APPLICATION OF A SPECTROPHOTOMETRIC METHOD TO THE DETERMINATION OF POTASSIUM PENICILLIN, PROCAINE PENICILLIN AND BENZATHINE PENICILLIN IN PHARMACEUTICAL PREPARATIONS

BY A. HOLBROOK

From the Pharmaceutical Department, Imperial Chemical Industries Limited, Pharmaceuticals Division, Hexagon House, Blackley, Manchester, 9

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A spectrophotometric method originally designed for the estimation of potassium penicillin has been applied to procaine penicillin and benzathine penicillin. A solution containing the penicillin salt is heated under controlled conditions with a sodium acetate-acetic acid buffer solution of pH 4·6 to which a trace of copper sulphate has been added. The optical absorption of the resulting penicillinic acid is measured on a spectrophotometer. The method has a standard error of 3 per cent and is applicable to a variety of penicillin preparations ranging in potency from 0·5 units/mg. upwards.

A method has been described by Herriot[1] for the determination of penicillin by its controlled degradation to penicillinic acid on heating with acetic acid-sodium acetate buffer of pH 4·6, followed by spectrophotometric measurement of the ultra-violet absorption at 322 mμ. It has since been shown by Stock[2] that traces of copper in the buffer solution play an important role in the reaction and that, in the absence of this element, reproducible results are unobtainable. Stock has successfully applied this method to the determination of penicillin in oral tablets. The object of this work was to study the extension of the method to formulated penicillin products other than oral tablets and to the procaine and dibenzylethylenediamine salts of penicillin, both of which are now widely used.

EXPERIMENTAL

Application to procaine penicillin. A standard solution was prepared containing 0·1 g./l. of Procaine Penicillin B.P. in 0·01M phosphate buffer. 20 ml. of this solution was diluted with 50 ml. acetate buffer (acetic acid-sodium acetate buffer of pH 4·6 containing copper sulphate equivalent to 0·45 p.p.m. of copper). Aliquots, each of 5 ml. of the diluted solution were heated in a water bath at 100° for varying periods of time, ranging from 5 to 35 minutes, cooled and made to volume in 10 ml. stoppered cylinders with acetate buffer. The optical absorptions of each of the solutions were measured at 322 mμ in 1 cm. silica cells with a spectrophotometer. A blank was done at the same time by proceeding exactly as in the preparation of the sample solution, but omitting the heating process. The results are shown in Figure 1. It was obvious from the outset that owing to the low solubility of procaine penicillin in water (0·4 per cent w/v) the method in its original form would be of little use in dealing with most procaine penicillin formulations because of the small weight of sample, and the uncertainty of securing complete extraction.

The above work was repeated using solutions covering the range of 5 to 25 per cent v/v methanol in water as solvent. The use of these concentrations of methanol did not produce any significant departure from the values obtained using water alone, and it was concluded that methanol, in which procaine penicillin is very soluble, would be suitable as an extracting solvent. Procaine absorbs strongly in the ultra-violet region of the spectrum exhibiting a maximum at 292 mμ. The absorption at 322 mμ (the wavelength maximum for penicillinic acid) is still significant and for pure procaine penicillin this irrelevant absorption amounts to 23 per cent of the total absorption at 322 mμ measured after heating on the water bath for 25 minutes. To ensure that the increase in absorption at 322 mμ was due solely to penicillin decomposition, a solution of the appropriate quantity of procaine base in M/100 phosphate buffer containing 20 per cent v/v methanol, was prepared and the absorption measured before and after heating with acetate buffer for 25 minutes. The optical densities of the two solutions were identical showing that, under the conditions of the determination, the contribution of the procaine component towards the total absorption is constant.

