Assay of Quaternary Ammonium Compounds in Various Dosage Forms by Acid-Dye Method

L. G. CHATTEN[▲] and K. O. OKAMURA

Abstract □ An organic dye salt partition technique was employed to estimate quantitatively some medicinally important quaternary ammonium compounds. Methyl orange, bromthymol blue, and orange IV were used as color-forming agents for the quaternary ammonium agents. The intensity of the color was measured spectrophotometrically. Linear relationships between concentration and absorbance were obtained for the majority of quaternary ammonium substances investigated. By using the calibration curves obtained, a quantitative procedure was developed for pharmaceutical dosage forms. Satisfactory results were obtained with the majority of dosage forms investigated. The tablet excipients studied had negligible effects on the procedure. The results obtained with this method were compared to results by an official procedure or the manufacturer's method, where possible.

Keyphrases □ Quaternary ammonium compounds (16) in 23 dosage forms—analysis, acid-dye technique □ Acid-dye technique—analysis, 16 quaternary ammonium compounds in 23 dosage forms □ Colorimetry—use of acid dyes as color-forming agents for analysis of quaternary ammonium compounds □ Complex formation—acid dyes–quaternary ammonium compounds, spectrophotometric analysis in dosage forms

In 1943, Auerbach (1) described a colorimetric method of analysis in which buffered solutions of quaternary ammonium compounds were treated with bromphenol blue and the ion-pair was extracted with ethylene dichloride. Colichman (2) analyzed two surfactants by a direct photometric titration, while Ballard *et al.* (3) determined several quaternary ammonium compounds by colorimetric measurement of the salt that the drugs formed with bromthymol blue.

Two photometric methods were presented by Helgren *et al.* (4) in 1957 for the analysis of hexocyclium methosulfate. Bromthymol blue was used as the color-producing agent in one instance, and ammonium cobaltothiocyanate was used in the other. Santoro (5) selectively determined isopropamide iodide in the presence of amine bases by treating it with methyl orange in a buffer at pH 10.2.

The most intensive work in this field has been by Schill and his coworkers. They published a series of papers on the photometric determination of amines and quaternary ammonium compounds with bromthymol blue (6–8). Results also were published on the determination of quaternary ammonium compounds with hexanitrodiphenylamine (9) and by their picrates (10). However, these methods were nonspecific because amines would react under the same conditions. In a more recent investigation, Irving and Markham (11) utilized bromcresol green as a color-forming agent in the analysis of long-chain, tertiary alkylamines and quaternary ammonium salts. Unfortunately, the blank is dependent both on pH and on concentration of the excess reagent.

UV spectroscopy has been employed by several authors as a means of analyzing certain quaternary ammonium salts and some of their dosage forms (12–15). In addition, the BP (16), USP XVIII (17), and NF XIII (18) employ this technique for a limited number of quaternary compounds.

In 1954, Carkhuff and Boyd (19) assayed cetylpyridinium chloride by titrating it with sodium lauryl sulfate in a two-phase system. NF XIII (18) describes a similar titrimetric method for benzethonium and cetylpyridinium chlorides, which also employs a two-phase system with sodium tetraphenylboron as titrant and bromphenol blue as indicator. NF XIII (18) also extensively employs nonaqueous titrimetry in glacial acetic acid as a means of assaying a substantial number of quaternary ammonium salts. In addition, a variety of titrimetric techniques are employed for various dosage forms. Billow and Baker (20) proposed a procedure for benzethonium chloride which is similar to that of NF XIII but which was carried out in a one-phase system. Eksborg *et al.* (21) reported the determination of emepronium bromide in urine by an ion-pair extraction method.

Although several methods have been employed in the analysis of quaternary ammonium substances, disadvantages were found to exist in all of them. The UV method is sensitive and rapid; however, interferences from tablet excipients occur in many instances. Most volumetric and gravimetric methods require large samples and cannot be applied to many dosage forms.

Therefore, the purpose of this investigation was to employ a sensitive and rapid method of analysis that could be applied to a large number of dosage forms whose active ingredient contains the quaternary ammonium functional group. A colorimetric method involving reaction of the medicinal agents with various acid dyes was selected.

