

Spectrophotometric Determination of Some Quaternary Compounds

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A procedure is described for the quantitative determination of several quaternary ammonium compounds. This method is based on the formation of a picric acid-quaternary ammonium complex in alkaline media and spectrophotometric analysis of this complex after chloroform extraction.

LONG-CHAIN quaternary ammonium compounds (QAC) are widely employed in pharmaceutical formulations as antiseptics and preservatives. In addition to their pharmaceutical use, they are being increasingly used in industry and agriculture. It is interesting to note here that frequently these QAC are used in the concentration range of parts per million. A number of methods for the determination of these QAC may be found in the literature. The methods are based on titration with an anionic surfactant (1), precipitation with potassium ferricyanide (2), precipitation with reineckate solution (3), precipitation with sodium tetraphenylboron (4), reaction with bromophenol (5) or other dyes (6), titration in nonaqueous solution or potentiometry (7), etc. Although several of these procedures have been applied to the analysis of small amounts of QAC, the majority of these methods do not readily lend themselves to the rapid determination of some QAC in concentrations of the order of 0.01–0.001% normally employed in pharmaceutical formulations. This report describes a rapid procedure for the determination of small amounts of several QAC alone and in combination with other medicinal agents. This method, a modification of the method of Sloneker *et al.* (8), is based on the reaction of picric acid with the QAC in an alkaline medium to form a colored complex which is measured spectrophotometrically after extraction with chloroform.

EXPERIMENTAL

Materials and Reagents.—Benzalkonium chloride U.S.P., benzethonium chloride U.S.P., cetylpyridinium chloride U.S.P., and picric acid, analytical reagent recrystallized, m.p. 122–123°.

Apparatus.—Beckman DB spectrophotometer with Sargent recorder model SRL and a Beckman DU spectrophotometer with 1-cm. silica cells.

Procedures.—*Spectra of Picric Acid in Chloroform.*—Picric acid was dissolved in water and extracted by chloroform in acid and alkaline medium. The spectra were taken from 300 to 500 $m\mu$.

Spectra of Picric Acid-QAC Complex in Chloroform.—An aqueous aliquot containing 1 mg. of QAC was added to a solution of picric acid made alkaline by the addition of ammonia T.S. The resulting complex was then extracted with chloroform and its spectrum taken from 300 to 500 $m\mu$.

Standard Curves.—All the standard curves were obtained by plotting the absorbance against the concentration of QAC. A 10-ml. aqueous aliquot containing the QAC was made alkaline with ammonia T.S., 2 ml. of a 0.1% picric acid solution was added, and the resulting complex extracted with 3 \times 10 ml. of chloroform. The absorbance of the chloro-

form extract was determined at a wavelength of 360 $m\mu$ using a Beckman DU spectrophotometer.

Analysis of QAC in Known Samples and in Dosage Forms.—Aliquots of the sample solution (tablet form was dissolved and additional water was added to volume) were taken, and the assay procedure was followed as described under *Standard Curves*.

RESULTS AND DISCUSSION

As has been pointed out already, picric acid reacts with QAC to form a complex which can be measured spectrophotometrically after extraction with chloroform. Picric acid because of its strong ultraviolet absorption spectrum and its strong single anionic functional group has also been the reagent of choice for the analysis of various organic cations. Although it has been reported (8) that the amount of picric acid extracted by chloroform from an acid aqueous solution is negligible, our data indicate that this is not the case. Curve B in Fig. 1 shows that a considerable amount of picric acid is extracted by chloroform from an acid solution. However, in a strongly basic medium, chloroform extraction of picric acid is practically negligible as illustrated by curve C. This basic medium has no significant effect on the interaction of picric acid with QAC to form a complex as shown by curve A of Fig. 1. For this reason it is essential that the pH of the aqueous solution containing the QAC be sufficiently high to assure that the noninteracted picric acid exists in solution as the anion so that it is not extracted into

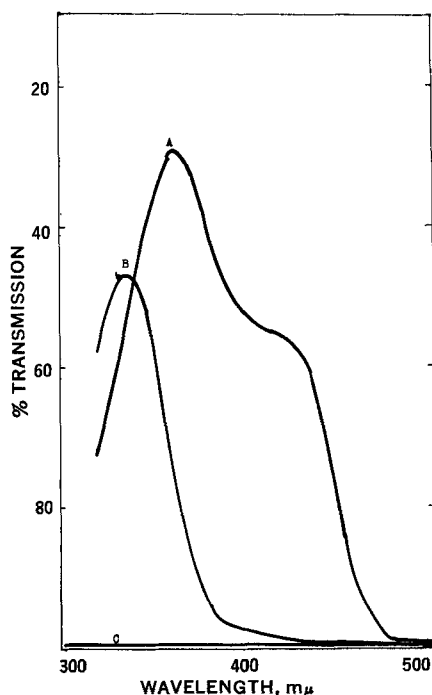


Fig. 1.—Spectra of picric acid and picric acid-QAC complex in chloroform. Key: A, picric acid-QAC complex; B, picric acid in acid medium; C, picric acid in basic medium.

