RESEARCH PAPERS

A TITRATION METHOD FOR THE DETERMINATION OF PROCAINE IN PROCAINE PENICILLIN AND ITS OILY SUSPENSIONS

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The essential characteristics of procaine benzyl penicillin ("procaine penicillin") have been described by Salivar *et al*[1]. It is a compound of one molecule each of procaine base and of benzyl penicillin (Penicillin G) with one molecule of water of crystallisation. Thus the theoretical composition is procaine 40·12 per cent., water 3·06 per cent. and penicillin potency 1008 I.U./mg., calculated on the basis of sodium benzyl penicillin as 1667 I.U./mg.

Procaine penicillin has been formulated for injection as a water-dispersible powder and also as a sterile suspension in arachis oil. Of these the latter is at present the more commonly used and normally contains 300,000 units of penicillin combined with 120 mg. of procaine base in each ml. This preparation may also contain 2 per cent. of aluminium monostearate as suspending agent[2]. The high dosage and the toxicity of free procaine required the development of a rapid and accurate method for determining the procaine content of procaine penicillin both in the dry state and also when in oily suspension. Published methods for the determination of procaine include bromination, titration of the base with standard acid following either extraction with chloroform or separation by distillation[3] and a colorimetric method for small amounts[4]. A spectrophotometric method for the determination of procaine in procaine penicillin G has recently been described[5]. It is well known, however, that most primary and secondary aromatic amines can be made to react quantitatively with nitrous acid. This method is used in the Pharmacopœial assay process for sulphanilamide and other sulphonamides but has apparently not been applied to procaine hydrochloride.

The object of the work described here was to investigate whether this reaction could be applied to the determination of procaine, particularly when in the forms already mentioned. The high cost of penicillin required that the amount used for each assay should be as small as possible and for this reason attention was directed to testing on a semi-micro scale.

Experimental

(1) *Titration of procaine hydrochloride.* Procaine hydrochloride B.P. was recrystallised twice from water and dried first at 60°C. and finally *in vacuo* over phosphorus pentoxide. Moisture content (Fischer reagent) 0·04 per cent., m.pt. 154·8° to 155·5°C. Found: C, 57·4; H, 7·72; N, 10·1*; ionisable chlorine, 13·00 per cent.

* I am indebted to Dr. F. R. Cropper for the results of all micro analyses quoted in this paper.

$C_{13}H_{20}N_2O_2$,HCl requires C, 57·23; H, 7·76; N, 10·27; ionisable chlorine, 12·99 per cent.

About 0·8 g. of the purified salt was dissolved in 15 ml. of 3N hydrochloric acid and the solution diluted to 150 ml. with distilled water. The solution was adjusted to 15°C. and titrated slowly (0·1 ml. at a time towards the end of the titration) with N/10 sodium nitrite solution. The end-point was taken when a drop of the titrated liquid gave an immediate blue colour on starch-iodide paper 2 minutes after the last addition of nitrite.

A blank determination omitting the procaine was carried out and the titre deducted. The results of a series of these titrations are given in Table I.

The determination was repeated on a semi-micro scale by dissolving about 0·1 g. of procain hydrochloride in 15 ml. of 3N hydrochloric acid and diluting to 50 ml. with water. After adjustment of temperature the solution was titrated as before with N/10 sodium nitrite added from a 5 ml. microburette in 0·02 ml. quantities towards the end of the titration. These results are also given in Table I.

TABLE I

TITRATION OF PURIFIED PROCAINE HYDROCHLORIDE WITH STANDARD SODIUM NITRITE SOLUTION

Procaine hydrochloride taken	N/10 sodium nitrite f.1·005	Procaine hydrochloride
g.	ml.	per cent.
0·8009	29·22	99·93
0·8547	31·18	99·91
0·7525	27·44	99·88
0·8055	29·40	99·95
0·9033	32·93	99·86
0·1104	4·04	100·2
0·1238	4·51	99·8
0·1168	4·28	100·3
0·1218	4·45	100·0
0·1203	4·385	99·8

