A DIRECT TITRIMETRIC METHOD FOR THE DETERMINATION OF SOME ORGANIC BASES IN PHARMACEUTICAL PREPARATIONS

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A rapid titrimetric method using an extractive end-point has been developed for the determination of atropine, codeine, methadone and pethidine in pharmaceutical preparations. Chloroform is added to the organic base dissolved in pH 2·8 buffer solution so that the ratio of chloroform to aqueous phase lies between 2 to 1 and 4 to 1. Titration is carried out with sodium dioctylsulphosuccinate using Dimethyl Yellow screened with Oracet Blue B as indicator; at the end-point the chloroform phase changes colour from green to pink. The method is accurate to ±1 per cent and has been applied to a number of injection, eye-drop, syrup and tablet preparations. Since little sample-preparation is required, most preparations may be assayed within 10 min., whilst the codeine content of complex compound tablets can be determined in 20 min.

Volumetric methods using extractive end-points with anionic surface-active agents as titrants have been described for the determination of certain tertiary bases (usually containing a terminal *N*-substituted side chain) in pharmaceutical preparations (Carkhuff and Boyd, 1954; Pellerin, Gautier and Demay, 1962). The extension of this type of titration to certain cyclic amines, notably codeine, has been investigated.

Experimental

Many cyclic amines form insoluble complexes with sodium lauryl sulphate or sodium dioctylsulphosuccinate but when Carkhuff and Boyd's method was used in an attempt to determine these compounds poor and premature end-points were obtained. Previous experience had shown us that better end-points could sometimes be obtained by replacing the mineral acid used in Carkhuff and Boyd's method with a weak acid such as acetic acid. This proved to be the case with codeine, the use of acetic acid giving stoichiometric end-points. In practice the use of an acetate buffer, pH 2·8, has been found to be convenient and satisfactory.

Detection of the end-point as recommended by Carkhuff and Boyd, by the colour of the drop of chloroform formed on shaking at the surface of the aqueous phase, is unsatisfactory in the titration of cyclic amines. As the ratio of chloroform to aqueous phase is progressively decreased below about 1·5 to 1, the volume of standard titrant required to titrate a given quantity of amine decreases. No such variation in the volume of titrant occurs if the ratio of chloroform to aqueous phase is maintained between 2 to 1 and 4 to 1; there is no drop formation and the end-point is

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judged by a change in colour of Dimethyl Yellow (the indicator used by Carkhuff and Boyd) from yellow to pink in the bulk of the chloroform. This colour change is greatly improved if the indicator is screened with Oracet Blue B. Under these conditions the titration may be conveniently carried out in a 500 ml. flask, provided that vigorous swirling is employed throughout.

Factors that might influence the results obtained have been examined. The volume of buffer solution used can be varied between 1 and 10 ml. without effect; above 10 ml., end-points are less sharp and titration values are slightly high. The ratio of chloroform to aqueous phase may be varied from 4 to 1 down to 1·5 to 1, whilst the volumes of chloroform and aqueous phases may lie between 20 to 150 ml. and 10 to 100 ml. respectively, provided that the correct ratio is maintained. A small titration blank is obtained and should always be allowed for; many batches of chloroform B.P. have been examined for use in this titration and all have given a blank that varies from about 0·13 ml. of titrant when 60 ml. of chloroform is used to 0·22 ml. when 150 ml. is used. The titration can be carried out equally well in daylight or under various forms of artificial lighting, and laboratory temperature variations between 15 and 26° have been shown to be without effect. The titrant appears to be very stable; no significant change in concentration was detectable over a period of 7 weeks.

Method

Reagents. Buffer solution pH 2·8: Dissolve anhydrous sodium acetate (4 g.) in water (approximately 830 ml.), add glacial acetic acid (approximately 155 ml.) until the pH reaches 2·8 and then dilute to 1 litre with water.

Sodium dioctylsulphosuccinate: Add "Manoxol O.T. 60 per cent solution" (15 ml.) to water (300 ml.) and warm to dissolve; cool to room temperature, then dilute to 2 litres with water. Store in an amber-glass bottle. Standardise by titration of codeine phosphate, atropine sulphate, pethidine hydrochloride or methadone hydrochloride, the purity of which has been determined by the official method of the British Pharmacopoeia. Then $E = \frac{A \times B}{C}$ where E = "mg./ml. equivalent"—the weight (mg.) of organic base equivalent to 1 ml. of sodium dioctylsulphosuccinate solution. A = weight (mg.) of organic base titrated. B = per cent purity of the organic base as determined by the official method of the British Pharmacopoeia. C = titre (ml.).

