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Short Communication

Spectrophotometric determination of amoxycillin and its dosage forms

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Introduction

Amoxycillin is a broad-spectrum antibiotic. Various methods for its analysis have been reported which include volumetric [1–3], UV-spectrophotometric [4, 5], colorimetric [6, 7], fluorimetric [8], polarographic [9], HPLC [10] and microbiological [11] techniques. Most of these methods are non-specific and time-consuming. A simple, time-saving and sensitive method for the assay of amoxycillin and its dosage forms is desirable.

In the present communication a new spectrophotometric method for the determination of amoxycillin and its dosage forms is reported.

Experimental

Instruments

Absorbance measurements were made on a Beckman Model 25 spectrophotometer using 1-cm quartz cuvettes. pH measurements were made with a Systronic digital pH meter, model 355, equipped with glass and calomel electrodes.

Materials and reagents

All chemicals were of Analar grade (BDH) unless otherwise specified.

Amoxycillin trihydrate BP and cloxacillin sodium BP were used. The dosage forms of amoxycillin were obtained locally.

A 0.005 M solution of amoxycillin was prepared by dissolving the requisite amount (210 mg) of the drug in distilled water and diluting to 100 ml with the same solvent. A freshly prepared solution was used.

The reagent solution was prepared by mixing freshly distilled acetylacetone (7.8 ml) and formaldehyde (15.0 ml) with the requisite amount of the appropriate sodium acetate–acetic acid buffer. The solution was kept at 100°C for 5 min, cooled, and the pH was adjusted to 4.3. The solution was diluted to 100 ml with distilled water. The reagent was freshly prepared.

Procedures

Preparation of calibration curve. One millilitre of a solution containing 2.1 mg ml^{-1} of amoxycillin trihydrate was mixed with 4.0 ml of the reagent solution in a 25-ml volumetric flask. The solution was kept at 35 ± 1°C for 30 min and diluted to 25 ml with water. The absorbance of the yellow solution was measured against a reagent blank at 400 nm. A calibration curve was plotted in the concentration range of 10–100 mcg ml^{-1} of amoxycillin.

Analysis of amoxycillin powder. Amoxycillin trihydrate (*ca* 200 mg) was weighed accurately, dissolved in water and diluted to 100 ml. The solution (0.5–1.5 ml) was mixed with the reagent solution (4.0 ml); the solution was kept at 35°C for 30 min and diluted to 25 ml with water. The absorbance of the solution was measured at 400 nm against a reagent blank.

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Table 1
Determination of amoxycillin as amoxycillin trihydrate and in pharmaceutical formulations

Drug/dosage form	Label claim*	Found† (% ±SD)	
		Official method‡	Proposed method
Amoxycillin trihydrate			
A		100.28 ± 0.60	100.50 ± 0.65
B		99.86 ± 0.40	99.46 ± 0.42
Tablets			
A	125 mg/tab	101.80 ± 0.45	101.60 ± 0.38
B		102.73 ± 0.58	102.08 ± 0.50
Capsules			
A	250 mg/cap	101.88 ± 0.39	102.00 ± 0.36
B	250 mg/cap	101.16 ± 0.31	101.22 ± 0.25
C	500 mg/cap	99.05 ± 0.49	99.20 ± 0.38
D	500 mg/cap	100.21 ± 0.42	100.20 ± 0.31
Injection			
A	100 mg/vial	105.00 ± 0.38	104.00 ± 0.35
B	250 mg/vial	100.86 ± 0.30	100.80 ± 0.21
C	250 mg/vial	100.85 ± 0.31	100.81 ± 0.25
Oral suspension			
A	125 mg/5 ml	104.80 ± 0.20	104.04 ± 0.18
B	125 mg/5 ml	105.24 ± 0.27	105.32 ± 0.23
C	250 mg/5 ml	106.82 ± 0.48	107.12 ± 0.40
Paediatric drops			
A	100 mg/ml	102.00 ± 0.30	102.40 ± 0.21
Amoxycillin–cloxacillin capsules	250 mg/cap	100.80 ± 0.58	101.12 ± 0.49
Amoxycillin content	250 mg/cap		

*Equivalent to amoxycillin anhydrous.
†Mean of five determinations.
‡From refs 13 and 14.

The amount of amoxycillin trihydrate was calculated by reference to the calibration curve (Table 1).

Analysis of amoxycillin tablets. Twenty tablets were weighed and powdered. The powder equivalent to *ca* 200 mg of amoxycillin was weighed accurately and extracted with water (3 × 20 ml) by stirring. Each extract was filtered through Whatman No. 41 filter-paper and the residue was washed thoroughly with water. The filtrate and washings were combined in a 100-ml volumetric flask and diluted to 100 ml with water. An aliquot (0.5–1.5 ml) of the solution was analysed as described under Analysis of amoxycillin powder (Table 1).

Analysis of amoxycillin capsules. The contents of twenty capsules were mixed and weighed. The powder equivalent to *ca* 200 mg of amoxycillin was analysed as described under Analysis of amoxycillin tablets (Table 1).