(II) *Titration of procaine penicillin.* (*a*) *Extraction of procaine from procaine hydrochloride.* When the above semi-micro procedure was applied to procaine penicillin fictitiously high results were obtained, apparently due to reaction of penicillin with nitrous acid. This was confirmed by titrating a sample of crystalline sodium penicillin in dilute hydrochloric acid solution with N/10 sodium nitrite. Slow absorption of nitrous acid occurred and no definite end-point was obtainable. Separation of procaine from penicillin was, therefore, necessary and an extraction technique was devised for this purpose. The procedure was first applied to procaine hydrochloride itself in order to check the efficiency of the extraction. The procaine hydrochloride used for this assayed 99·5 per cent. by the macro method given under (I).

About 100 mg. of procaine hydrochloride, accurately weighed, was dissolved in 50 ml. of water and the solution transferred to a separating funnel. 5 ml. of M ammonium hydroxide was added and the solution extracted successively with 20, 5, 5, and 5 ml. quantities of chloroform, previously washed by thorough shaking with an equal volume of

water.* Completion of extraction was checked by carrying out a fifth extraction with 5 ml. of chloroform, this being extracted with dilute hydrochloric acid and the acid solution tested for traces of procaine as described later. Each chloroform extract was run in turn into a 4 oz. wide-mouth glass stoppered bottle containing 35 ml. of water and 15 ml. of 3N hydrochloric acid. After adjusting the temperature to 15°C. the chloroform and acid layers were titrated with vigorous stirring with N/10 sodium nitrite solution in the manner described above until a reaction on starch-iodide paper was obtained. The stopper of the bottle was then inserted and the contents vigorously shaken for 30 seconds. After separation had occurred the titration of the aqueous layer was continued with stirring until an end-point reproducible after 2 minutes was obtained.

It was observed that any delay between addition of ammonia and extraction with chloroform led to low results. Decomposition of procaine in alkaline solution with the formation of *p*-aminobenzoic acid is well known[6] and this was thought to be the cause of the low results. To test this and to show that the presence of penicillin did not interfere with the extraction the following three separate procedures were adopted:— (i) Extraction after addition of ammonia was delayed for a series of time intervals. (ii) 50 mg. of *p*-aminobenzoic acid was added before making alkaline. (iii) An approximately equimolecular proportion of crystalline sodium benzyl penicillin was added before making alkaline. The results obtained are given in Table II.

TABLE II

RESULTS OBTAINED USING THE EXTRACTION PROCEDURE ON PROCAINE HYDROCHLORIDE

Procaine hydrochloride	Crystalline sodium penicillin G added	Delay in extraction	N/10 sodium nitrite (f. 0·994)	Procaine hydrochloride (Mol. Wt. 272·6)
mg.	mg.		ml.	per cent.
117·4	—	None	4·31	99·5
120·4	—	,,	4·44	99·9
122·5	—	,,	4·43 (f 1·009)	99·4
127·5	151	,,	4·61 (f 1·009)	99·4
123·7	158	,,	4·545	99·6
122·1	150*	,,	4·49	99·6
119·7	—	½ hr.	4·36	98·7
122·9	—	,,	4·37 (f 1·009)	97·8
123·2	—	1 hr.	4·33 (f 1·009)	96·6
116·0	—	,,	4·14	96·7
118·6	—	3 hrs.	3·99	91·1
115·1	—	,,	3·86	90·9

* 50 mg. of *p*-aminobenzoic acid also included in this assay.

(b) *Extraction from procaine penicillin.* Direct application of the method used in (a) was inapplicable to procaine penicillin on account of its low solubility in water. About 280 mg. of procaine penicillin was, therefore, dissolved by warming in 15 ml. of chloroform contained in

* NOTE: In the presence of chloroform B.P. slow absorption of nitrous acid occurred in the blank titration but this could be prevented by prior washing of the chloroform with distilled water.

a beaker and the solution poured into a separating funnel containing 50 ml. of water. A further 5 ml. of chloroform and 5 ml. of M ammonia were used in turn to rinse the beaker, each being added to the separating funnel. Extraction and titration procedure were as described above.

