

A SPECIFIC METHOD OF ASSAY OF STRONG SOLUTIONS OF BENZALKONIUM CHLORIDE AND OTHER QUATERNARY AMMONIUM GERMICIDES

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A method of assay of quaternary ammonium germicides in strong solutions or concentrated solid form is described. It can be used for solutions down to about 1 per cent in content. A known excess of iodide is added to the sample solution and the quaternary ammonium iodide is removed by shaking with chloroform. The excess iodide is titrated by an iodate method. When the chloroform extraction is made from a slightly alkaline solution only quaternary ammonium compounds are measured; if it is made from a slightly acid solution, non-quaternary cationic amine impurities are also included. The difference between assay results obtained from acid and alkaline extractions represents the non-quaternary amine content.

SEVERAL methods of assay exist for benzalkonium chloride and other similar quaternary ammonium germicides. Some of those designed for very dilute solutions are both convenient and selective, for example, the colorimetric method of Auerbach (1943), and adaptations of the titrimetric method of Barr, Oliver and Stubbings (1948), where solvent extraction is from an alkaline solution.

However, few satisfactory methods have been described for strong solutions such as the 50 per cent Benzalkonium Chloride Solution of the B.P.C. 1959, or the solid form of the U.S.P. XVI.

The available methods involve precipitation of an insoluble salt or complex, followed by filtration and determination of the excess precipitant, or gravimetric estimation of the precipitate. The precipitants include ferrocyanide (Lottermoser and Steudel, 1938), dichromate (Flotow, 1942), ferricyanide (Wilson, 1946), reineckate (Wilson, 1952, 1954), phosphotungstate (Yoshimura and Morita, 1955; Lincoln and Chinnick, 1956), phosphomolybdate (Yoshimura and Morita, 1955) and tetraphenylboron (Kirsten, Berggren and Nilsson, 1958). The Danish Pharmacopoeia (1954) adopts precipitation as the iodide in acid solution, then extracts the benzalkonium iodide with chloroform and titrates the separated chloroform solution with acetous perchloric acid. None of these methods distinguish the quaternary ammonium halide from tertiary long-chain alkyl amine hydro-halides which are the most likely organic impurities of the quaternary compound.

The ferricyanide method has been the one mostly used, having been the U.S.P. method since 1945. The B.P.C. has adopted a modified version. This method has proved its usefulness, but it is known to suffer from disadvantages. It involves filtration of the gelatinous benzalkonium ferricyanide which needs to stand for 1 hr., and a volume of the filtrate is titrated equivalent to half the volume of the precipitated mixture, thus

requiring a precipitate-volume correction. The B.P.C. version directs the complete washing of the precipitate free from ferricyanide, followed by titration of the whole of the filtrate. In addition, the end-point of the ferricyanide titration is not sharp and final, and the burette readings are small with the U.S.P. method while the B.P.C. employs a 5 g. sample, necessitating a volume of nearly 1 litre to be titrated to obtain a titration in the region of 20 ml.

The method to be described has been found satisfactory and convenient in routine use. It depends upon the ready extractability of benzalkonium iodide into chloroform in the presence of a small quantity of alkali. A known excess of potassium iodide solution is added to a dilution of the sample in a separator and the quaternary ammonium iodide removed by shaking with chloroform. In the alkaline conditions, any tertiary long-chain alkyldimethylamine hydrochlorides are converted to the amine bases having no effect upon the residual iodide which after acidifying with hydrochloric acid is then titrated in the separator with 0.05M potassium iodate solution by the method of Andrews (1903), with its sharp end-point. On a 1 g. sample of the 50 per cent solution, the final titration is about 15 ml., with a blank titration of approximately 30 ml.

In the presence of a small quantity of acid, tertiary cationic amine hydrochlorides are extracted as their hydriodides into the chloroform, giving high results. The difference between the titration readings of assays on the same quantity after both alkali and acid extractions quantitatively measures these non-quaternary amines (Table I).

