THE SPECTROPHOTOMETRIC DETERMINATION OF CERTAIN ALKALOIDS AND APPLICATION TO PHARMACEUTICAL PREPARATIONS

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A method is described for the determination of atropine, hyoscyamine, quinine, quinidine, brucine, strychnine and physostigmine by formation of the picrate of the alkaloid in an aqueous buffer at pH 7 followed by extraction of the picrate into chloroform. The picric acid is re-extracted into an alkaline buffer at pH 11 and the absorption measured at 3550 Å. The method has been applied to injections, tablets and other pharmaceutical preparations. The results are in agreement with those obtained by the official methods. The advantages of the method include its usefulness when dealing with small samples and the shorter assay time compared with classical methods.

Richter[1] devised a method for the estimation of higher amines and alkaloids based on the fact that picric acid gave little colour in 50 per cent chloroform-toluene or chloroform-light petroleum solution while amine picrates were strongly coloured, and used this method[2] to determine amphetamine, ephedrine and methylisomyn in urine. Gad[3] applied the method to the determination of amphetamine in organs, tissue and excreta after separation of the base by steam distillation from an alkaline preparation of the specimen. Page and Hopewell[4] applied the method to long-chain amines but obtained poor recoveries. Trautner, Neufeld and Rodwell[5] titrated atropine, hyoscyamine and hyoscine in chloroform solution with a standard solution of picric acid in chloroform and found that whereas atropine and hyoscyamine picrates were soluble, hyoscine picrate was only slightly soluble in chloroform. Semenicheva[6] determined atropine sulphate in eye drops by treating a neutral solution of the alkaloid with sodium picrate and extracting the alkaloid picrate into chloroform. After separation and removal of the chloroform, the residual picrate was reduced with sodium sulphide solution and the colour produced was compared with standards prepared from picric acid reduced in the same way. Cunningham, Dawson and Spring[7] determined the molecular weight of hydrocarbons and bases by preparation of the corresponding picrates and determination of the absorption in ethanolic solution at 3800 Å.

In the method now reported the picrates of certain alkaloids were formed in an aqueous buffered solution and then extracted into chloroform. After separation of the chloroform solution, the picrate was decomposed and the picric acid extracted into an alkaline aqueous buffer leaving the base in the chloroform. The absorption was measured at 3550 Å.

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METHOD

Reagents

1 *per cent w/v solution of sodium picrate in water.* Sodium picrate is prepared and freed from picric acid. "Analar" picric acid, received as a paste containing about 30 per cent of water for safety, was assayed and the calculated equivalent of sodium hydroxide was added to a hot solution of 50 g. of the paste in 500 ml. of water. The solution was evaporated to about 300 ml. and then allowed to cool. The precipitated sodium picrate was collected on a buchner funnel, was sucked dry and washed several times with chloroform. The solid was dried *in vacuo.*

pH 7 *Buffer solution.* 10 g. potassium dihydrogen phosphate was dissolved in 1 litre of water and adjusted to pH 7·0 $\pm$ 0·05 by the addition of 30 per cent w/v aqueous sodium hydroxide solution.

pH 11 *Buffer.* 1 g. of tribasic sodium phosphate was dissolved in water to 1 litre. The pH should be between 11·2 and 11·5.

To 25 ml. of chloroform (Reagent grade) in a 125 ml. separator was added exactly 20 ml. of a solution of the base in pH 7 buffer followed by 3 ml. of the 1 per cent sodium picrate solution. After vigorous shaking the layers were allowed to separate completely, making sure that no aqueous phase was trapped in the constricted portion of the separator. The chloroform layer was run into a second separator, leaving 0·2 to 0·3 ml. in the first to ensure that no aqueous phase was transferred. The extraction was repeated with two further 25 ml. portions of chloroform.

The bulked extracts were shaken with 40 ml. of pH 11 buffer solution and after complete separation, the chloroform layer was run off. The aqueous phase was transferred to a 100 ml. graduated flask, the separator rinsed with more buffer and the bulked extract and washings diluted to 100 ml. with buffer.