Analysis of amoxycillin injection. The contents of 10 vials of amoxycillin injection were mixed and weighed. The powder equivalent to *ca* 200 mg of amoxycillin trihydrate was analysed as described under Analysis of amoxycillin powder (Table 1).

Analysis of amoxycillin oral suspension (or paediatric drops). The powder equivalent to *ca* 200 mg of amoxycillin trihydrate was analysed as described under Analysis of amoxycillin tablets (Table 1).

Analysis of amoxycillin trihydrate in combination with cloxacillin sodium and excipients in synthetic mixtures. Synthetic mixtures of amoxycillin trihydrate with cloxacillin sodium as well as with common excipients, in the proportions usually encountered in the formulation of dosage forms, were prepared in the laboratory. The mixture equivalent to *ca* 200 mg of amoxycillin was analysed as described under Analysis of amoxycillin tablets (Table 2).

Analysis of amoxycillin–cloxacillin capsules. These capsules were analysed as described under Analysis of amoxycillin capsules (Table 1).

Table 2
Analysis of amoxycillin* in synthetic mixtures

Other drug/excipients (mg)		% Recovery† of amoxycillin
Cloxacillin sodium		
	250	100.02
	500	99.84
	750	99.49
	1000	99.82
	1500	99.60
Excipients		
Starch	1000	99.69
Dicalcium phosphate	1000	99.81
Talc	1000	100.02
Magnesium stearate	1000	99.95
Lactose	1000	99.34

*Amoxycillin trihydrate (500 mg).
†Mean of five determinations.

Results and Discussion

Absorption spectra

The yellow reaction product of amoxycillin with the acetylacetone–formaldehyde reagent had a maximum absorbance at 398–402 nm; hence all measurements were made at 400 nm. The molar absorptivity was 2.74×10^3 l mol^{-1} cm^{-1}. The Sandell's [12] sensitivity was 0.14 mcg cm^{-2}.

Effect of pH

The colour development of amoxycillin with the acetylacetone–formaldehyde reagent prepared in acetate buffer (pH range 3.5–5.8) was studied. The absorbance of the coloured product was maximum at pH 4.1–4.4. All subsequent measurements were made at pH 4.3.

Effect of reagent concentration

The effect of reagent concentration on the colour intensity was studied. It was found that 3.5–4.0 ml of the reagent was sufficient for maximum colour development. At higher concentrations the colour intensity remained constant.

Effect of temperature and time of reaction

The development of colour of amoxycillin with the acetylacetone–formaldehyde reagent was studied at various temperatures. It was found that the yellow product had a maximum absorbance when the contents were kept at 30–37°C for 30 min. However, the colour intensity was decreased when the solution was heated above 45°C. A reaction time of 30 min was sufficient for colour development.

Beer's law

The yellow reaction product of amoxycillin and the acetylacetone–formaldehyde reagent obeyed Beer's law at 400 nm in the concentration range of 10–100 mcg ml^{-1} of amoxycillin. The regression equation was $y = 0.5037x + 0.0443$ (correlation coefficient = 0.9989).

Accuracy of results

To evaluate the accuracy of the results recovery experiments were carried out. The results were compared with those obtained by the BP method [13, 14]. The accuracy of the present method was relatively greater than that of the BP method. Moreover, the present method can be used even for very low concentrations of amoxycillin, thus giving a wide concentration range for determination of the drug. The results are presented in Table 1.

Application of the method

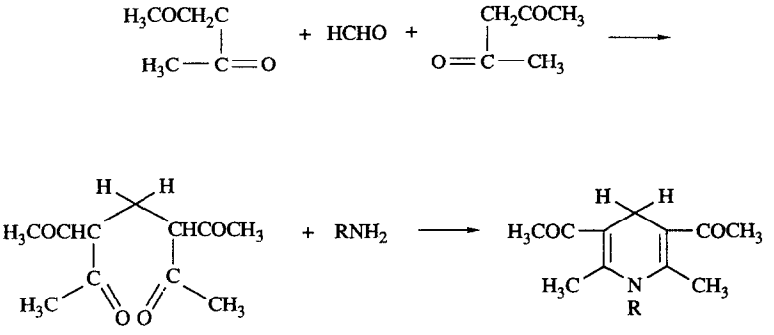
In order to test the suitability and specificity of the present method, amoxycillin was determined in synthetic mixtures containing cloxacillin sodium and commonly used excipients. The results in Table 2 show that neither the excipients nor cloxacillin sodium interfered with the determination.

The proposed method was successfully applied to the determination of amoxycillin in pharmaceutical preparations. The results are presented in Table 1.

The proposed procedure can detect impurities or distinguish cross-contamination from non-amino β-lactam penicillins. The procedure is not stability-indicating since it cannot detect the degradation products of amoxycillin.

Reaction mechanism

Based on published work and the findings of the present investigation, the following scheme is proposed for the formation of the chromogen by the reaction of acetylacetone and formaldehyde with amoxycillin (RNH_2):



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