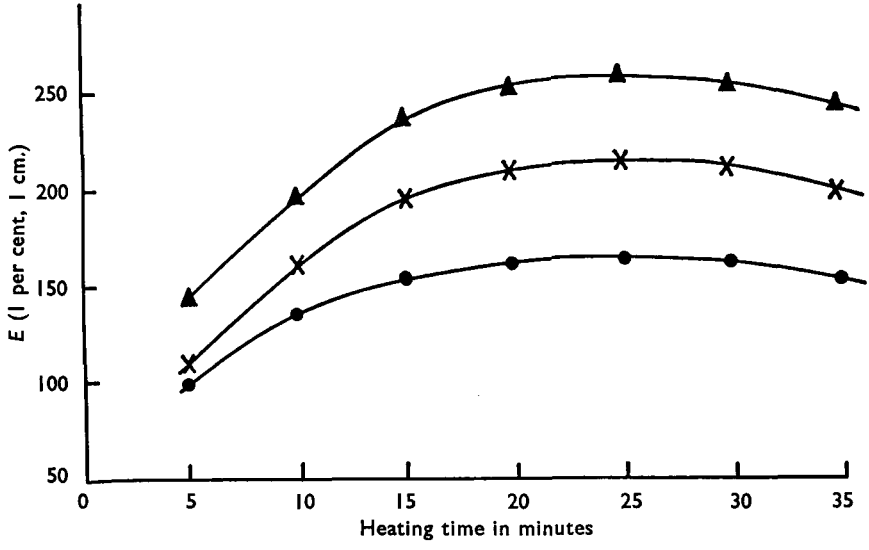

FIG. 1. Variation of E (1 per cent, 1 cm.) at 322 mμ with time of heating at 100°. ▲ Potassium penicillin, X benzathine penicillin, ● procaine penicillin.

Application to benzathine penicillin. This compound is virtually insoluble in water, sparingly soluble in most organic solvents, but significantly soluble in methanol and very soluble in dimethylformamide. A solution was prepared by dissolving 0·16 g. in 100 ml. of methanol and diluting to 500 ml. with 0·01M phosphate buffer and 20 ml. of the latter solution was diluted to 70 ml. with acetate buffer. Aliquots of this solution were heated for varying times as previously described. The results obtained were erratic, and this solvent was obviously unsatisfactory. Repetition of the work using aqueous 10 per cent v/v dimethyl

formamide (2·8 per cent v/v in the heated solution) as solvent gave good duplication of results, which are shown in Figure 1. The absorption of dibenzylethylenediamine at 322 mμ is negligible but to ensure that this obtained throughout the determination, a solution containing the appropriate quantity of the base was prepared in 10 per cent v/v dimethylformamide and its absorption measured at 322 mμ before and after heating for 25 minutes with acetate buffer. No difference in the absorption was observed showing that dibenzylethylenediamine at the concentration encountered in practice does not increase in absorption at 322 mμ. It has been shown[1] that the absorption produced at 322 mμ is a maximum when the pH of the solution is 4·68 and falls rapidly on either side of this value. It was confirmed (see Fig. 2) that the small quantity of copper in the buffer solution causes no change in this critical value and that for all practical purposes the results can be considered constant over the pH range 4·6–4·8. In the determination of benzathine penicillin the pH will fall between these limits, provided the concentration of dimethylformamide in the final solution does not exceed 5 per cent v/v.

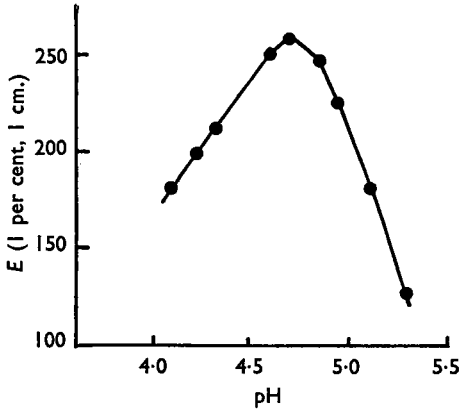

FIG. 2. Variation of E (1 per cent, 1 cm.) at 322 mμ with pH of the buffer solution.

RESULTS

The results shown in Table I are typical of many determinations carried out on pure samples of potassium penicillin, procaine penicillin, and samples of benzathine penicillin of known composition, and are compared with the microbiological potencies.