EXPERIMENTAL[1]

Chemicals—The following were used: chloroform, methylene chloride, glacial acetic acid, ether, hydrochloric acid, potassium chloride, potassium acid phthalate, sodium hydroxide, potassium phosphate, and boric acid. All were ACS grade.

[1] A Beckman model B spectrophotometer, a Beckman model DB spectrophotometer, a Beckman Zeromatic II pH meter, magnetic stirring apparatus, and conventional laboratory glassware were used.

Table I—Conditions for Quantitative Assay of Commercial Quaternary Ammonium Dosage Forms by the Organic Dye Salt Partition Method

Quaternary Ammonium Compound	Molecular Weight	Amount of Compound in Assay Solution Aliquot, μmoles	Dye Used	Optimum pH	Optimum Wavelength for Measurement, nm.	Procedure Used	Modification of General Procedure for Dosage Forms
Ambenonium chloride	608.5	0.24	Orange IV	6.0	410	B (tablets)	None
Benzethonium chloride	466.1	0.36	Methyl orange	8.0	415	A (solution)	See text
Cetylpyridinium chloride	358.0	0.42	Methyl orange	7.0	405	A (solution)	See text
Demecarium bromide	716.6	0.21	Orange IV	10.0	415	B (solution)	None
Domiphen bromide	414.5	0.36	Methyl orange	8.0	415	A (tablets)	Lozenges assayed by tablet method, powder treated as calibration curve
Edrophonium bromide	246.2	0.60	Bromthymol blue	7.0	410	C (solution)	None
Hexocyclium methosulfate	428.0	0.35	Methyl orange	8.0	410	A (tablets)	None
Isopropamide iodide	480.4	0.31	Methyl orange	8.0	415	A (tablets)	None
Methantheline bromide	420.3	0.35	Methyl orange	8.0	415	A (tablets)	None
Oxyphenonium bromide	428.4	0.35	Methyl orange	8.0	415	A (tablets)	None
Penthienate bromide	420.4	0.35	Orange IV	6.0	405	B (tablets)	None
Penthienate bromide			Orange IV	6.0	405	B (solution)	None
Pentolinium tartrate	538.6	0.27	Bromthymol blue	7.0	410	C (tablets)	See text
Pentolinium tartrate			Bromthymol blue	7.0	410	C (solution)	See text
Pipenzolate bromide	434.4	0.34	Orange IV	8.0	410	B (tablets)	None
Propantheline bromide	448.4	0.33	Methyl orange	8.0	410	A (tablets)	See text
Propantheline bromide			Methyl orange	8.0	410	A (solution)	See text
Pyridostigmine bromide	259.1	0.57	Bromthymol blue	7.0	410	C (tablets)	None
Trimethidinium methosulfate	490.7	0.30	Bromthymol blue	7.0	410	C (tablets)	None

Reagents—The following were used: 0.0001 *N* methyl orange[2], 0.0001 *N* orange IV[2] (tropaeolin OO), 0.0001 *N* orange II[2], 0.0001 *N* bromphenol blue[2], 0.0001 *N* bromthymol blue[2], 0.05 *N* perchloric acid in dioxane (standardized against potassium acid phthalate), 0.02 *M* sodium tetraphenylboron, 6% mercuric acetate, crystal violet indicator (0.5% in glacial acetic acid), methyl red indicator (0.1% in glacial acetic acid), and bromphenol blue solution (1 in 2000).

Quaternary Ammonium Substances—See Table I.

Analysis of Crystalline Quaternary Ammonium Materials—The purity of most of the crystalline materials was determined by a well-recognized, nonaqueous titrimetric technique.

The following substances were analyzed by a method similar to that employed in USP XVIII (17) for demecarium bromide: bethanechol chloride, demecarium bromide, isopropamide iodide, mepenzolate bromide, penthienate bromide, pentolinium tartrate, and pipenzolate bromide. For edrophonium bromide, methyl red was a more suitable indicator than crystal violet.