The sample* of procaine penicillin G used for this and replicate determinations assayed 1038 I.U./mg. (*Staphylococcus aureus*), 1016 I.U./mg. (*Bacillus subtilis*) water content (Fischer reagent) 3·4 per cent. Found: C, 59·15; H, 6·75 per cent.; N, 9·8; S, 5·31 per cent. $C_{29}H_{38}N_4O_6S,H_2O$ requires C, 59·17; H. 6·85; N, 9·52; S, 5·45 per cent.

As a check on possible interference by low potency penicillin one assay where this was added is included in Table III.

TABLE III

REPLICATE DETERMINATIONS OF PROCAINE IN PROCAINE PENICILLIN

Weight taken	N/10 sodium nitrite f. 1·006	Procaine
mg.		per cent.
290·0	4·90	40·1
279·5	4·71	40·0
292·1	4·91	39·9
281·5	4·72	39·8
274·6	4·60	39·8
283·1	4·755	39·9
291·6	4·92	40·0
271·0	4·54	39·8
280·2	4·73	40·0
273·1	4·57	39·8
282·2	4·73	39·8
270·1	4·54	39·9
290·1*	4·86	39·8

* Sodium penicillin 0·1 g. (potency 547 I.U./mg.) added.

(III) *Titration of Suspensions in Oil.*

Accurate weights of the procaine penicillin used in (II) (b) were converted into suspensions in oil, using:—(i) 300,000 units in 1 ml. of arachis oil, (ii) as above, but with 2 per cent. of aluminium monostearate.

The procaine in these preparations was determined using the same technique as that previously adopted for procaine penicillin itself. The results are given in Table IV.

TABLE IV

DETERMINATION OF PROCAINE IN PROCAINE PENICILLIN WITH ADDED ARACHIS OIL AND ALUMINIUM MONOSTEARATE

Procaine penicillin taken	N/10 sodium nitrite f. 1·006	Procaine
mg.	ml.	per cent.
With arachis oil ... ... {290·5 · ...	4·89	40·0
{293·8 ...	4·93	39·9
With arachis oil containing 2 per cent. w/w aluminium monostearate ... ... {284·1 ...	4·78	39·9
{281·7 ...	4·71	39·7

The same procedure was then applied to several routine manufactured batches of procaine penicillin suspension in arachis oil (with and without aluminium monostearate).

* I am indebted to Mr. D. H. Geard for this sample and to Mr. C. R. Bond for the results of all penicillin assays quoted.

These results are given in Table V.

TABLE V

REPLICATE DETERMINATIONS ON PROCAINE PENICILLIN OILY SUSPENSION (300,000 I.U./ML.)

Sample	Density at 20° C.	Penicillin potency I.U./ml. (Iodimetric assay)	Replicate procaine determinations (calculated to Mol. Wt. 236·1)
			per cent. w/w
A	0·994	295,000	12·0
			12·0
			12·0
			12·0
			12·0
B	0·996	303,000	12·2
			12·1
			12·2
			12·1
C	1·001	309,100	12·6
			12·6
			12·6
			12·4
D*	1·002	304,600	12·4
			12·3
			12·3
			12·3

* Containing 2 per cent. w/w of aluminium monostearate.

CONCLUSIONS

Results given in Table I show that procaine may be determined satisfactorily under macro and semi-micro conditions by direct titration in acid solution with standard sodium nitrite.

By employing an extraction procedure, interference by penicillin may be eliminated with no loss of accuracy. The results in Table II show that extraction should follow immediately after addition of ammonia, otherwise, low results, due to decomposition of procaine, are obtained. For the same reason the chloroform extracts should be run directly into dilute acid.

The presence of chloroform in the final solution for titration in no way affects the results, but the emulsion formed by stirring or shaking during titration should be allowed to separate before removing a drop of the aqueous layer for spotting on to starch-iodide paper. In the presence of penicillin the first chloroform extract is normally turbid, but this has not been observed to affect the results. Vigorous shaking may produce emulsions that separate slowly and should, therefore, be avoided.