TABLE I

EXTINCTION COEFFICIENTS AT 322 Mμ COMPARED WITH MICROBIOLOGICAL POTENCIES

Compound		E (1 per cent, 1 cm.) 322 mμ	Microbioloigcal Potency u./mg.
Procaine penicillin	1	158	1000
	2	159	1000
	3	161	1000
	Average	159	
Benzathiene penicillin	1	212	1290
(Results calculated	2	218	1310
to Mol. Wt. 909)	3	214	1300
	4	215	1300
Average calculated to potency of 1300 u./mg.		214	
Potassium penicillin	1	253	1600
	2	255	1600
	3	256	1600
	Average	255	

Discussion

The spectrophotometric method previously developed for potassium penicillin can be applied to both the procaine and dibenzylethylenediamine salts provided a suitable solvent is employed, and the pH of solution is carefully controlled by suitable buffer solutions. The absorption at 322 mμ reaches a maximum after 25 minutes heating for these two salts, and this was subsequently found to be so for potassium penicillin itself. Earlier workers have used a heating time of 15 minutes, but in view of the above findings it was decided to increase this time to 25 minutes for all future work. It was also decided to use 0·01M phosphate buffer in place of water wherever possible as penicillin is more stable in this medium. A general method is described below and this is followed by applications of the technique to formulated preparations.

General Method

Reagents

Copper sulphate. Dissolve 0·392 g. of copper sulphate ($CuSO_4 \cdot 5H_2O$) in 100 ml. of distilled water. This solution contains 1 mg./ml. of copper. *Acetate buffer.* Mix equal volumes of 0·4 M acetic acid and 0·4 M sodium acetate and add 0·9 ml. of copper sulphate solution per 2 litres of mixed solution (equivalent to 0·45 p.p.m. of copper as Cu). 0·5 *M Phosphate buffer.* Dissolve 78 g. of sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$) and 179·1 g. disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) in distilled water and make to 2 litres. Adjust the pH to lie between 6·45 and 6·55 by the addition of 2N sodium hydroxide or 2N phosphoric acid. 0·01*M Phosphate buffer.* Dilute 10 ml. of 0·5 M phosphate buffer to 500 ml. with distilled water.

Procedure

Prepare an extract of the preparation under examination by an appropriate method so that it will contain between 160–190 units/ml. Take a 20 ml. aliquot of this solution and add, by pipette, 50 ml. of acetate buffer. Pipette 5 ml. aliquots of the above solution into each of five 6 × 1 inch boiling tubes and simultaneously place four of these into a 1500 ml. beaker of boiling water in which the level of water is above the level of liquid in the boiling tubes, and the bases of the tubes are protected for example by a wire gauze supported by 1 inch legs, from direct contact with the base of the beaker. Allow the tubes to remain in the beaker for 25 minutes and maintain the water at boiling point during the whole of this period.

Dilute the contents of the fifth tube to 10 ml. by the addition of 5 ml. of acetate buffer and immediately read the absorption of the resultant solution in a 1 cm. silica cell at 322 mμ against a cell containing distilled water. The figure so obtained represents the blank.

After exactly 25 minutes heating, remove the four tubes from the water bath, cool rapidly and transfer the contents of each in turn to four 10 ml. stoppered cylinders, rinsing out each tube with 3 ml. of acetate buffer and adding the washings to the appropriate cylinder. Adjust the volume

of each cylinder to 10·0 ml. by the addition of acetate buffer and measure the absorption of each solution at 322 mμ against a cell containing distilled water. Calculate the average of the four readings and subtract from this the figure obtained in the blank determination. Calculate the *E* (1 per cent, 1 cm.) value for the sample and use this figure to evaluate the potency in the manner indicated.

$$\text{Potency} = \frac{E\ (1\%, 1\text{ cm.})\ 322\ \text{m}\mu\ \text{sample}}{E\ (1\%, 1\text{ cm.})\ 322\ \text{m}\mu\ \text{pure penicillin salt}} \times 10 \times \text{A units/g.}$$

Where A = 1600 for potassium penicillin
1000 for procaine penicillin
1300 for benzathine penicillin.