For the following substances, chloroform was the solvent and methyl red the indicator: benzethonium chloride, cetylpyridinium chloride, domiphen bromide, hexocyclium methosulfate, methantheline bromide, oxyphenonium bromide, propantheline bromide, pyridostigmine bromide, and valethamate bromide.

Ambenonium chloride and trimethidinium methosulfate were assayed by their respective NF XIII (18) procedures.

Selection of Color-Forming Agent—Solutions of 0.0001 *N* were prepared from each dye and were buffered at pH values of 2, 4, 6, 8, and 10, respectively. Five milliliters (0.5 mmole) of the quaternary ammonium substance was added to 10 ml. of each buffered dye solution contained in a 250-ml. separator. The ion–ion colored species was extracted with 10 ml. of methylene chloride, and the organic layer was drawn into a cell. The cells were stoppered, and absorbance measurements were taken at regular intervals for 120 min., at the wavelength of maximum absorbance. This wavelength was employed for all subsequent determinations of that particular substance.

[2] These dye solutions were buffered with Clark and Lubs phosphate buffers to the appropriate pH.

Calibration Curves—*General Procedure*—Fifty milligrams of the quaternary ammonium compound was accurately weighed into a 100-ml. volumetric flask and dissolved in distilled water. The solution was made up to volume with distilled water, and 10 ml. of it was diluted to 100 ml. to produce a 0.05-mg./ml. solution.

To five 250-ml. separators, each containing 10 ml. of a 0.0001 *N* solution of the dye, were added 1, 2, 3, 4, and 5 ml. of the 0.05-mg./ml. solution of the quaternary ammonium compound. Sufficient distilled water was added to make the volume constant in each separator. Twenty-five milliliters of methylene chloride was then added to each separator from a 50-ml. buret. The separator was shaken vigorously for 1 min., and the system was allowed to equilibrate for 10 min. The inside of the stem was wiped clean with adsorbent paper. The organic phase was then drawn into a cell, and the absorbance was measured at the optimum wavelength, using a methylene chloride blank. The procedure was repeated five times and the calibration curve was constructed from the average.

Procedure A—The general procedure was employed at pH 8.0 with methyl orange as the color-forming agent for those substances so designated in Table I. For cetylpyridinium chloride and propantheline bromide, only 20 ml. of methylene chloride was used to extract the colored species.

Procedure B—This procedure employed orange IV as the color-forming agent and was followed for those substances so designated in Table I. For ambenonium, 20 ml. of orange IV at pH 6.0 was used. The equilibration time, however, was 30 min. and the organic phase was drawn into a 25-ml. volumetric flask and the contents were shaken.

Demecarium required a 20-min. equilibration time and also 10 min. to stabilize the color in the cell prior to taking absorbance measurements. The general procedure was followed for penthienate, and again a 10-min. period was allowed for color stabilization prior to measuring the absorbance.

Procedure C—The general procedure was employed with 20 ml. of bromthymol blue at pH 7.0 as the color-forming agent for the substances so designated in Table I.

Analysis of Pharmaceutical Dosage Forms—*General Procedure for Tablets*—Ten tablets were weighed and finely powdered. A sample of powder equivalent to 5 mg. of the quaternary ammonium compound was weighed into a 150-ml. beaker and stirred magnetically

for 15 min. with 40 ml. distilled water. This solution was suction filtered through Whatman No. 1 paper into a 125-ml. flask. The beaker and residue were washed with a further 30 ml. of distilled water, and the filtrate was made to volume in a 100-ml. volumetric flask. Three milliliters of this solution (theoretically 0.05 mg./ml.) and 2 ml. of distilled water were added to the dye solution employed in the preparation of its calibration curve. The remainder of the procedure was identical to that described for the calibration curve of each particular substance.

General Procedure for Injections and Solutions—A suitable volume of the injection was diluted to yield a theoretical concentration of 0.05 mg./ml. Three milliliters of this solution was added to the appropriate dye solution. The remainder of the procedure was identical to that described for the calibration curve of each particular substance.

For many of the dosage forms, the general procedures, as previously outlined, can be followed. The conditions regarding dye, pH, wavelength, *etc.*, are presented in Table I. The following are modifications required for the assay of certain pharmaceuticals.