Screened Dimethyl Yellow indicator: Dissolve Dimethyl Yellow (15·0 mg.) and Oracet Blue B (15·0 mg.) in chloroform, B.P. (500 ml.).

Sample preparation. Syrup, linctus and simple aqueous preparations. Titrate directly the quantity of preparation specified in Tables III and V, diluted to 25 ml. with water.

Tablets (containing lactose basis). Dissolve the number of powdered tablets specified in Tables III and V in water (25 ml.), warming gently if

necessary. Add pH 2·8 buffer solution (5 ml.) and continue by the general procedure below, beginning with the words "a known volume of chloroform . . .".

Compound Codeine Tablets. Add N sodium hydroxide (20 ml.) to the powdered tablets (2·5 g.) contained in a 150 ml. separator; shake for 2 min. then extract by shaking with one 50 ml., two 20 ml. and one 10 ml. portions of chloroform, washing each extract in turn with the same 10 ml. of water. To the combined chloroform extracts add water (25 ml.), pH 2·8 buffer solution (5 ml.) and continue by the general procedure below, beginning with the words "and screened Dimethyl Yellow indicator (5 ml.)".

General procedure. To a solution of the active material in a total volume of 25 ml. contained in a 500 ml. conical flask, add pH 2·8 buffer solution (5 ml.), a known volume of chloroform (between 60 and 120 ml.) and screened Dimethyl Yellow indicator (5 ml.). Titrate with sodium dioctylsulphosuccinate solution, adding the titrant fairly rapidly until nearing the end-point and swirling vigorously throughout. Then add the titrant dropwise, again swirling vigorously; after each addition allow the two phases to separate and then gently swirl for about 5 sec. The end-point is detected by a colour change from green to pinkish-grey in the bulk of the chloroform. Carry out a blank determination under the same conditions; the difference between the two titration values is equivalent to the amount of base present.

Results and Discussion

Of the substances examined, codeine phosphate (3·5 to 38 mg.), atropine sulphate (6 to 30 mg.), pethidine hydrochloride (7 to 24 mg.) and methadone hydrochloride (7 to 31 mg.) are all satisfactorily titrated. Hyoscine, aneurine, morphine, procaine, pilocarpine, lignocaine and dexamphetamine also titrate, but the end-points are very sluggish. The pink dimethyl yellow-sodium dioctylsulphosuccinate complex formed at the end-point tends to concentrate at the interface between the chloroform and aqueous phases and is best extracted into the bulk of the chloroform by very gentle swirling. It is for this reason that the titration technique described under "General Procedure" has been adopted. It has proved satisfactory even to operators new to the method. The precision of the titration, as applied to codeine phosphate (between 3·5 and 38 mg.) by two operators on different days, is shown in Table I. Possible interference from commonly occurring tablet excipients, bacteriostats, etc., was examined by titrating codeine phosphate in their presence (Table II).

TABLE I

Precision of the titration when applied to codeine phosphate, B.P.

	No. of determinations	Mean	Standard deviation
Analyst A*	9	3·95	0·026
Analyst B	26	3·96	0·015

* Analyst A had no previous experience of the method.

TABLE II

INTERFERENCE FROM OTHER MATERIALS IN THE TITRATION OF 36·3 MG. OF CODEINE PHOSPHATE, B.P.

Material	Quantity	Codeine phosphate found (mg.)	Recovery per cent
Syrup, B.P.	10 g.	36·2; 36·2	99·7; 99·7
Lactose, B.P.	1 g.	36·4; 36·1	100·3; 99·4
Stearic Acid, B.P.C.	10 mg.	36·5; 36·2	100·6; 99·7
Solution for Eye-drops, B.P.C.	10 ml.	36·3; 36·5	100·0; 100·6
Starch, B.P.	20 mg.	36·3; 36·5	100·0; 100·6
*Sodium Chloride, B.P.	0·1 g.	37·0; 36·7	101·9; 101·2
0·5 per cent w/v Phenol, B.P.	20 ml.	36·3; 36·3	100·0; 100·0
0·5 per cent w/v Cresol, B.P.	20 ml.	36·4; 36·6	100·4; 100·9
Salicylamide	1 g.	36·7; 36·6	101·2; 100·9
Caffeine, B.P.	0·75 g.	36·4	100·3
†Soluble Compound Codeine Tablets B.P. Add. 1960, but containing no codeine	2·5 g.	36·3; 36·3	100·0; 100·0
†Polyvinylpyrrolidone	10 mg.	36·5; 37·1	100·6; 102·3
Gelatin, B.P.	10 mg.	Titrates with emulsification of the two phases	

*End-point sluggish; this quantity should not be exceeded.
† Chloroform extract made from 20 ml N sodium hydroxide.