Applications

Preparations containing Potassium Penicillin

Penicillin Ointment B.P. and Penicillin Eye Ointment B.P. (1953). Transfer a weight of sample expected to contain about 8000 units of penicillin to a 150 ml. flask fitted with a ground glass stopper and add 20 ml. of carbon tetrachloride. Stopper tightly and shake the flask until all excipient material is in solution. Add, from a pipette, 50 ml. of 0·01 phosphate buffer and shake vigorously for 15 minutes. Allow the layers to separate and, by decantation, transfer the aqueous layer as completely as possible to a 100 ml. centrifuge tube. Centrifuge at 2000 r.p.m. for 10 minutes. Filter the aqueous layer through a No. 42 Whatman filter paper and determine the penicillin content of 20 ml. of the filtrate by the general method. *Note.* In order to test the reproducibility of the method in the hands of different operators, and to compare the results obtained with the microbiological method, a series of ointments of varying potencies were prepared in the laboratory from potassium penicillin of known purity and these were analysed on successive days by three operators. The results are shown in Table II.

TABLE II

COMPARISON OF RESULTS OBTAINED ON LABORATORY PREPARED PENICILLIN OINTMENTS

Sample No.	Theoretical potency u/g.	Spectrophotometric potency u./g.			Average spectrophotometric potency u./g.	Microbiological potency u./g.
		Operator 1	Operator 2	Operator 3		
1	750	730 740	740 750	750 700	735	760
2	1140	1140 1120	1130 1140	1120 1070	1120	1130
3	1250	1280 1230	1260 1210	1180 1190	1225	1220
4	1320	1390 1360 1380	1390 1300 1320	1280 1290 1350	1340	1300
5	1640	1650 1590 1660	1680 1650 1580	1690 1630 1580	1630	1600

Statistical evaluation of the results in Table II gives a standard error of 3 per cent for a single determination. The method is therefore comparable to the microbiological method in accuracy.

Penicillin Lozenges B.P. Transfer to a stoppered 100 ml. centrifuge tube a weight of powdered lozenges expected to contain 8000 units. Add 50 ml. of 0·01 M phosphate buffer and shake vigorously for 10 minutes. Centrifuge at 2000 r.p.m. for 10 minutes and filter through a No. 42 Whatman filter paper. Determine the penicillin content on 20 ml. of the filtrate by the general method. *Note.* With some formulations of lozenges the phosphate buffer extract is almost impossible to filter due to the presence of binding agents, tragacanth for example, which form viscous solutions. This difficulty can be overcome by adding 5 ml. of chloroform to the phosphate buffer extract, shaking vigorously and centrifuging at high speed (e.g., 4000 r.p.m.). The insoluble matter from the lozenge separates at the chloroform/water interface leaving the supernatant liquor sufficiently clear to use without filtration.

Procaine Penicillin and Preparations containing Procaine Penicillin

Procaine Penicillin, Injection of Procaine Penicillin, and Injection of Procaine Penicillin Fortified with Potassium Penicillin. Dissolve an accurately weighed quantity of between 0·13 and 0·14 g. of sample in 100 ml. of methanol and make to volume in a litre graduated flask with 0·01 M phosphate buffer. Determine the total penicillins on a 20 ml. aliquot of this solution by the general method.

Procaine Penicillin Oily Injection B.P. 300,000 *units/ml.* Accurately weigh between 0·3–0·4 g. of the sample into a 100 ml. beaker and add 50 ml. of light petroleum (b.p. 40° to 60°). Stir until all fatty material is in solution and filter with the aid of suction through a No. 4 sintered glass crucible. Wash the beaker and the residue on the filter with a further 50 ml. portion of light petroleum. Dissolve the residue in 150 ml. of methanol and transfer to a litre flask, washing the beaker and filter with a further 50 ml. of methanol and adding this washing to the bulk of the solution in the litre flask. Adjust the volume to 1 litre with 0·01 M phosphate buffer and determine the penicillin on a 20 ml. aliquot of this solution by the general method.

Procaine Penicillin Premix, Veterinary. (A mixture of Procaine Penicillin with an inert base). Transfer a quantity of sample, expected to contain about 16,000 units of penicillin, to a 250 ml. flask fitted with a ground glass stopper and extract the procaine penicillin by shaking for 5 minutes with 100 ml. of 0·01 M phosphate buffer containing 20 per cent v/v methanol. Filter through a Whatman No. 42 filter paper and determine the penicillin content of 20 ml. of the filtrate by the general method.

