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Application of Azure A in the Spectrophotometric Determination of Penicillin Drugs

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ABSTRACT

A simple, accurate, and rapid method for the quantitative analysis of two penicillin drugs is developed. The method is based on the formation of a blue penicillin-azure A ion pair that can be extracted into chloroform in phosphate-citric acid buffer of pH 2.6-6.8 at room temperature (25 $\pm$ 2ºC). The molar absorptivities for sodium penicillin G and potassium penicillin V at 660 nm are 6.74 x $l0^3$ and 3.87 x $l0^4$ M^{-1} cm^{-1}, respectively. Beer's law was obeyed over the concentration range of 3.5 - 75 ppm for sodium penicillin G and 3.0 - 50 ppm for potassium penicillin V. The optimum concentration ranges evaluated by Ringbom's method and the Sandell's sensitivities for sodium penicillin G and for potassium penicillin V were 7.0 - 68 ppm and 6.0 - 45 ppm, and 76 ng cm^{-2} and 24 ng cm^{-2}, respectively. Maximum absorbance of the solution was obtained almost instantaneously and was stable for several days. The Job's method of continuous variations indicated that a single l:l ion pair species was extracted. The relative error was $\pm$2%.

INTRODUCTION

The continuing introduction of new penicillin drugs has resulted in an extensive literature on their determination. The assays of antibiotics have been reviewed by Fairbrother (l). Most chemical assays for the penicillins are based on the titration of unconsumed iodine after incubation with hydrolyzed penicillin (2). One of the methods is based on the reaction of penicillins with an excess of N-bromosuccinimide (3). Adams et al. (4) have reported a novel enzymatic penicillin assay based on a pH-stat instrument incorporating coulometric generation of the titrant. Ibrahim et al. (5) have determined penicillins by

oxidizing with iodine monochloride and titrating the residual iodine monochloride with standard potassium iodate. Grime and Tan (6) have determined some selected penicillins by direct titration with potassium iodate.

The available spectrophotometric methods for penicillins have been based on the determination of the hydroxamic acid formed by reaction with hydroxylamine (7) or penicillenic acid-mercury(II) mercaptides formed by reaction with imidazole in the presence of mercury(II) (8). A method based on the formation of a chromogen (λ_{max} = 750 nm) by boiling penicillins and ammonium vanadate solution in sulfuric acid has also been reported (9). The purpose of this work was to apply the reagent Azure A to the spectrophotometric determination of microgram amounts of penicillin drugs. Azure A was found to form ion pairs with sodium penicillin G and potassium penicillin V extractable to an organic layer. The extraction of these ion pairs provided a very sensitive spectrophotometric method which is applicable over a wide range of pH with good selectivity.

MATERIALS AND METHODS

Apparatus
A Beckman DB spectrophotometer and stoppered silica cells of l-cm optical path were used for all absorbance measurements. An Elico Ll-l0 pH meter was used for pH measurements.

Penicillins
Pharmaceutical-grade benzylpenicillin sodium (sodium penicillin G) and phenoxymethylpenicillin potassium (potassium penicillin V) were obtained in sealed vials (Fisher Scientific Company). Elemental analysis revealed no significant impurities. Standard stock solutions of penicillins were prepared by dissolving appropriate amounts of the solid sample in l00 mL phosphate-citric acid buffer, pH 4.8.

Azure A solution
A 0.05% solution of Azure A (Fisher Scientific Company) was prepared in doubly distilled water.

Disodium hydrogen phosphate-citric acid buffer
Fresh Mcllvaine buffer solutions in the pH range 2.2 - 8.0 were prepared from 0.2 M disodium hydrogen phosphate and 0.l M citric acid (l0).

As all other reagents were of analytical grade, they were used without further purification.

Procedure
An aliquot of the analyte solution (containing 35.0 μg - 750 μg of sodium penicillin G or 30.0 - 500 μg of potassium penicillin V) and 5 mL of buffer, pH 4.8, were taken in a 50-mL separatory funnel and the solution was diluted to 20 mL with doubly distilled water. Two mL of 0.05% azure A solution and l0 mL of chloroform were added. The separatory funnel was shaken vigorously for 2 min. to extract the ion pair formed between azure A and penicillin to the organic phase. The solution was allowed to stand for a fixed time (l0-30 min.), the organic layer was transferred to a l5-mL glass stoppered tube containing

some anhydrous sodium sulfate and it was shaken vigorously until transparent. The transparent extract was transferred to a l0-mL volumetric flask, made up to the mark with chloroform, and the absorbance was measured at 660 nm against a reagent blank. The amount of penicillin was determined from the calibration curve prepared under the same experimental conditions.

CAUTION. Since chloroform is a carcinogen, extreme care should be taken in handling the same.

For the analysis of pharmaceutical preparations, an appropriate amount (approx. 20 mg) of the analyte was dissolved in phosphate-citric acid buffer (l00 mL) to obtain an approximately 200 ppm solution of the penicillin drug, which was filtered, if necessary. The above procedure was then applied.

RESULTS AND DISCUSSION

Azure A is an important thiazine dye. It is soluble in water, forming a blue solution, and is almost insoluble in chloroform. A detailed investigation of the reaction between azure A and penicillins in various buffer media showed that azure A reacts with penicillins quantitatively in disodium hydrogen phosphate-citric acid buffer at room temperature, forming a blue ion-pair that can be extracted into chloroform phase. The color of the chloroform extract shows no loss of absorbance even after several days of standing.

