

Modified Iodimetric Determination of Echothiophate Iodide

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Abstract □ Kinetic and chemical data are used to increase the precision and specificity of the previously described iodimetric method for echothiophate iodide. Hydrolysis is effected at pH 12.0 to ensure quantitative cleavage of the S—P bond of the echothiophate ion while minimizing interference by probable impurities. The product of hydrolysis—thiocholine—is determined by iodimetric titration under acidic conditions. The coefficient of variation for the raw material and the smallest dosage form is less than 1.0%.

Keyphrases □ Echothiophate iodide—analysis, iodimetric titration of thiocholine □ Iodimetric titration—thiocholine, echothiophate iodide analysis □ Thiocholine, iodimetric titration—analysis, echothiophate iodide □ Coulometry, standardization—iodine titrant, echothiophate iodide analysis

Echothiophate iodide¹ (I), a long-acting cholinesterase inhibitor for the control of glaucoma, was analyzed by two different procedures (1, 2). These methods differ in detail but are based upon the same fundamental chemistry (Scheme I) (3). The thiocholine iodide (II), the product of the alkaline hydrolysis (Step 1), is determined by titration with an oxidant (Step 3). This titer is stoichiometrically related to the quantity of I that has been hydrolyzed.

This report describes the reexamination of Steps 1 and 3 to establish the reagents and conditions for optimum specificity and accuracy. The fundamental kinetic studies by Hussain *et al.* (3) of the chemistry of I in aqueous solutions provided the theoretical data needed to improve the specificity of Step 1. Coulometric techniques were used to develop a standardization procedure for the dilute iodine titrant, which is very unstable. A TLC procedure (3) may be used to detect the presence of impurities.

EXPERIMENTAL

Reagents—The following were used: echothiophate iodide²; arsenic trioxide solution, 0.006 *N*, prepared from NBS primary standard as described in USP (2) and diluted to proper volume; iodine titrant, 0.004 *N*, 0.1 *N* iodine³, diluted to proper titer with distilled water and standardized by titration of 0.006 *N* arsenic trioxide in pH 7 phosphate buffer (2); phosphate buffer, pH 12.0, prepared by dissolving 54.4 g. anhydrous dibasic sodium phosphate in 1 l. of distilled water and adjusting to pH 12.0 with 50% aqueous sodium hydroxide.

Deoxygenated distilled water was used throughout; it was prepared by boiling and cooling under a stream of nitrogen.

Apparatus—All iodimetric titrations were performed potentiometrically with a combination Pt—Ag/AgCl electrode. A Heath Malmstadt-Enke pH and millivolt recording electrometer was synchronized with a Sargent model C constant-rate buret (Catalog No. S-11120-10). A Beckman model 40495 high-alkalinity pH electrode

Table I—Titration of Possible Impurities of Echothiophate Iodide

Compound	Percent Titration ^{a, b}		
	This Method	USP XVII	USP XVIII
$[(\text{CH}_3)_3\text{N}^+-\text{CH}_2-\text{CH}_2-\text{S}]_2^{2I^-}$ III	8	90	57
$(\text{CH}_3)_3\text{N}^+-\text{CH}_2-\text{CH}_2-\text{S}-\text{P} \begin{matrix} \text{O} \\ \text{O}-\text{C}_2\text{H}_5^c \\ \text{O}-\text{H} \end{matrix}$ IV	—	—	—
$(\text{CH}_3)_2\text{N}-\text{CH}_2-\text{CH}_2-\text{S}-\text{P} \begin{matrix} \text{O} \\ \text{O}-\text{C}_2\text{H}_5 \\ \text{O}-\text{C}_2\text{H}_5 \end{matrix}$ V	35	88	90

^a Relative to an equal weight of echothiophate iodide as 100%. ^b The hydrolytic procedure for the raw material was used in each case. ^c No hydrolysis detected.

was calibrated with a pH 12.45 buffer and then used to monitor the pH 12.0 buffer.

Coulometric titrations were performed with a Sargent coulometric current source S-30974. The generating and counter electrodes were fabricated from 18-gauge platinum wire. The general procedure was described previously (4). The iodine titrant was generated at 12 microequivalents/min. Potentiometric end-point detection was used.

Titration of Echothiophate Iodide—All reagents, which were not prepared with deoxygenated distilled water, were deoxygenated with a stream of nitrogen.

A sample of echothiophate iodide, weighing 125 mg., was dissolved in distilled water and diluted to 100 ml. A 10.0-ml. aliquot of this solution was added to the titration vessel along with 30 ml. distilled water and 10 ml. pH 12.0 buffer. A stream of nitrogen was directed into the titration vessel and onto the liquid during transfer. The headspace of the titration vessel was flushed with nitrogen and capped to reduce the amount of oxygen in the solution. [Note: A number of experiments indicated that exclusion of oxygen is not a critical step, but it was deemed advisable because the thiocholine is oxidized by oxygen under basic conditions (5).] After 20 min. at 25 ± 3°, the reaction was quenched by the addition of 2 ml. glacial acetic acid. The solution was titrated with 0.004 *N* iodine to an end-point as indicated by the potential change of the combination Pt—Ag/AgCl electrode.

