THE ASSAY OF BENZATHINE PENICILLIN BY TITRATION IN A NON-AQUEOUS SOLVENT

By C. Knight and W. H. Stephenson

From The Chemical Division, Standards Department, Boots Pure Drug Co., Ltd., Beeston

Received July 23, 1954

Since the description of *NN'*-dibenzylethylenediamine dibenzylpenicillin (benzathine penicillin) by Szabo, Edwards and Bruce[1], this material has become widely used as a repository-type penicillin. Since it is only very slightly soluble in aqueous media, although this property is made use of in therapeutics, its chemical assay is somewhat difficult. As has been pointed out by Parker and Donegan[2] it is impossible to obtain an aqueous solution of sufficient strength for a trustworthy assay by the normal iodimetric method. Since benzathine penicillin contains four secondary nitrogen atoms, it was thought likely that these would be readily titratable, in a suitable non-aqueous system. This was found to be the case, a solution of the amine in glacial acetic acid titrating readily with 0·1N perchloric acid in glacial acetic acid.[3]

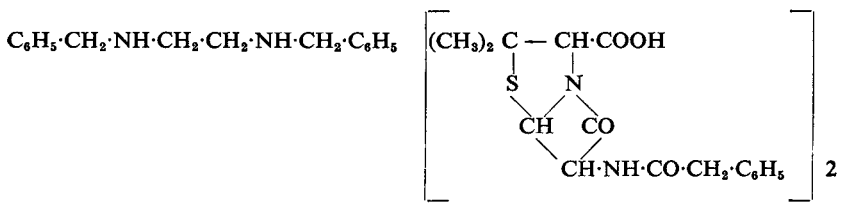
Method

Standardisation of the perchloric acid solution

Dissolve about 0·4 g. of A.R. potassium hydrogen phthalate, accurately weighed, in 70 ml. of glacial acetic acid containing 2 per cent. of acetic anhydride, by gently refluxing. Cool the solution, add 0·2 ml. of 0·1 per cent. aqueous solution of crystal violet and titrate with 0·1N perchloric acid to the first disappearance of the violet tinge.

Determination of the purity of the sample

Transfer 500 mg. to a conical flask and dissolve in 70 ml. of glacial acetic acid containing 2 per cent. of acetic anhydride. Titrate with 0·1N perchloric acid in glacial acetic acid, using crystal violet as indicator and titrating to the same colour as obtained in the standardisation of the perchloric acid. The formula for benzathine penicillin is



and its molecular weight 909·1 so that 1 ml. of 0·1N perchloric acid is equivalent to $\frac{909{\cdot}1}{40{,}000}$ g. of anhydrous benzathine penicillin. To calculate the purity of the sample as anhydrous compound:—

$$\text{Percentage} = \frac{\text{ml. of 0·1N perchloric acid} \times 909{\cdot}1 \times 100}{\text{g. of sample} \times 40{,}000}$$

The potency in I.U./mg. is given by:—percentage purity × 1307 (theoretical potency 1307 I.U./mg.).

A series of comparative determinations was run using this method, the two methods of Parker and Donegan[2], a method based on the determination of the optical density in absolute methanolic solution at 264 mμ[4] and a biological assay using *Staphylococcus aureus*. The results given in

