Analytica Chimica Acta, 134 (1982) 411—415
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

Short Communication

SPECTROPHOTOMETRIC DETERMINATION OF MICROGRAM AMOUNTS OF PENICILLINS BY SOLVENT EXTRACTION WITH AZURE B

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(Received 14th April 1981)

Summary. Penicillins are determined by means of ion-pair formation with azure B and extraction into chloroform: the absorbance of the extract is stable for several days. The apparent molar absorptivities for sodium penicillin G and potassium penicillin V at 634 nm are 3.91×10^3 and 1.25×10^4 l mol^{-1} cm^{-1}, respectively. Calibration graphs are linear over the range 60—950 μg of sodium penicillin G and 40—600 μg of potassium penicillin V in 10 ml. The method is successfully applied to pharmaceutical preparations.

The continuing introduction of new penicillin drugs has resulted in an extensive literature on their determination. The assay of antibiotics has been reviewed by Fairbrother [1]. Most chemical assays for the penicillins are based on the titration of unconsumed iodine after incubation with hydrolyzed penicillin [2]. An adaptation of this method is based on the reaction of penicillins with an excess of *N*-bromosuccinimide [3]. Adams et al. [4] proposed a novel enzymatic penicillin assay based on a pH-stat instrument incorporating coulometric generation of the titrant. Ibrahim et al. [5] determined penicillins by oxidizing with iodine monochloride and titrating the residual iodine monochloride with standard potassium iodate. Grime and Tan [6] determined some selected penicillins by direct titration with potassium iodate.

Spectrophotometric methods for penicillins have been based on the determination of the hydroxamic acid formed by reaction with hydroxylamine [7], or the penicillenic acid mercury(II) mercaptides formed by reaction with imidazole in the presence of mercury(II) chloride [8]. A method based on the formation of a coloured compound (λ_{max} = 750 nm) by boiling penicillins with ammonium vanadate solution in sulphuric acid has been reported [9].

Azure B forms ion-pairs with various anions; such reactions have been

0003-2670/82/0000-0000/$02.75

used for extraction—spectrophotometric determinations of rhenium [10], antimony [11], uranium [12], etc. In a comprehensive investigation of analytical methods for penicillins, azure B was found to give extractable ion-pairs with sodium penicillin G and potassium penicillin V. The extraction of these ion-pairs provides a very sensitive spectrophotometric procedure which is applicable over a wide pH range with good selectivity.

Experimental

Apparatus and reagents. A Beckman DB spectrophotometer with 10-mm silica cells and in Elico L1-10 pH meter were employed. All chemicals were of analytical grade and were used without further purification. An azure B stock solution (5×10^{-3} M) was prepared by dissolving 340.5 mg of azure B in redistilled water, and diluting to 250 ml.

Penicillins. Pharmaceutical-grade benzyl penicillin sodium (sod. pen. G) and phenoxymethyl penicillin potassium (pot. pen. V) were obtained in sealed vials. Elemental analysis revealed no significant impurities. Standard stock solutions of penicillins were prepared by dissolution of appropriate amounts of solid in 100 ml of 0.1 M phosphate buffer solution, pH 5.91.

Procedure. Pipette an aliquot of sample or standard solution containing 60–950 μg of sod. pen. G or 40—600 μg of pot. pen. V into a 50-ml separating funnel. Add 5 ml of buffer pH 5.0 (14.74 g of Na_2HPO_4 and 10.20 g of citric acid per litre) and dilute to 20 ml with redistilled water. Add 1 ml of 1×10^{-3} M azure B solution and 10 ml of chloroform. Shake the funnel for 2 min to extract the ion-pair formed between azure B and penicillin. After standing for a fixed time between 5 min and 2 h, transfer the organic phase to a 15-ml glass-stoppered tube containing some anhydrous sodium sulphate. Shake the mixture vigorously until transparent and measure the absorbance at 634 nm against a reagent blank. Construct a calibration graph from the standard measurements.