The blank titration was carried out exactly as already described, except the glacial acetic acid was added first, followed immediately by the pH 12.0 buffer. The 20-min. hydrolysis step was omitted and the sample was titrated immediately. This titer corrected for the amount of free thiol present in the sample. Because the normality of the iodine solution is altered by dust, stopcock grease, and light, it is advisable to restandardize periodically during a series of determinations.

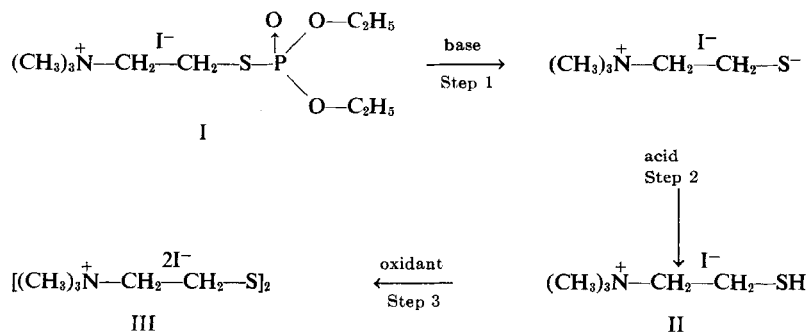
RESULTS AND DISCUSSION

Hydrolysis of S—P Bond—Previous work (3) established that the hydrolysis of I in aqueous media within pH range 10–12 proceeds exclusively by cleavage of the S—P bond to yield II. At successively lower pH's, a greater and greater proportion of I hydrolyzes with

¹ Phospholine iodide, Ayerst Laboratories.

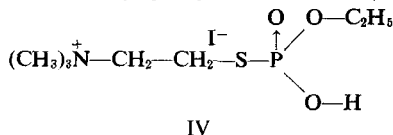
² Prepared by Dr. Gordon Myers, Ayerst Research Laboratories, Montreal, Canada.

³ Anachemia Limited, Champlain, N. Y.



Scheme 1

cleavage of the C—O bond to produce (2-mercaptoethyl)trimethylammonium iodide *O*-ethyl phosphorothioic acid (IV). This sub-



stance will not, under the conditions of the analysis, hydrolyze further to yield II (3). Thus, to ensure quantitative generation of II, the solution must be more alkaline than pH 9.

The necessity of careful pH control is clearly apparent from previous work (3). At pH 12.0, the half-life of echothiophate is 1.1 min.; therefore, the reaction is essentially complete in the 20-min. reaction time required by the procedure described in this report. A reduction in pH of only 1 unit increases the half-life to 10 min.; therefore, after 20 min., only 75% of I is converted to thiocholine. The present method requires a phosphate buffer, in contrast to the previously published procedures (1, 2) which depend upon the addition of sodium hydroxide for pH adjustment. These methods provide a sufficiently basic solution by the addition of a large excess of sodium hydroxide. The result is that while I is completely hydrolyzed, there is a loss of specificity (Table I). The results given in Table I illustrate that the method described in this paper minimizes the interference by probable impurities, e.g., III, IV, and V.

TLC—The presence of excessive amounts of III, IV, and V, which would cause an unacceptably high level of interference, can be detected by TLC (3).

Statistical Evaluation of the Method—A collaborative study, involving three different laboratories with a total of five workers, was performed. Each analyst randomly selected 20 vials from a common stock, pooled the samples, and made one, and only one,

Table II—Collaborative Study of the Echothiophate Iodide Method

Sample	Laboratory			Percent Co-efficient of Variation
	1	2	3	
Vial ^a , mg./vial	1.68	1.69	1.65	0.78 ^b
Raw material, mg./g.	96.74	97.05	96.07	0.81 ^c

^a Lyophilized Product No. 1062, Batch No. 1NI, input 1.65 mg./vial. Based upon 21 determinations. ^b Based upon 23 determinations.

determination per day. The same sample of raw material was analyzed at each laboratory. To test interlaboratory agreement, Laboratories 1 and 2 analyzed the same solution of raw material which was made up daily. Each laboratory was limited to one vial and one raw material assay per day.

Each analyst prepared his own reagents and calibrated his own instrumentation; however, all drew from a common supply of oxidimetric standard, which had been standardized by coulometry.

Because there were no significant differences in the standard deviations obtained by the five analysts, the results were pooled according to the following relationship (6):

$$(\Sigma n - m)s^2 = \Sigma \Sigma (x - \bar{x})^2 \quad (\text{Eq. 1})$$

where *m* equals the number of collaborators and *n* equals the total number of determinations. The other symbols have the usual significance. The resulting standard deviation and the overall mean were used to calculate the percent coefficients of variation (Table II).

The results given in Table I prove that the new procedure is more specific than the USP XVIII method. Careful attention to standardization of reagents is necessary to achieve the precision noted in this work.

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