To establish the optimal pH range necessary for the reaction, penicillin was allowed to react with azure A in aqueous solutions buffered to pH 0.7 - 8.0 and the complex formed was extracted into chloroform for measurement. Sulfuric acid was used for adjustment to below pH 2.2 and Mcllvaine buffers were used for pH values in the range 2.2 - 8.0. Constant absorbances were obtained over the pH range 2.6 - 6.8. The decrease in absorbance below pH 2.6 could be attributed to a decrease in the concentration of free penicillin ion caused by protonation. The increased absorbance above pH 6.8 was caused by the formation of an extractable azure A species. In all subsequent work, a pH of 4.8 was used.

Spectral Characteristics

The absorption spectra of the azure A-penicillin ion pairs extracted into chloroform showed that the maximum absorption (λ_{max}) of the blue ion pairs was at 650-670 nm. The absorption spectrum of the reagent blank under similar conditions showed little absorption at and around the λ_{max} and, therefore, the analytical conditions were excellent. All subsequent studies were carried out at 660 nm. The λ_{max} did not shift with a change in buffer composition at a given pH, when acetate and Mcllvaine buffers were tested.

The effect of reagent concentration was investigated by measuring at 660 nm the absorbance of chloroform extracts obtained by the reactions of 40 μg/mL of sodium penicillin G or 25 μg/mL of potassium penicillin V with various amounts of azure A. A 3-fold molar excess of azure A was required for maximum absorbance for both the penicillins. Therefore, 2 mL of 0.05% reagent solution sufficed for less than 80 μg/mL of sodium penicillin G or 55 μg/mL of potassium penicillin V. The Job's method of continuous variations (ll) indicated that a single l:l ion pair species was extracted.

Optimum extraction conditions were established by treating penicillin solution containing 40 μg of penicillin with azure A as recommended and then extracting with successive l0 mL portions of chloroform. The absorbance of the organic phase after each extraction was measured against chloroform at 660 nm. The absorbance of the fourth extract was very similar to that of the reagent blank, indicating that three l0-mL portions of chloroform suffice for complete extraction. However, one l0-mL portion of chloroform was used in the recommended procedure. A test made on sodium penicillin G (40 μg/mL) with single extraction gave a mean result of 39.84 ppm with standard deviation of 0.228 (n = l0). Other organic solvents were tested, but chloroform was found to be the most suitable one.

Shaking times of 0.5 - 5 min. produced constant absorbance, and hence 2 min. shaking was used throughout. In contrast, increasing standing times (5-60 min.) after shaking produced a slight increase in absorbance for both the reagent blank and the ion pair. The reproducibility was good over a fixed period. For example, measurements on 30 μg/mL solutions after a l0-min. standing time showed standard deviations of approximately 0.26 ppm (n = l0). The absorbances of the separated extracts were, however, stable for a week in a glass-stoppered tube at room temperature.

Beer's law was obeyed over the concentration range of 3.5-75 μg/mL for sodium penicillin G and 3.0-50 μg/mL for potassium penicillin V with optimal range of 7.0-68 μg/mL for sodium penicillin G and 6.0-45 μg/mL for potassium penicillin V. The molar absorptivities ∈ were 6.74 x $l0^3$ M^{-1} cm^{-1} and 3.87 x $l0^4$ M^{-1} cm^{-1} for sodium penicillin G and potassium penicillin V, respectively, at 660 nm. For log I_0/I = 0.00l (where I_0 is the intensity of the incident light and I is the intensity of the transmitted light), the Sandell sensitivities for sodium penicillin G and for potassium penicillin V were 76 ng cm^{-2} and 24 ng cm^{-2}, respectively.

Effect of Concomitant Substances and Applications to Penicillin Drugs

The effects of some compounds that often accompany penicillin in pharmaceutical products were studied. The compounds were added to 30 μg/mL penicillin solutions and their effects on the penicillin determination were studied by the recommended procedure. The tolerance limits are given in Table l. 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) compared favorably with the quoted values, and with those obtained using the standard method of British Pharmacopoeia (l2).

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Table 1. Effect of concomitant substances on the determination of penicillins (penicillin taken, 30 μg/mL).

Compound Added	Tolerance Limit* (μg/mL)	
	Sodium penicillin G	Potassium penicillin V
Ascorbic acid	150	150
Benzoic acid	300	350
Citric acid	5000	5000
4-Hydroxybenzoic acid	425	400
Sorbic acid	550	600
Stearic acid	1000	950
Tartaric acid	9000	9500
Lactose	5500	6000
Maltose	7500	7500
Sucrose	10000	10000
Barbitone	2500	2800
Gelatin	6000	6500
Gum cilacia	10000	10000
Reserpine	400	400
Sodium alginate	950	1000
Sodium bicarbonate	1750	2000
Starch	5000	5000
Talc	5600	5600

*Amount causing an error of ±2%

Table 2. Determination of penicillins in commercial pharmaceutical preparations.

Preparation	Percent Recovery[a] (S.D.)	
	B.P. Method[b]	Proposed Method
Potassium penicillin V[c]		
(125 mg/tab)	102.3 (1.2)	102.8 (1.3)
(250 mg/tab)	98.8 (0.8)	99.2 (0.6)
Sodium penicillin G[c]		
(500,000 U/vial)	98.2 (1.1)	98.4 (1.4)
(1,000,000 U/vial)	101.6 (0.8)	101.4 (0.6)
Sodium penicillin G[d]		
(200,000 U/vial)	102.4 (0.6)	102.0 (0.8)
(500,000 U/vial)	96.8 (1.5)	97.2 (1.4)
(1,000,000 U/vial)	101.2 (1.2)	100.6 (1.2)

[a]Mean of ten determinations with standard deviation (S.D.).
[b]British Pharmacopoeia (1973).
[c]Marketed by Hindustan Antibiotics, Ltd.
[d]Marketed by Squibb.