For the analysis of pharmaceutical preparations, dissolve an appropriate amount of sample in the phosphate buffer to obtain a ca. 200-ppm solution of penicillin and filter if necessary. Then apply the above procedure.

Results and discussion

Figure 1 shows the absorption spectra of the azure B-penicillin ion-pairs extracted into chloroform, and of the reagent blank. All these spectra have their absorption maxima at 634 nm and this wavelength was used in all subsequent measurements. The wavelength of maximum absorption did not shift with a change in buffer composition at a given pH, when acetate and McIlvaine buffers were tested.

To establish the optimum pH range, penicillin was allowed to react with azure B in aqueous solutions buffered to pH 0.7—8.0 and the complex formed was extracted into chloroform for measurement. Sulphuric acid was used for adjustment to below pH 2.2, and McIlvaine buffers [13] for pH values in the range 2.2—8.0. Constant absorbances were obtained over the pH

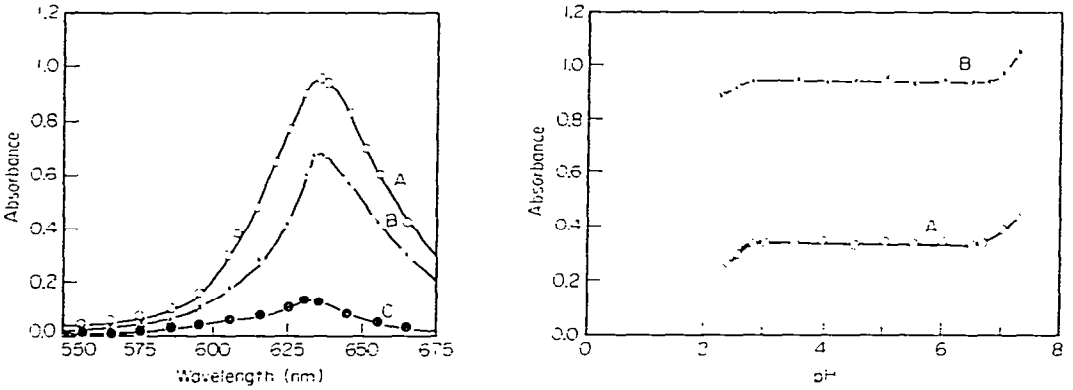

Fig. 1. Absorption spectra of azure B ion-pairs in chloroform: (A) 30 ppm potassium V (reagent blank subtracted); (B) 60 ppm sodium penicillin G (reagent blank subtracted); (C) reagent blank against chloroform.

Fig. 2. Effect of pH on the formation of the ion-pairs of azure B with (A) sodium penicillin G; (B) potassium penicillin V (reagent blank subtracted).

range 2.8—6.6 (Fig. 2). The decreased absorbance below pH 2.8 can be attributed to a decrease in the concentration of free penicillin ion caused by protonation. The increased absorbance above pH 6.6 is caused by the formation of an extractable azure B species. In all subsequent work, a pH of ca. 5.0 was used.

The extraction of the ion-pair was affected by the concentration of azure B. To establish the optimal amount, 1-ml aliquots of 10^{-4}—2×10^{-3} M azure B solutions were used in the conditions given above. Calibration graphs with 1 ml of 1×10^{-4} or 2.5×10^{-4} M azure B were not linear but the reagent blanks were low. The graphs with 5×10^{-4}—2×10^{-3} M azure B solutions were linear, but the more azure B used, the higher the reagent blank. In subsequent work 1 ml of 1×10^{-3} M azure B was employed. The method of continuous variations indicated that a single 1:1 ion-pair species was extracted.