Benzathine Penicillin and Preparations containing Benzathine Penicillin

Benzathine Penicillin. Accurately weigh about 0·08 g. of sample; dissolve in 30 ml. of dimethylformamide in a 50 ml. volumetric flask and make to volume with water. Dilute 10 ml. of this solution to 100 ml. with 0·01 M phosphate buffer and, by the general method, determine the benzathine penicillin in 20 ml. of the solution so obtained.

Benzathine Penicillin Oral Suspension. Transfer a weight of sample, expected to contain about 100,000 units of penicillin, to a 50 ml. volumetric flask with the aid of 30 ml. of dimethylformamide, shake for 5 minutes and dilute to volume with distilled water. Using these quantities with the samples available, the sugars, suspending and dispersing agents remained in solution. Dilute 10 ml. to 100 ml. with 0·01 M phosphate buffer and, by the general method, determine the penicillin content of a 20 ml. aliquot of the solution so obtained.

TABLE III

COMPARISON OF RESULTS OBTAINED ON SAMPLES OF VARIOUS PENICILLIN FORMULATIONS

Sample		Nominal potency	Operator 1	Operator 2	Operator 3	Average spectrophotometric potency	Microbiological potency	Potency calculated from total base determination
Penicillin lozenges	1	5000 units/ lozenge	4840	5000	5030	5000	5200	
	2		5300	5200	5300	5300	5200	
	3		5970	5810	5790	5860	5700	
	4		5820	5850	5910	5860	5910	
	5		5740	5750	5920	5830	5920	
Procaine penicillin aqueous suspension	1	330,000 units/ml.	341,000	323,000	344,000	336,000	346,000	
	2		358,000	316,000	351,000	342,000	333,000	
	3		348,000	317,000	338,000	334,000	356,000	
	4		359,000	338,000	350,000	349,000	347,000	
	5		356,000	343,000	355,000	351,000	343,000	
	6		358,000	347,000	357,000	354,000	351,000	
Procaine penicillin oily injection	1	300,000 units/ml.	322,000	306,000	280,000	303,000	301,000	
	2		295,000	296,000	282,000	291,000	292,000	
	3		296,000	283,000	282,000	287,000	300,000	
	4		306,000	315,000	293,000	305,000	308,000	
	5		298,000	280,000	295,000	291,000	295,000	
	6		290,000	290,000	286,000	289,000	300,000	
Procaine penicillin fortified	1	1060 units/ mg.	1030	1020	1050	1035	1060	
	2		1070	1090	1060	1075	1085	
	3		1020	1075	1050	1050	1075	
	4		1080	1030	1060	1065	1065	
	5		1040	1070	1030	1050	1090	
	6		1050	1030	1060	1050	1095	
Procaine penicillin Premix	1	3000 units/ g.	2900	2900	—	2900	3100	2800
	2		2900	3100	—	3000	3000	2900
	3		2900	2900	—	2900	3200	3300
	4		2900	3100	—	3000	3100	2900
	5		3000	3000	—	3000	3200	3200
	6		2800	2900	—	2850	3000	2900
	7		3000	2900	—	2950	3200	2900
	8		3200	3100	—	3150	3100	2800
Benzathine penicillin oral suspension	1	65,000 units/ml.	70,800	70,200	70,300	70,400	69,300	67,500
	2		68,000	66,400	69,200	57,800	65,600	65,500
	3		75,400	76,400	77,100	76,300	72,600	75,300
	4		64,700	67,800	68,200	66,900	65,500	66,900
	5		65,400	67,600	67,500	66,800	65,200	67,300
	*6		55,100	57,600	56,200	56,300	55,700	64,400
	7		63,700	66,200	64,000	64,600	64,000	—

* This sample was originally of correct potency but decomposition, due to adverse storage conditions, had subsequently taken place. This decomposition is shown by both the spectrophotometric and microbiological determinations. The total base figure result indicates that the preparation originally contained the correct amount of benzathine penicillin.

RESULTS

Using the techniques outlined in the previous sections, the results shown in Table III were obtained. The estimations of Total Base were carried out by a modification of the method of Knight and Stephenson[3].

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