Benzethonium Chloride—Ten milliliters of benzethonium chloride tincture (1:500) was pipeted into a 150-ml. beaker containing 10 ml. of distilled water. This solution was heated on a water bath to evaporate the acetone and alcohol. After cooling, this solution was made to 100 ml. in a volumetric flask. Twenty-five milliliters of the resulting solution was diluted to 100 ml., yielding a theoretical concentration of 0.05 mg./ml. of benzethonium. Three milliliters of this solution and 2 ml. of distilled water were added to 10 ml. of 0.0001 *N* methyl orange (pH 8.0). The remainder of the procedure was identical to that described for the calibration curve beginning with: "Twenty-five milliliters of methylene chloride. . . ."

Cetylpyridinium Chloride—

1. Ten milliliters of cetylpyridinium chloride solution (1:2000) was pipeted into a 150-ml. beaker and heated over a steam bath to evaporate the ethanol. The solution was cooled to room temperature and then made to 100 ml. in a volumetric flask. Three milliliters of this solution and 2 ml. of distilled water were added to 10 ml. of 0.0001 *N* methyl orange at pH 8.0 in a 250-ml. separator. The remainder of the procedure was identical to that described for the calibration curve beginning with: "Twenty-five milliliters of methylene chloride. . . ."

2. Five cetylpyridinium chloride lozenges were weighed and finely powdered. A sample equivalent to 2.5 mg. of cetylpyridinium chloride was weighed in a beaker and stirred magnetically for 30 min. with 25 ml. distilled water. The solution was made to volume in a 50-ml. volumetric flask. Three milliliters of this solution and 2 ml. of distilled water were added to 10 ml. of 0.0001 *N* methyl orange at pH 8.0 in a separator. The remainder of the procedure was identical to that described for the calibration curve beginning with: "Twenty-five milliliters of methylene chloride. . . ." A blank was also determined, replacing the dye solution with 10 ml. of distilled water.

Pentolinium Tartrate—

1. The general procedure for tablets was used for pentolinium tartrate tablets (40 mg.), except a solution of theoretical concentration 0.5 mg./ml. was prepared first.

2. Five milliliters of pentolinium tartrate injection was pipeted into a 250-ml. volumetric flask and made to volume with distilled water. To 20 ml. of 0.0001 *N* bromthymol blue at pH 7.0 were added 1.5 ml. of the diluted injection and 3.5 ml. of distilled water. The remainder of the procedure was identical to that described for the calibration curve beginning with: "Twenty-five milliliters of methylene chloride. . . ."

Propantheline Bromide—

1. Ten propantheline bromide tablets (7.5 mg.) were weighed and finely powdered. Fifty milligrams of the powdered material was weighed in a 150-ml. beaker and stirred magnetically for 30 min. with 25 ml. of methylene chloride. The solution was filtered through Whatman No. 1 paper, and the residue was washed with a further 10 ml. of methylene chloride. The filtrate was evaporated to dryness and the residue was dissolved in water and made to volume in a 100-ml. volumetric flask. The remainder of the procedure was identical to that described for its calibration curve.

2. The procedure for 15-mg. propantheline bromide tablets was identical to that described for the 7.5-mg. tablets, except that only 25 mg. of the powdered tablets was used.