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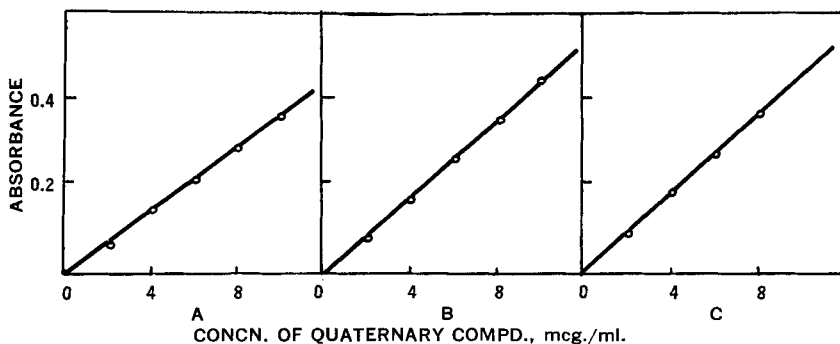


Fig. 2.—Standard curves of quaternary ammonium compound - picric acid complex. Key: A, benzethonium chloride; B, cetylpyridinium chloride; C, benzalkonium chloride.

TABLE I.—ANALYSIS OF KNOWN SAMPLES

Components	Concn.	% Re-covered
Benzalkonium chloride	1:5,000	100.2
	1:10,000	99.0
	1:100,000	95.5
Benzethonium chloride	1:5,000	99.5
	1:10,000	100.4
	1:100,000	99.2
Cetylpyridinium chloride	1:1,000	100.2
	1:5,000	100.1
	1:10,000	102.0

TABLE II.—ANALYSIS OF COMMERCIAL FORMULATIONS

Items	QAC Concn.	% Re-covered
Throat lozenges ^a	Cetylpyridinium chloride, 1:1,500	101 ± .9
	Mouthwash ^a	Cetylpyridinium chloride, 1:4,000
OTC soln. ^b	Benzethonium chloride, 0.02%	102 ± 0.5
	Benzalkonium chloride ophth. soln. ^c	Benzalkonium chloride, 1:10,000
Ophth. soln. ^d	Benzalkonium chloride, 0.02%	78 ± 3

the chloroform phase along with the QAC-picric acid complex.

Satisfactory data were obtained under these conditions, and the absorbance of the QAC-picric acid complex was found to be linear with respect to QAC concentration as shown in Fig. 2. These plots were used for determination of the unknown concentration throughout this investigation. The plot of absorbance as a function of concentration of QAC passed through the origin, indicating complete extraction of the QAC-picric acid complex by the chloroform in this procedure.

Table I shows the quantitative recovery of several QAC in the concentration ranges normally employed in pharmaceutical dosage forms. The good reproducibility obtained indicates that this method is reliable even in concentrations as low as 1:100,000. Table II lists data representing an average of three determinations of each dosage form performed on commercial formulations, again indicating the applicability of this method to various preparations.

^a The Wm. S. Merrell Co. ^b Wampole Laboratories. ^c College of Pharmacy, University of Iowa. ^d Madland Laboratories.

Although only three QAC and a limited number of commercial formulations were investigated, the data presented do indicate that the method would be adaptable to other QAC and other formulations containing this type preservative.

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Amino Acid Derivatives of Aminosalicic Acids

By W. HOEK, J. EBELS, and J. STRATING

A simple method for the synthesis of *p*-amino-salicylglycine ester is given.

TO TEST the antitubercular effect of amino acid derivatives from aminosalicic acids, Foye and Hull (1) prepared a number of these peptide-like

derivatives of 3-, 4-, and 5-aminosalicylic acids with α -amino acids. A simpler method of synthesis became available when Sheehan and Hess (2) introduced dicyclohexyl carbodiimide as a condensation agent for the formation of peptide bonds.

For a research project of the Laboratory of Pharmacotherapy and Pharmaceutical Compounding, University of Groningen, involving the estimation of *p*-aminosalicylic acid and its metabolites

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