THE CHEMICAL ASSAY OF BENZATHINE PENICILLIN

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Introduction

The widespread clinical use of procaine penicillin for producing lasting concentrations of penicillin in the blood has led to the search for other sparingly soluble penicillin salts. One such compound, benzathine penicillin, *NN'*-dibenzylethylenediamine dibenzyl penicillin, has recently been described[1]. This salt is characterised by an extremely low solubility in water (0·015 per cent.), which makes it impossible to obtain aqueous solutions sufficiently concentrated to assay by the usual iodimetric procedure of Alicino.[2] As it recently became necessary to have a rapid chemical procedure for determining the purity of preparations of benzathine penicillin, the present paper describes a modification of the procedure of Alicino developed for this purpose. The results obtained by this method are compared with those obtained by estimation of the benzethamine content, and with those given by the normal biological assay procedure.

Iodimetric Assay

Initially, attempts were made to find a solvent in which benzathine penicillin is soluble to an extent sufficient to give solutions of concentrations high enough for iodimetric assay (i.e. about 1 mg./ml.). Formamide, the best solvent for this substance, cannot be used as it reacts with iodine solutions. The same objection applies to acetone and ethanol. The solubility in butanol is insufficient to allow of accurate assay. Aqueous methanol solutions of the required concentration can be prepared, but it is known that, by causing gradual penicillin breakdown,[3] the presence of methanol will affect the blank titration of an iodimetric assay. This was, in fact, confirmed for benzathine penicillin: the potency of a sample, assaying biologically at 1207 I.U./mg., was found by iodimetric assay to be only 1039 I.U./mg., dropping to 883 I.U./mg., after the methanol solutions had stood for 2 hours at room temperature.

Attempts were then made to decompose aqueous suspensions with phosphoric acid, and to extract the free penicillin into butyl acetate solution and then back into phosphate buffer. Iodimetric assays of such extracts in trial experiments with sodium penicillin showed practically quantitative recoveries of the penicillin, but when applied to benzathine penicillin the method showed recoveries of approximately 80 per cent. only.

It was eventually found that satisfactory assays were obtained by dissolving the solid directly in alkali, making up to a standard volume and, after standing for 15 minutes, iodinating a suitable aliquot. As the rate of degradation of penicillin by alkali is exceedingly rapid, it is not possible to run a blank by neutralising a portion of the alkaline solution

immediately after preparation. It was, however, found that a correct blank titration was obtained by treating an aqueous suspension with iodine solution in the normal manner, the total reaction volume then being sufficient to dissolve completely the benzathine penicillin.

Method

Approximately 500 mg. is accurately weighed, treated with 1 to 2 ml. of approximately N sodium hydroxide and washed into a 500-ml. flask with water. The flask is shaken till solution is complete (2 to 3 minutes) and then diluted to 500 ml. with water. 3 aliquots, each of 5 ml., are pipetted into 200-ml. bottles carrying ground-glass stoppers and treated with 5 ml. of N sodium hydroxide. After standing for at least 10 minutes, each sample is treated successively with 5 ml. of 1·1N hydrochloric acid and 20 ml. of 0·01N iodine, and is then allowed to stand for exactly 15 minutes. The excess of iodine is then titrated against 0·01N sodium thiosulphate.

The blank is prepared by weighing 4 to 6 mg. of benzathine penicillin, washing with 10 to 15 ml. of water into a stoppered reaction bottle, treating with 20 ml. of 0·01N iodine and then immediately titrating against the standard thiosulphate solution.

Then if B = blank thiosulphate titre (ml.), S = average of sample thiosulphate titres (ml.), N = normality of the thiosulphate solution, and W = weight of sample taken (g.), the potency of the sample (I.U./mg.),

$$= \frac{(B - S) \times N \times 6615}{W}$$

and the purity,

$$= \frac{\text{Potency (I.U./mg.)} \times 100}{1307} \text{ per cent.}$$

Estimation of Purity from the Dibenzylethylenediamine Content

The dibenzylethylenediamine content of benzathine penicillin preparations is readily determined by treating a suspension of the salt with excess of alkali in presence of sodium chloride, and extracting the free base into ether. The extract is then shaken with an excess of hydrochloric acid, and the residual acid determined by titration with standard sodium hydroxide solution to *p*H 3·5. Trial experiments with solutions of dibenzylethylenediamine sulphate and dihydrochloride, and with benzathine penicillin suspensions, showed that 99 per cent. of the base was removed by a single extraction with ether, two extractions removing over 99·5 per cent. A single extraction therefore appears to be sufficient for assay purposes.

Method

Approximately 100 mg. is accurately weighed out, decomposed with 1 ml. of approximately N sodium hydroxide and washed into a 100-ml. separating funnel with 15 to 25 ml. of water. 1 to 2 g. of solid sodium

chloride is added, and the solution is then shaken with 30 to 40 ml. of ether for 2 to 3 minutes. The aqueous layer is allowed to settle and is then discarded. The ether solution is washed 3 times with 10-ml. portions of 10 per cent. sodium chloride solution, and the washings discarded. The ether solution is then shaken with 25 ml. of 0·01N hydrochloric acid for 2 to 3 minutes. The acid layer is carefully run off, and the ether layer is washed once with about 20 ml. water. This wash is added to the original acid extract, 3 drops of bromophenol blue are added, and the mixture titrated with 0·01N sodium hydroxide, the end-point being the disappearance of the green tinge.

A blank is run by titrating 25 ml. of the 0·01N hydrochloric acid, containing approximately 50 mg. of dibenzylethylenediamine dihydrochloride, with the sodium hydroxide solution, to the same end-point.

Then if B = blank sodium hydroxide titre (ml.), S = sample sodium hydroxide titre (ml.), N = normality of the sodium hydroxide solution, and W = weight of sample taken (g.), the purity

$$= \frac{(B - S) \times N \times 12}{W} \text{ per cent.}$$

Comparison of Results Obtained by the two Methods

The purities of a series of preparations were determined by each of the preceding methods. The results are given in Table I, which also lists the purities obtained by biological assay against *Staphylococcus aureus*. In the biological assays, the samples were dissolved in formamide, diluted with *p*H 7, 0·07 M phosphate buffer and compared with standards of pure benzathine penicillin in a cup-plate 4-point assay.

TABLE I

Sample number	Moisture, per cent.	Iodimetric assay, I.U. mg.	Dibenzylethylenediamine base, per cent.	Biological assay, I.U./mg.	Percentage on a dry weight basis from:		
					Iodimetric assay	Dibenzylethylenediamine assay	Biological assay
Standard	7·00	1213	24·63	—	99·8	100·2	100·0
24	5·44	1164	24·02	1169	92·2	95·8	94·5
32	3·77	1071·5	23·09	1096	85·2	90·9	85·5
33	3·65	1121·5	22·41	1108	89·1	87·9	87·9
34	6·80	1106	22·47	1152	90·8	91·3	94·5
35	2·70	1022	22·07	1059	80·3	85·9	83·2
36	3·90	1138·5	23·03	1124	90·6	90·9	89·4
37A	4·42	1195·5	24·25	1200	96·2	96·1	96·0
37B	7·81	1047·5	20·99	1058	86·9	86·3	87·7

Each chemical assay shows reasonable agreement with the biological results. Moreover, both methods are rapid and easy to perform. A single determination by either of the methods described can be completed in 45 minutes, and in both methods it is possible to assay several samples simultaneously.

Summary

1. A modified iodimetric assay for benzathine penicillin, based upon the method of Alicino, is described.

2. Typical results are detailed, and compared with values obtained by estimation of the organic base content, and by biological assay.

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References

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