TABLE I

COMPARATIVE ASSAYS OF BENZATHINE PENICILLIN

Batch	Water (Karl Fischer)	100-Water	Microbiological		Iodimetric		Optical density		Base extraction		Non-aqueous titration	
			Potency, I.U./mg.	Purity, per cent.	Potency, I.U./mg.	Purity, per cent.	Potency, I.U./mg.	Purity, per cent.	Potency, I.U./mg.	Purity, per cent.	Potency, I.U./mg.	Purity, per cent.
414	7·5	92·5	1284	98·2	1203	92·0	1177, 1139	90·0, 87·1	1188, 1191	90·9, 91·1	1207	92·3
415	7·0	93·0	1283	98·2	1204	92·1	1177, 1233	90·0, 94·3	1190, 1195	91·0, 91·4	1188	90·9
416	7·2	92·8	1240	94·9	1194	91·4	1215, 1233	93·0, 94·3	1200, 1203	91·8, 92·0	1196	91·5
59	7·3	92·7	1208	92·4	1183	90·5	1195	91·4	1185, 1177	90·7, 90·0	1164	89·2
60	7·2	92·8	1268	97·0	1203	92·0	1195	91·4	1191, 1186	91·1, 90·7	1169	89·4
61	7·3	92·7	1234	94·4	1186	90·7	1203	92·0	1199, 1187	91·7, 90·8	1172	89·7
62	7·5	92·5	1243	95·1	1191	91·1	1139	86·5	1195	91·4	1189	91·0
63	7·4	92·6	1220	93·3	1207	92·3	1225, 1207	93·7, 92·3	1202	92·0	1193	91·3
65	7·4	92·6	1220	93·3	1203	92·0	1225, 1233	93·7, 94·3	1204	92·1	1195	91·4
66	7·5	92·5	1216	93·0	1204	92·1	1233, 1215	94·3, 93·0	1198	91·7	1198	91·7
67	6·8	93·2	1218	93·2			1232, 1194	94·3, 91·4			1194, 1187	91·4, 90·9
68	7·0	93·0	1156	88·4			1262	96·6			1191, 1203	91·2, 92·1
69	6·6	93·5	1220	93·3			1266	96·9			1195	91·5
70	6·0	94·0	1220	93·3			1240	94·9			1179	90·3
71	7·1	92·9	1201	91·9			1240	94·9			1211	92·7
72	7·1	92·9	1220	93·3			1157, 1164	88·6, 89·1			1198	91·7
73	6·3	93·7	1213	92·8			1194, 1202	91·4, 92·0			1198	91·7
74	6·9	93·1	1220	93·3			1194, 1176	91·4, 90·0			1204	92·3
411	7·3	92·7	1255	96·0			1194	91·4			1183	90·6
412	7·3	92·7	1207	92·4			1325	101·4			1195	91·5
413	7·1	92·9	1264	96·7			1150, 1145	88·0, 87·7			1211	92·7
410	7·3	92·7	1235	94·5			1204	92·2			1203, 1207	92·1, 92·4
409	6·8	93·2	1196	91·5			1194	91·4			1191, 1194	91·2, 91·4
58	7·0	93·0	1286	98·4			1187	90·9			1178, 1173	90·2, 89·8

Table I show that the proposed method gives results which are in reasonable agreement with those given by other methods, and is rapid.

SUMMARY

1. A method for the assay of benzathine penicillin using titration with perchloric acid in glacial acetic acid is described.

2. Results are set out and show favourable comparison with four other methods.

REFERENCES

1. Szabo, Edwards and Bruce, *Antibiotics and Chemotherapy*, 1951, I.8, 499.
2. Parker and Donegan, *J. Pharm. Pharmacol.*, 1954, 6, 167.
3. Fritz, *Acid-Base Titrations in Non-Aqueous Solvents*, Frederick G. Smith, Chemical Co., 1952.
4. F.D.A. Regulations.

DISCUSSION

The paper was presented by MR. W. H. STEPHENSON.

Mr. F. A. J. TALMAN (Liverpool) asked whether the authors had any information on the use of this assay for preparations such as suspensions and tablets.

DR. F. HARTLEY (London) said that penillic acid had about the same strength as penicillin acid, and asked what would happen if they were dealing with a partly degraded benzathine penicillin. Iodimetric determination of the degradation product would then appear to be advantageous over the recommended titration method.

DR. A. H. BECKETT (London) said that 2 per cent. of acetic anhydride had been added to glacial acetic acid. Knowing that excess acetic anhydride immediately acylated secondary or primary amines, it was possibly a little dangerous to have this amount present. Usually it was customary, when titrating amines, secondary or primary, to make sure that a trace of water was present. Possibly the lower results in Table 1 by titration, as compared with the optical density method, could be explained in terms of a trace of acylation. The end-point colour for the standardisation using potassium hydrogen phthalate and the colour in the actual determination were stated to be the same. Had this been checked potentiometrically? When potassium ions were present, there was precipitation of potassium perchlorate, which altered the colour at the end-point.

DR. G. E. FOSTER (Dartford) said that some analysts had difficulty in determining the end-point with crystal violet. He had used quinaldine red. Had the authors used any other indicators?

MR. STEPHENSON, in reply, said their method was used with benzathine penicillin itself. In suspensions it was necessary to carry out a blank on the suspending gel. They were in the experimental stage of their work on tablets. He had no analytical evidence with penicillic acid. He agreed that the 2 per cent. excess of acetic anhydride might be responsible for the somewhat lower results. A potentiometric titration had been carried out to check the colorimetric end-point. This might be preferable, since crystal violet was not an ideal indicator. They had not used quinaldine red.