %			1.017		1.168	

[a] See footnotes to Table II.

partially extracted with halogenated solvents from neutral or basic media, especially when present as the halide salts. In this respect, they are similar to cationic surfactants and other quaternary ammonium compounds (17–19).

This situation resulted in their incomplete extraction into ethylene dichloride with the alkaloidal base and the partial formation of their trihalide charge-transfer band at 280 nm (4, 5), leading to interference. The problem was overcome by the addition of excess iodide ion to the alkaline buffer, which resulted in the quantitative recovery of the quaternized alkaloids as iodide salts into ethylene dichloride with the alkaloidal bases. This finding is in line with the reported facile extraction of many quaternary ammonium compounds into chloroform as their iodide salts (17, 20).

The charge-transfer spectrum of the quaternary iodide with iodine was different from the spectrum previously found for the quaternary bromide salt (4, 5). Instead of the single band at 280 nm and the shoulder at about 355 nm, two well-defined bands at 295 and 375 nm were found (Fig. 5). The spectrum of the quaternary iodide salt was practically identical with the common charge-transfer spectra of alkaloidal bases with iodine in ethylene dichloride (Table I and Fig. 3). This finding reflects the similar electron-donating ability of both the iodide ion and the *n*-electron lone pair of the nitrogen of the alkaloids and their difference from the bromide ion. This evidence also conforms with the spectra of precipitated triiodide salts of other quaternary ammonium compounds and betaines when dissolved in ethylene dichloride, with reported maxima at 295 and 365 nm (21). All of these spectra bear close resemblance to the spectrum of iodine in aqueous potassium iodide, with λ_{max} at 295 and 360 nm (22).

The appearance of the major band at 295 nm for the iodide salts simplified the analysis of mixtures of these weak UV-absorbing quaternary compounds with strong absorbing alkaloids by making possible the adoption of Eqs. 1 and 2 without any modifications. The slope β of the calibration curve of homatropine methylbromide was 0.075 (Fig. 2), determined by the method of least squares. The results

Table VI—Assay of Commercial Dosage Forms

Preparation[a]	Strong Absorbing Alkaloid				Weak Absorbing Alkaloid			
	Label Claim, mg/Unit	Found[b], mg	Added, mg	Recovered, mg	Label Claim, mg/Unit	Found[b], mg	Added, mg	Recovered, mg
Papaverine–ephedrine tablets	30	29.56	30	59.98	10	9.91	10	19.78
Reserpine–ephedrine tablets	0.25	0.261	0.50	0.776	8	8.13	8	16.06
Papaverine–homatropine methylbromide injection	50	49.41	25	75.14	2.5	2.68	5	7.65
Papaverine–codeine pulvules	15	15.33	15	30.51	15	14.73	15	30.0
Ergotamine–total belladonna alkaloids tablets	0.3	0.305	0.6	0.894	0.1	0.117	0.5	0.603
Sparteine sulfate injection	—	—	—	—	150	148.60	100	249.72

[a] Detailed composition is given in *Experimental* section. [b] Average of three determinations.

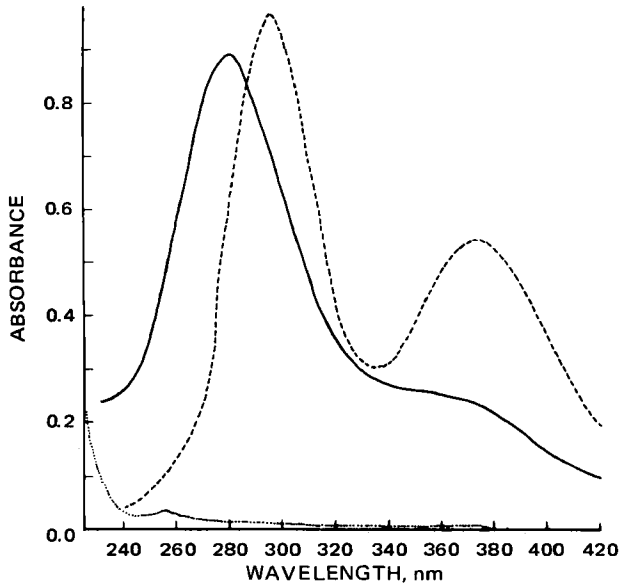

Figure 5—*Spectra of iodine-complexed and uncomplexed homatropine methylhalide salts in ethylene dichloride. Key: —, homatropine methylbromide (12.5 μg/ml) with iodine (25.5 μg/ml); - - - -, homatropine methyliodide (12.5 μg/ml) with iodine (25.5 μg/ml); and . . . . , homatropine methylbromide (12.5 μg/ml).*

of analysis of injection of papaverine hydrochloride with homatropine methylbromide are shown in Table VI.