TABLE III

APPLICATION TO SIMPLE CODEINE PREPARATIONS

Preparation	Quantity taken	Codeine phosphate content found by: Proposed method	Codeine phosphate content found by: Official method	Results expressed as
Syrup of Codeine Phosphate B.P.C. 0·5 per cent w/v	6 g.–9·5 g.	0·495; 0·500; 0·495; 0·501; 0·495 (Mean = 0·497)	0·49	Per cent w/v of monohydrate
Linctus of Codeine B.P.C. 0·375 per cent w/v	9 g.–11 g.	0·372; 0·372 (Mean = 0·372)	0·369	Per cent w/v of *monohydrate*
Codeine Phosphate Tablets B.P. 16·2 mg./tab.	2 tablets	16·7; 17·0; 16·7; 16·8 (Mean = 16·8)	16·9	mg./tab. of $1\frac{1}{2}$ H_2O
Codeine Phosphate Tablets B.P. 32·4 mg./tab.	1 tablet	33·2; 32·6; 32·7 (Mean = 32·8)	32·8	*mg./tab.* of $1\frac{1}{2}$ H_2O

TABLE IV

APPLICATION TO COMPOUND CODEINE TABLETS

Preparation	Declared mg./tab.	mg./tab. Codeine Phosphate B.P. found by: Proposed method	mg./tab. Codeine Phosphate B.P. found by: Official method
Soluble Compound Codeine Tablets B.P. Add. 1960	8·1	1. 7·6; 7·6 2. 7·7; 7·7; 7·7 3. 7·1; 7·3 4. 7·8; 7·9 5. 9·3; 9·3†	8·0 7·6 7·3 7·7 9·3; 9·0
Compound codeine tablets (modified formula)	8·1	1. 8·1; 8·1	8·0
Compound Codeine Tablets B.P.	8·1	1. 8·2; 8·2 2. 8·1; 8·4	— —
Soluble compound codeine tablets (modified formula)	8·1	1. 8·1; 8·0	—
*Codeine, caffeine and aspirin tablets	16·2	1. 16·1; 16·1	—

* Weight of powdered tablets equivalent to two tablets used in the determination.
† Rejected batch.

TABLE V

APPLICATION TO THE DETERMINATION OF OTHER ORGANIC BASES IN MISCELLANEOUS PHARMACEUTICAL PREPARATIONS

Preparation	Quantity taken	Declared	Compound found by: Proposed method	Compound found by: Alternative method	Results expressed as
Injection of Atropine Sulphate B.P.	25 ml.	0·65	0·67; 0·68	0·67*	mg./ml. Atropine Sulphate B.P.
Hypodermic tablets of atropine sulphate ..	30 tablets	0·65	0·61	0·62†	mg./tab. Atropine Sulphate B.P.
Eye-drops of atropine sulphate	2 or 3 ml.	1·00	1·00; 0·99; 0·99	0·99§	per cent w/v Atropine Sulphate B.P.
Atropine Sulphate Tablets B.P.	40 tablets	0·65	(*a*) 0·67; 0·68 (*b*) 0·68; 0·67 (*c*) 0·69	0·66* 0·62* 0·69†	mg./tab. Atropine Sulphate B.P.
Methadone Tablets B.P...	5 tablets	5·0	4·89; 4·89	4·87‡; 4·93‡	mg./tab. Methadone Hydrochloride B.P.
Methadone Tablets—rejected batch	5 tablets		4·43; 4·56; 4·41	4·45†	mg./tab. Methadone Hydrochloride B.P.
Methadone Injection B.P.	3 ml.	1·00	1·03; 1·02; 1·04; 1·03	1·03*	per cent w/v Methadone Hydrochloride B.P.
Pethidine Tablets B.P. ..	1 tablet	25·0	23·9; 23·8; 23·9	23·9* 23·9†	mg./tab. Pethidine Hydrochloride B.P.
Pethidine Injection B.P...	Dilute 2 ml. to 50 ml. with water; take 10 ml.	50·0	50·7; 50·5; 50·9; 51·1; 51·1	50·8*	mg./ml. Pethidine Hydrochloride B.P.

* Official method of the British Pharmacopoeia.
‡ Ultra-violet spectrophotometric method.
† Tetraphenylboron method (Johnson and King, 1962).
§ Laboratory-prepared sample.

Table III lists the results obtained by the proposed method and other methods on simple codeine preparations, and Table IV gives results for a number of compound tablets. Table V shows the results obtained for the determination of other organic bases, by the proposed and alternative methods, in various pharmaceutical preparations. Linctus, syrup, simple tablet, injection solution and aqueous eye-drop preparations may be assayed within 10 min., and the codeine content of compound tablets can be determined in 20 min.

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