3. The general procedure for tablets was used for 30-mg. propantheline bromide tablets.

Table II—Analysis of Commercial Pharmaceutical Dosage Forms

Compound	Average Recovery, %	Average Recovery, % Comparative Method
Ambenonium chloride tablets		
10 mg.	102.9 ± 0.9	95.20[a]
25 mg.	100.2 ± 1.3	94.52[a]
Benzethonium chloride:		
Solution	98.9 ± 0.9	101.26[b]
Tincture	98.8 ± 0.7	95.00[a]
Cetylpyridinium chloride:		
Solution	97.2 ± 1.0	Not available
Lozenge	102.7 ± 1.9	
Demecarium bromide ophthalmic solution	115.3	
Domiphen bromide:		
Lozenge	97.1 ± 0.9	103.33[a]
Powder	100.2 ± 1.1	99.81[c]
Edrophonium bromide injection	100.8 ± 1.1	99.57[b]
Hexocyclium methosulfate tablets with phenobarbital	102.0 ± 1.3	101.45[a]
Methantheline bromide tablets, 50 mg.	102.9 ± 0.9	Not available
Oxyphenonium bromide tablets		
5 mg.	100.1 ± 1.2	98.80[a]
10 mg.	100.5 ± 1.1	100.00[b]
Penthienate bromide:		
Tablets, 5 mg.	102.1 ± 0.8	102.40[b]
Elixir	98.4 ± 0.9	108.00[a]
Pentolinium tartrate:		
Tablets, 40 mg.	101.4 ± 0.7	Not available
Injection	102.8 ± 1.4	100.76[a]
Pipenzolate bromide tablets, 5 mg.	95.8 ± 1.3	Not available
Propantheline bromide tablets		
7.5 mg.	96.4 ± 1.0	Not available
15 mg.	101.7 ± 1.4	
30 mg.	99.4 ± 0.7	
Injection	103.7 ± 0.1	
Pyridostigmine bromide tablets, 60 mg.	97.8 ± 0.4	96.22[b]
Trimethidinium methosulfate tablets, 20 mg.	86.5 ± 2.0	Not available

[a] Analytical data supplied by manufacturer. [b] Analysis by official procedure. [c] Analysis by nonaqueous titration.

4. The contents of the propantheline bromide injection ampul were transferred to a beaker and weighed. Distilled water was added to dissolve the powder, and the solution was transferred to a 100-ml. volumetric flask and made to volume. Appropriate dilutions were made to ensure that the absorbance would fall within the limits of the calibration curve. The remainder of the procedure was identical to that described for its calibration curve.

RESULTS AND DISCUSSION

The results for the analyses of the crystalline materials indicated that they were of high purity, and all were used without further purification.

The dyes chosen for the investigation were bromphenol blue, bromthymol blue, orange IV, orange II, and methyl orange. In most instances, the wavelength of maximum absorbance was the same for all ion-ion-pairs involving one particular dye. Methyl orange was found generally to give colored species with high absorbances and greatest stability. Therefore, it was employed where possible.

Methylene chloride was found to be a more suitable solvent than chloroform. In most instances, 25 ml. was found to be a sufficient volume to extract completely the ion-ion-pair species. Blank determinations on the dyes alone indicated negligible absorbances.

When the most suitable dye for each quaternary compound was selected, the optimum wavelength for each colored species and the optimum pH of the aqueous media were determined (Table I). Linear calibration curves were obtained for all pure substances except bethanechol, isopropamide, and mepenzolate.

Table III—Molar Absorptivities of Quaternary Ammonium Compounds

Compound	Molecular Weight	$M \times 10^{-5}$	Absorbance	Absorptivity ($\times 10^4$)
Benzethonium[a]	466.09	1.28	0.408	3.19
Cetylpyridinium[a]	357.99	2.09	0.593	2.84
Domiphen[a]	414.46	1.45	0.470	3.24
Hexocyclium[a]	428.61	1.40	0.330	2.36
Isopropamide[a]	480.42	1.25	0.321	2.57
Methantheline[a]	420.34	1.43	0.394	2.76
Propantheline[a]	448.42	1.67	0.504	3.02
Oxyphenonium[a]	428.41	1.40	0.346	2.47
Echothiophate[b]	383.23	2.09	0.264	1.26
Edrophonium[b]	246.15	3.25	0.704	2.17
Pentolinium[b]	538.58	1.49	0.597	4.01
Pyridostigmine[b]	261.14	3.06	0.457	1.49
Trimethidinium[b]	490.67	1.63	0.695	4.26
Ambenonium[c]	608.50	1.31	0.497	3.79
Pipenzolate[c]	434.39	1.84	0.483	2.63
Penthienate[c]	420.41	1.90	0.557	2.93
Demecarium[c]	716.61	1.12	0.541	4.83

[a] Reacted with methyl orange. [b] Reacted with bromthymol blue. [c] Reacted with orange IV.