TABLE I

COMPARISON OF TITRATION VOLUMES OBTAINED (BLANK MINUS TITRE) ON THE SAME APPROX. 2 PER CENT BENZALKONIUM CHLORIDE SOLUTION U.S.P. BY KIO₃ AND U.S.P. XVI METHODS, BEFORE AND AFTER ADDITION OF TERTIARY ALKYLDIMETHYLAMINE HYDROCHLORIDES. AVERAGE MOL. WT. 264, TO ASSAY MIXTURE

Method	KIO ₃	KIO ₃	U.S.P. XVI
Conditions	Alkaline (0.5 ml. N NaOH)	Acid (0.5 ml. N HCl)	—
Amount of sample soln. taken . .	25 ml.	25 ml.	50 ml.
Titrant	0.05 M KIO ₃	0.05 M KIO ₃	0.1 N Na ₂ S ₂ O ₈
1. Sample soln. without addition	12.89 ml.	13.34 ml. (i)	4.38 ml.
2. As 1. with 48.5 mg. tertiary amine HCl's added	12.88 ml.	15.18 ml. (ii)	4.70 ml.
3. As 2. with 97.0 mg. tertiary amine HCl's added	12.89 ml.	17.04 ml. (iii)	5.04 ml. (iv)

- (i) Non-quaternary amine HCl originally present calculated = 11.9 mg. (in 25 ml. of 2 per cent sample solution)
(ii) Added tertiary amine HCl recovered = 48.6 mg.
(iii) Added tertiary amine HCl recovered = 97.6 mg.
(iv) Added tertiary amine HCl recovered = 105 mg.

By applying the technique to pharmaceutical benzalkonium chloride solutions it should, therefore, be possible to detect the presence of and determine any such non-quaternary cationic amines. Examination of samples of varying origins showed these amines to form from almost nil

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to about 2.5 per cent of the total weight as anhydrous benzalkonium chloride (Table II).

TABLE II

NON-QUATERNARY AMINE HYDROCHLORIDE CONTENTS FOUND IN PHARMACEUTICAL QUALITY BENZALKONIUM CHLORIDE SAMPLES OF VARIOUS ORIGINS, CALCULATED ON THE ANHYDROUS BENZALKONIUM CHLORIDE CONTENT

Sample	Acid and alkaline extraction titration difference, (A-B) ml. of 0.05 M KIO ₃ on 25 ml. of a 2 per cent sample solution (ml.)	Non-quaternary amine HCl content (calc. on anhydrous) (per cent)
1	0.40	2.12
2	0.37	1.96
3	0.25	1.32
4	0.05	0.26
5	0.10	0.53
6	0.45	2.32

The method has been found to be accurate for crystalline, pure, benzyl-dimethylmyristylammonium chloride dihydrate, recrystallised from water until shown to be free from other long-chain alkyl homologues by paper chromatography, the moisture content being determined by drying *in vacuo* over phosphorous pentoxide giving an anhydrous mol. wt. of 368.0. Using the proposed method the result (mean of 14 determinations) was 91.03 ± 0.088 per cent while the U.S.P. method gave 90.87 ± 0.30 per cent. The assay figures obtained from both alkaline and acid iodide extractions were identical showing freedom from non-quaternary amines. The moisture content found was 8.92 per cent.

Samples of pure, tertiary long-chain alkyldimethylamines and their salts could be assayed by the method incorporating chloroform extraction of the hydriodides. From sufficiently alkaline extraction conditions, there was no iodide uptake. From slightly acid conditions, full recovery of the amines was obtained; more acid conditions gave slightly lower results. Table III gives results of a tertiary alkyldimethylamine hydrochloride analysis. A sample of pure primary dodecylamine hydrochloride

TABLE III

ANALYSIS OF AN APPROXIMATELY 96 PER CENT PURE, MIXED N-LONG CHAIN ALKYLDI-METHYLAMINE HYDROCHLORIDES, AVERAGE MOL. WT. 264, BY KIO₃ METHOD UNDER VARIOUS ALKALINE AND ACID EXTRACTION CONDITIONS

Wt. of amine HCl (100 per cent) taken for assay (mg.)	Acid or Alkali amount added (N)	Amine HCl found (mg.)
1. 100	0.5 ml. NaOH	Nil
2. 200	0.5 ml. NaOH	10.6
3. 200	1.0 ml. NaOH	Nil
4. 400	2.0 ml. NaOH	Nil
5. 400	0.5 ml. HCl	396

was also found to respond similarly to the two assay processes, except that only about 80-90 per cent recovery was obtained in the acid extraction of the hydriodide. This was also found to be influenced by the amount of excess acid and best recovery occurred near neutrality.