To establish the optimal extraction conditions, solutions containing 400 μg of penicillin in redistilled water were treated with azure B as recommended and then extracted with successive 10-ml portions of chloroform, the absorbance of the organic phase after each extraction being measured against chloroform at 634 nm. The absorbance of the fifth extract was very similar to that of the fifth reagent blank, indicating that four 10-ml portions of chloroform suffice for complete extraction. However, the reproducibility with a single extraction was so good that, for simplicity, one 10-ml portion of chloroform was used in the recommended procedure. Tests made on sodium penicillin G (40 ppm) with the single extraction gave a mean result of 39.8 ppm with a standard deviation of 0.27 (n = 5). Other organic solvents were tested, but chloroform was found to be the most suitable.

Shaking times of 0.5—5 min produced constant absorbance, and so shaking for 2 min was used throughout. In contrast, increasing standing times (5 min—3 h) after shaking produced a slight increase in absorbance for both the reagent blank and the ion-pair. The reproducibility was good on standing for a fixed period; for example, measurements on 40-ppm solutions after 10-min standing times showed standard deviations of about 0.3 ppm (n = 10). The absorbances of the separated extracts were, however, stable for 5 days in a glass-stoppered tube at room temperature.

Calibration graphs showed a linear dependence of absorbance on concentration over the ranges 6—95 ppm of sodium penicillin G and 4—60 ppm of potassium penicillin V. For log I_0/I = 0.001, the Sandell sensitivities were 0.091 and 0.031 $\mu g\ cm^{-2}$, respectively, and the corresponding apparent molar absorptivities were 3.91×10^3 and 1.25×10^4 $l\ mol^{-1}\ cm^{-1}$.

Effect of concomitant substances and applications to penicillin drugs. The effects of some compounds which often accompany penicillin in pharmaceutical products were studied. The compounds were added to 40-ppm penicillin solutions and studied by the recommended procedure. The tolerance limits are given in Table 1. The proposed method has the advantage of virtual freedom from interference and should be of value in the trace determination of penicillins in many samples.

The proposed method was successfully applied to the determination of penicillins in various pharmaceutical preparations. The results of the assays of tablets and vials (Table 2) compare favourably with the quoted values, and with those obtained by the official method of the British Pharmacopoeia [14].

The authors thank Hindustan Antibiotics Ltd., India, for the supply of pharmaceutical-grade penicillins.

TABLE 1

Effect of concomitant substances on the determination of penicillin (40 ppm)

Compound added	Tolerance limit[a] (ppm)		Compound added	Tolerance limit[a] (ppm)	
	Sod. pen. G	Pot. pen. V		Sod. pen. G	Pot. pen
Ascorbic acid	120	130	Sucrose	6500	6300
Benzoic acid	250	275	Barbitone	2000	2200
Citric acid	4500	4300	Gelatin	5500	5800
4-Hydroxybenzoic acid	325	300	Gum acacia	10000	9500
Sorbic acid	500	550	Reserpine	300	300
Stearic acid	850	800	Sodium alginate	700	800
Tartaric acid	7800	8000	$NaHCO_3$	1400	1500
Dextrose	5500	5800	Starch	4000	4200
Lactose	4500	4500	Talc	4500	4600
Maltose	5000	5000			

[a]Amount causing an error of ± 2.5%.

TABLE 2

Determination of penicillins in commercial pharmaceutical preparations

Preparations	Mean recovery[a] (%)	
	B.P. method	Proposed method
Pot. pen. V[b]		
(125 mg/tab)	102.3 ± 1.2	102.7 ± 1.4
(250 mg/tab)	98.8 ± 0.8	99.4 ± 0.7
Sod. pen. G[b]		
(500000 U/vial)	98.2 ± 1.1	98.6 ± 1.3
(1000000 U/vial)	101.6 ± 0.8	102.0 ± 0.6
Sod. pen. G[c]		
(200000 U/vial)	102.4 ± 0.6	102.1 ± 0.7
(500000 U/vial)	96.8 ± 1.5	96.4 ± 1.6
(1000000 U/vial)	101.2 ± 1.2	100.8 ± 0.8

[a]Average of five determinations with standard deviation. [b]Marketed by Hindustan Antibiotics Ltd. [c]Marketed by Squibb.

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