Before the pharmaceutical dosage forms were analyzed, a study on the effect of common tablet excipients was performed. In this investigation, a quantity of the tablet excipient was added to 10 ml. of the appropriate dye and extracted with methylene chloride. The absorbance of the organic phase was measured, and the results showed that the absorbance due to these tablet excipients was negligible. Other common ingredients used as tablet excipients are kaolin, starch, magnesium stearate, and talc. However, all of these compounds are water insoluble and would be filtered out in the procedure. One of the products contained phenobarbital as an active constituent. This drug was also treated with the appropriate dye (methyl orange) and extracted with methylene chloride. The result produced negligible absorbance.

The results for the analysis of the dosage forms are presented in Table II. Where official procedures existed, they were used as a comparative means of analysis. Good results were obtained with the majority of dosage forms, and generally good agreement was noted with the comparative method.

A general procedure could be employed for most analyses, but slight modifications were required in some instances. For the majority of tablets, extraction of the powdered material was made by distilled water. For 7.5- and 15-mg. propantheline bromide tablets, which are sugar coated, methylene chloride was utilized as the extracting solvent, since extraction with distilled water gave poor recoveries.

In some analyses, blank determinations of the sample without the dye were required because the sample was colored. Both benzethonium chloride tincture and penthienate bromide elixir gave no absorbance when extracted with methylene chloride. This indicated that the absorbance would be entirely due to the colored species formed between the quaternary ammonium and the dye in these instances.

Satisfactory results could not be obtained with the dosage forms of demecarium, isopropamide, and trimethidinium. The high results obtained for the dosage forms of isopropamide may be attributed to the calibration curve, which was not entirely linear, or to decomposition of the drug.

The high recoveries obtained for demecarium bromide ophthalmic solution (0.25%) can be explained by the fact that this preparation contained benzalkonium chloride as a preservative[3]. Since this method cannot differentiate between quaternary ammonium compounds, the benzalkonium would also react with the dye. The result would be overestimation.

An explanation for the low recoveries obtained with trimethidinium methosulfate tablets is not apparent, because the calibration curve for the crystalline material was linear and reproducible.

[3] D. F. Solometo, Merck Sharp & Dohme Laboratories, Philadelphia, Pa., personal communication.

A study of the molar absorptivities of the quaternary ammonium compounds shown in Table III gave more indication of the stoichiometry of the reaction. In those instances where methyl orange was the dye of choice, the cationic portion contains one quaternary nitrogen and shows approximately the same molar absorptivity. With bromthymol blue and orange IV, the bisquaternary compounds show molar absorptivities that are approximately twice that of the monoquaternary compounds. This would, therefore, indicate a dye–quaternary stoichiometry of 2:1 for the bisquaternary compounds.

SUMMARY AND CONCLUSIONS

1. A study on various acid dyes as color-forming agents for quaternary ammonium compounds was presented. Methyl orange, orange IV, and bromthymol blue were the most satisfactory.

2. Linear and reproducible calibration curves were obtained for 16 quaternary compounds. Of the compounds investigated, only bethanechol, isopropamide, and mepenzolate failed to show a linear relationship.

3. The effect on the procedure by common tablet excipients was found to be negligible.

4. A quantitative method of analysis was developed for 23 pharmaceutical dosage forms containing a quaternary ammonium compound. The procedure is very sensitive and was adaptable to the various types of dosage forms investigated.

5. On the basis of the present investigation, it is concluded that the proposed method is generally superior to existing methods. It is recognized that the UV method is equally satisfactory except that it is more prone to error by interferences than is the colorimetric technique.

REFERENCES

(1) M. E. Auerbach, *Anal. Chem.*, **15**, 492(1943).

(2) E. L. Colichman *ibid.*, **19**, 430(1947).

(3) C. W. Ballard, J. Isaacs, and P. G. W. Scott, *J. Pharm. Pharmacol.*, **6**, 971(1954).

(4) P. E. Helgren, J. G. Theivagt, and D. J. Campbell, *J. Amer. Pharm. Ass., Sci. Ed.*, **46**, 639(1957).