The absorption of the resulting solution was determined at 3550 Å in a Hilger Uvispek in a 1 cm. silica cell using a slit setting 9 (equivalent to 5 Å bandwidth) and a similar cell containing pH 11 buffer as the blank.

A standard curve relating concentration to absorption at 3550 Å was prepared for each base by carrying known amounts of base dissolved in 20 ml. of pH 7 buffer through the process described. Blank values obtained by using 20 ml. of pH 7 buffer in place of the base solution were very low (0·002 to 0·006 absorption in a 1 cm. cell).

When a greater sensitivity was required, the final extract was diluted to 50 ml. and either 2 or 4 cm. cells were used for the measurement of the absorption.

Various preliminary manipulations were used to enable alkaloids to be determined in pharmaceutical preparations. Simple solutions such as injections or guttae were diluted with pH 7 buffer so that 20 ml. contained an appropriate quantity of alkaloid. With tablets containing alkaloids of adequate solubility at pH 7, a suitable weight was shaken with buffer and diluted to a known volume. After filtration and dilution of the filtrate with buffer, a 20 ml. aliquot was used in the assay.

Tablets of quinine or quinidine salts were powdered and shaken with 40 ml. of 0·5 N hydrochloric acid for 20 minutes. After dilution to 500

ml. with water, a 10 or 20 ml. aliquot of the filtrate was diluted with pH 7 buffer, and sufficient N sodium hydroxide solution was added to just neutralise the hydrochloric acid present before making up to 100 ml. with more buffer. 20 ml. of the final solution was used for the assay.

TABLE I

APPLICATION OF THE METHOD TO ALKALOID BASES OR SALTS

Base	mg. used (range)	Number of results	Mean O.D.* per mg.	Standard deviation
Atropine (sulphate)	0·412–1·648	4	0·244	0·0014
Hyoscyamine (sulphate)	0·441–1·765	4	0·239	0·0020
Quinine (base) hydrochloride and sulphate	0·842–2·148	8	0·422	0·0056
Quinidine (sulphate)	0·823–3·291	5	0·421	0·0028
Brucine (base)	0·669–2·674	4	0·341	0·0057
Strychnine (base)	0·303–1·211	4	0·365	0·0030
Physostigmine (salicylate)	0·404–1·616	4	0·401	0·0022

* Optical density.

TABLE II

RESULTS OBTAINED WITH PHARMACEUTICAL PREPARATIONS USING THE METHOD

Preparation	Claim	Assay†	Picrate method	Samples examined
Tab. Atrop. Sulph. B.P.	0·01 gr.	0·009 gr.	0·0097–0·0099	3
Inject. Atropine Sulph. B.P.	0·6 mg./ml.	—	0·59 mg./ml.	3
Atropine Eye Ointment B.P.	1*	0·95*	1·00–1·04*	4
Gutta Atropine Sulph. B.P.C.	1*	1·00*	0·97–0·98*	4
Guttae Physostigmine B.P.C.	0·5* Physos. Salicyl.	—	0·44–0·45*	4
Liq. Strych. Hyd. B.P.	1·0*	1·01*	0·99–1·00*	4
Ammon. Tinct. Quin. B.P.C.	1·56–1·76* quinine	—	1·62*	1
Tab. Quin. Sulph. B.P.	5 gr.	4·63 gr.	4·66 gr.	1
Tab. Quin. Hydrochlor. B.P.	5 gr.	4·86 gr.	4·75–4·81 gr.	4
Tab. Quin. Bisulph. B.P.	(*a*) 5 gr. (*b*) 5 gr.	— 4·75 gr.	4·54–4·66 gr. 4·90–4·91 gr.	3 2
Tab. Quin. Bisulph. B.P.	(*a*) 2 gr. (*b*) 2 gr.	— 1·98 gr.	1·91 gr. 1·98 gr.	1 1
Tab. Quinidine Sulph. B.P.	5 gr.	5·09 gr.	4·93–5·07 gr.	4
Proprietary tonic Ref—2284	10·9 mg. strychnine per 100 ml.	—	10·9–11·2 mg.	3
Ref—2285	,,	—	10·6–10·8 mg.	3
Ref—2319	,,	10·5 mg.	11·2–11·3 mg.	2
Ref—2329	,,	10·4 mg.	11·1–11·3 mg.	2

* All per cent.
† The assay method used was either the official one or extraction of the alkaloid was by a "classical" technique.