METHOD

Reagents

Potassium iodate 0.05M. Prepare a 0.05M solution by dissolving exactly 10.702 g. of potassium iodate (AR) previously dried at 105° for 1 hr. in water to 1000 ml.

Potassium iodide 5 per cent. Prepare w/v. Use only if colourless.

Method for Solutions and Solids Containing 30 to 100 per cent Benzalkonium Chloride

Accurately weigh a sample containing about 0.5 g. of anhydrous benzalkonium chloride, and transfer with the aid of water (35 ml.) to a 500 ml. glass-stoppered, conical separator containing chloroform (25 ml.). Add N sodium hydroxide solution (0.5 ml.) followed by the 5 per cent potassium iodide solution (exactly 10 ml.). Stopper the separator and shake well. Allow the two layers to separate and run off the lower chloroform layer through a loosely packed plug of about 0.5 g. of absorbent cotton wool,¹ placed in a small glass funnel, to absorb any traces of entrained aqueous liquid. (The chloroform filtrate, which can be discarded, should be clear and dry). Repeat the extraction with chloroform (3 × 10 ml.) running the chloroform layers each time through the same cotton wool filter. Finally, wash the filter with a further 5 ml. of chloroform, and allow the filter to drain.

Add to the separator, concentrated hydrochloric acid (40 ml.), preferably previously chilled,² and titrate the mixture in the separator with 0.05M potassium iodate until the solution becomes only light brown in colour; add chloroform (5 ml.), stopper, and shake. Continue the titration, with shaking, until the chloroform layer becomes colourless and the supernatant liquid is clear yellow. Remove the cotton wool filter from the glass funnel and add the cotton wool directly into the contents of the separator. Wash the glass funnel with water (2 or 3 ml.), receiving the washings in the separator. Stopper, shake, and complete the titration if necessary.

Perform a blank by titrating a mixture of water (20 ml.), 5 per cent potassium iodide solution (exactly 10 ml.), concentrated hydrochloric acid (40 ml.), and adding chloroform (5 ml.) when the mixture becomes light brown in colour.

The difference between the two titrations represents the 0.05M potassium iodate equivalent to the anhydrous benzalkonium chloride contained in the amount of solution taken for the assay.

Each ml. of 0.05M KIO₃ is equivalent to 0.0354 g. of anhydrous benzalkonium chloride, average mol. wt. 354³.

¹ The use of the cotton wool filter to retain entrained aqueous liquid does not normally increase the burette reading by more than about 0.05 ml. No benzalkonium iodide remains adsorbed on to the cotton wool under the conditions described.

² The use of hydrochloric acid, chilled by storage of the stock bottle in a refrigerator reduces the temperature rise due to dilution of the acid. Otherwise, cool the mixture sufficiently, if necessary, before titrating.

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Method for Solutions Containing 1 to 30 per cent Benzalkonium Chloride

Into a 500 ml. glass stoppered, conical separator containing chloroform (25 ml.), transfer an accurately measured volume, or weight, of the solution not exceeding 35 ml., containing as near as possible 0.5 g. of anhydrous benzalkonium chloride. If the volume of solution taken is less than 35 ml., add water to make the total aqueous liquid 35 ml. Proceed as above method commencing at the words: "Add N sodium hydroxide solution (0.5 ml.)."

Combined Assay Method and Determination of Non-quaternary Long-chain Alkylamine Hydrochlorides

Sample dilution: Transfer an accurately weighed amount, or accurately measured volume, of the sample, containing about 2 g. of anhydrous benzalkonium chloride, to a 100 ml. volumetric flask and dilute with water to 100 ml. Mix well.

Place in each of two glass-stoppered, conical separators labelled A and B respectively, 25 ml. of chloroform (25 ml.) and water (10 ml.). Transfer exactly 25 ml. of the sample dilution into each separator. To separator A add N sodium hydroxide solution (0.5 ml.); to separator B add N hydrochloric acid (0.5 ml.). To each separator add 5 per cent potassium iodide solution (exactly 10 ml.); stopper, and shake well.

Complete the assay method on each separator by the above method, commencing with the words: "Allow the two layers to separate . . ."

Let the burette reading (ml. 0.05M) of separator A be: A.

Let the burette reading (ml. 0.05M) of separator B be: B.

Let the burette reading (ml. 0.05M) of blank titration be: Blank.