(5) R. S. Santoro, *ibid.*, **49**, 666(1960).

(6) G. Schill and M. Marsh, *Sv. Farm. Tidskr.*, **67**, 385(1963).

(7) G. Schill, *Acta Pharm. Suecica*, **1**, 101(1964).

(8) *Ibid.*, **1**, 169(1964).

(9) G. Schill, *Anal. Chim. Acta*, **21**, 341(1959).

(10) K. Gustavii and G. Schill, *Acta Pharm. Suecica*, **3**, 241 (1966); through *Chem. Abstr.*, **67**, 102815v(1967).

(11) H. M. Irving and J. J. Markham, *Anal. Chim. Acta*, **39**, 7 (1967).

(12) M. Pernarowski and L. G. Chatten, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 211(1958).

(13) J. Kracmar and J. Zyka, *Cesk. Farm.*, **10**, 449(1961).

(14) L. Varcel, *Pharmazie*, **23**, 19(1968); through *Chem. Abstr.*, **68**, 88908b(1968).

(15) L. Chafetz, *J. Pharm Sci.*, **53**, 1192(1964).

(16) "The British Pharmacopoeia," The Pharmaceutical Press, London, England, 1968, pp. 368, 432, 783, 838, 850, 851, 1050, 1051.

(17) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, pp. 170, 222, 527, 528, 764.

(18) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, pp. 37, 246, 381, 530.

(19) E. D. Carkhuff and W. F. Boyd, *J. Amer. Pharm. Ass., Sci Ed.*, **43**, 240(1954).

(20) J. A. Billow and H. W. Baker, *J. Pharm. Sci.*, **55**, 1446 (1966).

(21) S. Eksborg, B. A. Persson, J. Vessman, and B. Enell, *ibid.*, **60**, 475(1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 1, 1972, from the *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, T6G 2H7, Alberta, Canada.*

Accepted for publication February 21, 1973.

Presented at the 19th Canadian Conference on Pharmaceutical Research, Edmonton, Alberta, Canada, August 1972.

Supported by the Medical Research Council of Canada.

The quaternary ammonium substances and their dosage forms were generously supplied by the following pharmaceutical manufacturers: Ayerst Laboratories, Montreal, Canada; Abbott Laboratories Ltd., Montreal, Canada; Ciba Co. Ltd., Dorval, Quebec, Canada; Hoffmann-La Roche Ltd., Montreal, Canada; Lakeside Laboratories, Lincoln, Neb.; Merck Sharp & Dohme Ltd., Montreal, Canada; Wm. S. Merrell Co., Cincinnati, Ohio; Parke, Davis & Co., Brockville, Ontario, Canada; Poulenc Ltd., Montreal, Canada; G. D. Searle & Co., Bramalea, Ontario, Canada; Smith Kline & French Ltd., Montreal, Canada; Winthrop Laboratories, Aurora, Ontario, Canada; and John Wyeth & Brother, Windsor, Ontario, Canada.

▲ To whom inquiries should be directed.

Microdetermination of Hyoscyamine and Scopolamine in Mixtures

SAWSAN EL-MASRY and SALEH A. H. KHALIL▲

Abstract □ Following the acid-dye technique, hyoscyamine (or atropine) could be selectively determined in the presence of scopolamine, using bromcresol purple at pH 6.6. Total alkaloids were determined using bromthymol blue at pH 5.6. By referring to calibration curves of the two alkaloids with the appropriate dye, the concentration of each alkaloid in the mixture could be computed. The suggested procedure was adopted to analyze the two alkaloids in synthetic mixtures. As low as 0.05 mg. of each alkaloid could be estimated, with average percentage recoveries of 98.6 and 101.5 for hyoscyamine and scopolamine, respectively. Tincture of belladonna was assayed following a modification of the suggested method. The results obtained were comparable with those obtained following the BP method.