Comments on Procedure—It is common practice in alkaloid analysis (1–3) first to isolate these basic nitrogenous compounds in one fraction by extracting the sample, buffered to alkaline pH, with suitable organic solvent. The extract is then subjected to further fractionation or directly to analysis. For extracts containing binary mixtures of weak and strong UV-absorbing alkaloids, the charge-transfer spectrophotometric procedure seems to represent a convenient method of analysis without prior separation. In view of the disproportionate amounts of alkaloids of the two types in dosage forms due to differences in potency and the difficulty in achieving clearcut separations of mixtures of alkaloids having close pKa values and solubility parameters, the method may be considered of practical value. Its applicability is not restricted to the specific examples analyzed, since the same technique could be applied to combinations of other strong UV-absorbing synthetic amines capable of charge-transfer formation with iodine such as many antihistamines in combination with weakly absorbing alkaloids or nonalkaloidal drugs[10].

The ratio of weak to strong absorber seems to be limited to the 1:10–1:20 range. More extreme ratios are expected to yield poor recoveries and unacceptable precision. This range, however, adequately covers many popular binary mixtures of alkaloids.

Since the different amines may vary in the rate of attaining maximum intensity of the charge-transfer band and also in the rate of change of absorbance due to the conversion of outer charge-transfer complexes to inner complexes (8), the proper temperature and time lapse before the UV measurement should be carefully controlled and investigated for each new system. Similarly, in the construction of calibration curves, care should be devoted to avoid possible anomalies from the presence of termolecular complexes or the formation of contact charge-transfer pairs in the different concentration ranges.

[10] A case in point is the combination of tripelennamine hydrochloride, 25 mg [λ_{max} 239 and 314 nm, ϵ 14,500 and 8100 (3)], with methylphenidate hydrochloride, 5 mg [λ_{max} 257 nm, ϵ 185 (3)], present in Plimasin (Ciba).

REFERENCES

(1) "Pharmaceutical Analysis," T. Higuchi and E. Brochmann-Hanssen, Eds., Interscience, New York, N.Y., 1961, pp. 313–543.

(2) "Official Methods of Analysis of the Association of Official Analytical Chemists," 11th ed., W. Horwitz, Ed., Association of Official Analytical Chemists, Washington, D.C., 1970, pp. 620–649.

(3) "Isolation and Identification of Drugs," E. G. C. Clarke, Ed., The Pharmaceutical Press, London, England, 1969.

(4) A. M. Taha, A. K. S. Ahmad, C. S. Gomaa, and H. El-Fatatry, *J. Pharm. Sci.*, **63**, 1853(1974).

(5) C. S. Gomaa and A. M. Taha, *ibid.*, **64**, 1398(1975).

(6) R. Foster, "Organic Charge-Transfer Complexes," Academic Press, London, England, 1969.

(7) H. Yada, J. Tanaka, and S. Nagakura, *Bull. Chem. Soc. Jpn.*, **33**, 1330(1960).

(8) C. N. R. Rao, S. N. Bhat, and P. C. Dwivedi, in "Applied Spectroscopy Reviews," vol. 5, E. G. Brame, Ed., Dekker, New York, N.Y., 1972, pp. 1–170.

(9) P. H. Emslie, R. Foster, C. A. Fyfe, and I. Horman, *Tetrahedron*, **21**, 2843(1965).

(10) D. W. Tanner and T. C. Bruice, *J. Phys. Chem.*, **70**, 3816(1966).

(11) G. D. Johnson and R. E. Bowen, *J. Am. Chem. Soc.*, **87**, 1655(1965).

(12) S. Carter, *J. Chem. Soc., A*, **1968**, 404.

(13) D. P. Stevenson and G. M. Koppinger, *J. Am. Chem. Soc.*, **84**, 149(1962).

(14) L. E. Sutton, *Arch. Pharm. Chem., Sci. Ed.*, **2**, 105(1974).

(15) A. L. Hayden, O. R. Sammul, G. B. Selzer, and J. Carol, *J. Assoc. Offic. Agr. Chem.*, **45**, 797(1962).

(16) P. D. Lark, B. R. Craven, and R. C. L. Bosworth, "The Handling of Chemical Data," Pergamon Press, Oxford, England, 1968, p. 107.

(17) "Cationic Surfactants," E. Jungermann, Ed., Dekker, New York, N.Y., 1970, pp. 420–422.

(18) P. A. Lincoln and C. C. T. Chinnick, *Analyst*, **81**, 100(1956).

(19) M. J. Rosen and H. A. Goldsmith, "Systematic Analysis of Surface Active Agents," 2nd ed., Wiley–Interscience, New York, N.Y., 1972, pp. 39–52.

(20) R. Reiss, *Arzneim.-Forsch.*, **6**, 77(1956).

(21) J. S. Wall, D. D. Christianson, R. J. Dimler, and F. R. Senti, *Anal. Chem.*, **32**, 870(1960).

(22) "Colorimetric Determination of Nonmetals," D. F. Boltz, Ed., Wiley–Interscience, New York, N.Y., 1958, pp. 218–220.

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