Sufficient 0·5 N hydrochloric acid to neutralise the ammonia was added to 5 ml. of the ammoniated tincture of quinine before dilution to 100 ml. with water. 10 ml. of the dilution was made up to 100 ml. with pH 7 buffer and 20 ml. of this solution was used for the determination.

Atropine eye ointment (0·5 g.) was dissolved in chloroform to 100 ml. and a 25 ml. aliquot was transferred to a separating funnel. 20 ml. of pH 7 buffer and 3 ml. of 1 per cent sodium picrate solution were added. After shaking and separation of the chloroform layer, the extraction of the alkaloid picrate was continued with a further 2 × 25 ml. of chloroform. The assay was completed by the general method.

A similar modification was applied to 100 ml. of a proprietary strychnine tonic which also contained sodium and calcium glycerophosphates and phosphates and colouring agents. Sufficient sodium hydroxide solution was added to make the mixture alkaline, and the alkaloid was extracted with 3 × 25 ml. of chloroform. 20 ml. of pH 7 buffer and 3 ml. of 1 per cent sodium picrate solution were added to the bulked chloroform extracts and after vigorous shaking, the separated chloroform layer was shaken with 40 ml. of pH 11 buffer. The assay was completed as before.

RESULTS

The method was applied to several alkaloids using either the bases or salts as set out in Table I.

Table II lists the results obtained with some pharmaceutical preparations. Most preparations were also assayed by the official method or a "classical" extraction method ending with either the weighing or titration of the extracted alkaloid was used.

DISCUSSION

Any base whose picrate is largely undissociated at pH 7 and for which the partition coefficient between the aqueous phase and chloroform favours extraction into chloroform should be amenable to determination by the technique described. It has proved especially useful for the determination of small quantities of atropine, hyoscyamine and strychnine in a variety of pharmaceutical forms, and has given more reproducible

TABLE III

APPARENT MOLECULAR EXTINCTION COEFFICIENTS FOR ALKALOIDAL PICRATES

Alkaloids	Apparent ϵ of picrate
Atropine	14,080
Hyoscyamine	13,800
Quinine	13,700
Quinidine	13,660
Brucine	13,410
Strychnine	12,200
Physostigmine	11,050

results than similar techniques based on the sulphonphthalein dye complex method of Brodie[8] or the many published modifications thereof. Hyoscine did not give satisfactory recoveries, probably because of the very low solubility of its picrate in chloroform reported by Trautner and others[5]. The method was also applied to nicotine, emetine and ephedrine but the results did not warrant its use for the determination of these alkaloids. For the alkaloids reported, the extraction of the base-picrates into chloroform was checked by assaying separately each of the three

25 ml. extracts and in every case more than 95 per cent of the extractable picrate was removed in the first 25 ml. The picric acid was completely extracted from the chloroform solution by one extraction with 40 ml. of the alkaline buffer leaving the base in the chloroform layer.

The apparent molecular extinction coefficients for the alkaloid picrates listed in Table III may be calculated. Picric acid has a molecular extinction coefficient of 14,140 in pH 11 buffer. The dissociation of the alkaloid picrates of atropine, hyoscyamine, quinine, quinidine and brucine, was largely depressed in the presence of the large excess of picrate ion. For strychnine, only about 86 per cent and for physostigmine only 78 per cent of the alkaloid was present as the picrates.

Provided the stated conditions for the assay were adhered to this did not represent a drawback since the degree of dissociation was constant. By using a higher concentration of sodium picrate in the method the dissociation of the picrates could be further suppressed, but the blank values obtained were too large.

References

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After Mr. Cross presented the paper there was a DISCUSSION. The following points were made.

A mixture of alkaloids which formed picrates would give a total assay figure, but the number of alkaloids forming picrates in chloroform is limited. In general, phenolic preservatives would not interfere with the method, as their picrates would favour the aqueous phase. The method was satisfactory for the determination of small quantities of the atropine alkaloids, but was especially designed for forensic analysis.