Keyphrases □ Hyoscyamine mixtures with scopolamine—acid-dye analysis without prior separation, effect of pH and dye type □ Scopolamine mixtures with hyoscyamine—acid-dye analysis without prior separation, effect of pH and dye type □ Tropane alkaloid mixtures—acid-dye analysis of hyoscyamine and scopolamine □ Acid-dye technique—analysis of hyoscyamine and scopolamine in mixtures

Most reported methods for the determination of tropane alkaloids present in mixtures necessitate separation of the individual alkaloids (1–3). The acid-dye technique reported for the estimation of microquantities of atropine (4, 5) can be adopted to determine the individual alkaloids, without preliminary separation, if a proper choice is made regarding the dye used and the pH of the medium. Khalil and El-Masry (6) found that tropine did not interfere in the assay of atropine when using bromthymol blue at pH 3. The present article examines the effects of pH and selection of the dye on the assay of microquantities of hyoscyamine and scopolamine without prior separation.

EXPERIMENTAL

Materials—Both hyoscyamine sulfate[1] and scopolamine hydrobromide[1] were BP quality. For the dye solutions, bromcresol purple and bromthymol blue were separately dissolved in chloroform to produce 2×10^{-4} *M* solutions. For the buffer solutions, McIlvaine's buffer solutions of various pH values (3–7.5 ± 0.05) were used. Chloroform was reagent grade[1] and freshly distilled. Two lots[2] of belladonna tincture BP were used.

Method—The procedure described by Khalil and El-Masry (6) was followed. Into a 50-ml. separator containing 10 ml. of the buffer solution, a volume of the sample corresponding to about 0.05–0.1 mg. of either alkaloid was added. Then 10 ml. of the chloroformic dye solution was added. After manual shaking for 1 min., the layers were left for complete separation and the chloroformic layer was transferred into a 25-ml. volumetric flask. The aqueous layer was further extracted with 10 and 5 ml. of chloroform, and the color intensity of the combined chloroform extracts was measured at 420 nm. using a spectrophotometer[3] and a blank similarly prepared.

RESULTS AND DISCUSSION

Figure 1 shows the effect of pH on the extractability of the alkaloid–bromcresol purple complex. At all pH values studied, the hyoscyamine–dye complex gave comparatively higher absorbance values at 420 nm. compared with scopolamine. The latter was not assayable at pH values higher than 6.6 since the chloroformic phase was colorless. Due to the relatively high absorbance readings obtained at pH 6.6, hyoscyamine was thus selectively determined at this pH using bromcresol purple. A linear relationship was obtained over the concentration range used (Fig. 2). When using bromthymol blue, both alkaloids were complexed at all pH values studied. However, the calibration curve for hyoscyamine using bromthymol blue at pH 5.6 and that with bromcresol purple at pH 6.6 were almost identical. The slopes were 2.564 and 2.578, respectively (Fig. 2). Therefore, to simplify calculation, pH 5.6 was chosen for the

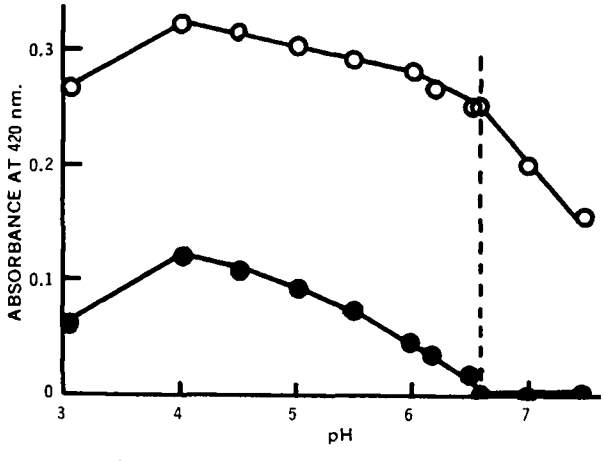

Figure 1—*Effect of pH on extraction of the alkaloid–bromcresol purple complex. Key: ○, hyoscyamine base; and ●, scopolamine base. The amount of the alkaloidal base used was 0.1 mg.*

[1] British Drug Houses Ltd., Poole, England.
[2] William Ransom & Son Ltd., Hitchen, Hartfordshire, England.
[3] Unicam SP 500.