Calculate the benzalkonium chloride content from $4(\text{Blank} - \text{A})$ ml. which represents the anhydrous benzalkonium chloride contained in the original weight or volume taken to make the sample dilution.

Calculate the non-quaternary long-chain alkylamine hydrochlorides from $4(\text{A} - \text{B})$ ml. which represents the non-quaternary amine hydrochlorides contained in the original weight or volume taken to make the sample dilution. Calculate the percentage found on the anhydrous benzalkonium chloride content.

Each ml. of 0.05M KIO_3 is equivalent to 0.0264 g. of non-quaternary long-chain alkylamine hydrochlorides, average mol. wt. 264.³

DISCUSSION

In the alkaline assay extraction it is necessary to ensure that there is at least sufficient alkali present to ensure complete liberation of the amine bases from the hydrochlorides, otherwise some amine hydriodide will be extracted into the chloroform layer, as illustrated by example 2 of Table III. Reference to Tables II and III shows that 0.5 ml. N sodium hydroxide is sufficient for the quantity of non-quaternary long-chain alkyl amine hydrochlorides likely to be encountered in samples of pharmaceutical

³ If the actual average molecular weight of the quaternary ammonium sample is known, then the equivalent weight used should be adjusted accordingly.

quality. Similarly, the amount of acid used must be sufficient to convert any free amine bases to their hydrochlorides; 0.5 ml. N hydrochloric acid is sufficient for about 100 mg. of bases. In general, the alkaline aqueous liquid extracted should be about pH 12. More strongly alkaline conditions can be used without affecting the result, but it is advisable to keep the amount of alkali used to a minimum to avoid neutralising some of the hydrochloric acid used in the final titration, as this generates heat. For the acid conditions, it is advisable to keep excess hydrochloric acid to a minimum, and on many samples less than 0.5 ml. of N hydrochloric acid can be used.

It may be noted that the specificity of the assay performed from alkaline extraction conditions parallels the finding of Auerbach (1943). In describing his colorimetric method for dilute quaternary solutions, in which the bromophenol blue dye salt of the quaternary is extracted by solvent from sodium carbonate solution, he pointed out that the basic medium gave the method its selectivity. Of about fifty non-quaternary amines which he tested, including dodecyldimethylamine, all responded negatively to his assay method.

The general iodide-iodate method was found to be applicable to cetrimide and cetylpyridinium halides.

It was also established experimentally that assay methods based on the Danish Pharmacopoeia (1954) method of non-aqueous perchloric acid titration of the chloroform extract, did not distinguish between quaternary and non-quaternary long-chain alkylamines when these are extracted from either acid or alkaline iodide solution. This is because both the non-quaternary amine hydriodides and bases respectively are extracted into the chloroform, and both titrate similarly with perchloric acid in non-aqueous conditions in the presence of mercuric acetate. Results are shown in Table IV.

TABLE IV

ACETOUS-PERCHLORIC ACID 0.1 N TITRATION OF CHLOROFORM EXTRACT FROM ACID AND ALKALINE CONDITIONS OF EQUIVALENT OF 0.675 G. ANHYDROUS BENZALKONIUM CHLORIDE, WITH AND WITHOUT ADDITION OF TERTIARY ALKYL DIMETHYLAMINE HYDROCHLORIDES, MEAN MOL. WT. 264

	MI. 0.1 N Acetous-HClO ₄ required	Added Amine HCl's recovered
Extraction from acid conditions		
Sample	19.10	—
Sample + 27.2 mg. amine HCl	20.20	26.4 mg.
Extraction from alkaline conditions		
Sample	19.10	—
Sample + 27.2 mg. amine HCl	20.21	28.0 mg.

It was found, however, that the method could be made indicative of non-quaternary amine content of quaternaries by titrating the chloroform extract from the alkaline medium with acetous-perchloric acid to the first end-point before addition of mercuric acetate. When this end-point, indicating non-quaternary amine bases, is reached, mercuric acetate

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solution is then added and the titration completed. The final end-point represents total amine and quaternary compounds. The first end-point was considered to be poor using Oracet Blue B or B.Z.L. Blue as indicator, presumably due to the small amount of amine bases involved and the presence of a large excess of quaternary iodide. The accuracy of attempts to determine the true quaternary content by difference by this method was thus affected.

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