Although with a suitable microburette the volume of titrant used may be read to 0·002 ml., experience has shown that the end-point cannot be estimated to better than 0·02 ml., corresponding to an error of about 0·5 per cent. on a volume of 4 to 5 ml. This is borne out by the results quoted in Table III. Whilst this error would be correspondingly reduced by taking a larger sample for assay, it was considered tolerable for routine application.

It will be noted that starch-iodide papers have been used throughout

in place of the starch iodide paste recommended in the British Pharmacopœia for use in the assay of sulphanilamide. Papers have been found much more convenient, and they are reasonably stable if prepared and stored as described below.

Recommended Method

Special Reagents.

(a) *Chloroform.* Wash chloroform B.P. by shaking thoroughly with an equal volume of distilled water. Allow complete separation to take place and run off the chloroform for use.

(b) *Starch-iodide papers.* (i) Dissolve 4 g. of cadmium iodide in 50 ml. warm water.

(ii) Make 5 g. of soluble starch into a thin smooth paste with a little distilled water. Pour the suspension into 450 ml. of boiling distilled water. Boil for 1 minute, add solution (i) and boil again for 1 minute. Cool the solution to 70° to 80°C. and impregnate strips of suitable filter paper (e.g., embossed filter paper No. 633 made by Evans, Adland and Co., Postlip Mills) by immersion in the solution and then removing as much surplus liquid as possible by means of a glass rod. Dry the impregnated filter paper in a warm atmosphere free from fumes. Cut off the edges of the strips and cut the remainder into strips for use. Store in a well-stoppered amber-coloured bottle.

Procedure.

Weigh accurately 0·27 to 0·29 g. of procaine penicillin (or about 0·9 g. of procaine penicillin suspension in oil, 300,000 I.U./ml.) and dissolve in 15 ml. of chloroform by warming. Pour the solution into a separating funnel containing 50 ml. of water. Rinse the beaker with 5 ml. of warm chloroform, then with 5 ml. of M ammonia, adding each in turn to the separator. Shake the contents of the separator gently for 2 minutes. Allow to separate, ignoring a turbidity of the chloroform layer and run the lower layer into a 4-oz. wide-mouthed bottle (provided with a well-fitting stopper) containing 35 ml. of water and 15 ml. of 3N hydrochloric acid.

Extract with 3 further portions each of 5 ml. of chloroform, running each in turn into the bottle. Adjust the temperature of the contents of the bottle to 15°C. and titrate with N/10 sodium nitrite with vigorous stirring until a drop of the aqueous portion of the titrated solution yields an immediate blue colour when spotted on starch-iodide paper. Insert the stopper of the bottle and shake vigorously for 30 seconds. Allow the layers to separate and complete the titration of the aqueous layer with gentle stirring. The end-point must be reproducible after allowing the titrated liquid to stand for two minutes after the last addition of nitrite, added 0·02 ml. at a time towards the end of the titration. Carry out a blank determination omitting the sample and deduct the titration figure. 1 ml. of N/10 sodium nitrite is equivalent to 0·02361 g. of procaine base.

Note 1: The extraction procedure described normally extracts the procaine quantitatively, the following test for traces of procaine may, however, be applied for confirmation.

Extract 5 ml. of chloroform solution with 4 ml. of water containing 0·2 ml. of N/10 hydrochloric acid. Allow to separate, discard the chloroform layer, warm the aqueous layer gently to expel chloroform, cool and add 1 ml. of N/10 iodine. No turbidity should be produced.

NOTE 2: Unless preparations of approximately known strength are being tested it is advisable to carry out a preliminary titration in order to establish the approximate end-point.

SUMMARY

1. A semi-micro method for the determination of procaine in procaine-penicillin and in its suspensions in arachis oil is given.

2. The method is based upon extraction of procaine base with chloroform followed by acidification of the extract and titration with sodium nitrite solution.

3. No interference has been encountered in the presence of low potency penicillin or aluminium monostearate.

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