Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, No. 1, pp. 43–47, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00

Determination of ampicillin in the presence of cloxacillin

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Abstract: A spectrophotometric method is described for the assay of ampicillin in the presence of cloxacillin in pharmaceutical preparations. It involves the reaction of hydrolysed ampicillin with formaldehyde in an acidic phosphate buffer (pH 2.5) and measurement of the absorbance of the product at 373 nm after dilution with 2 M sulphuric acid. The method is selective for ampicillin in the presence of cloxacillin. The absorbance had a linear relationship with concentration for the range studied (5–35 μg ml^{-1}). By this method the ampicillin content of combined preparations of ampicillin and cloxacillin has been successfully determined in capsules, injections and a syrup.

Keywords: *Ampicillin; cloxacillin; combined preparations; capsules; injections; syrups.*

Introduction

Because of the similarity in the structures of ampicillin and cloxacillin (due to the presence of β-lactam and thiazolidine rings), a method for their analysis in combined preparations must be designed to achieve a high degree of selectivity. Many analytical methods have been reported [1–11] but only the liquid chromatographic methods are selective. Although the difference spectrophotometric method of Davidson and Stenlake [12] is applicable to combined preparations, the λ_{max} of 268 nm where the absorbance of ampicillin is measured is subject to interference from cloxacillin and additives in pharmaceutical preparations. The British Pharmacopoeia [13] gives a difference spectrophotometric method for the assay of ampicillin, cloxacillin and amoxycillin at λ_{max} 325, 348 and 325 nm, respectively. The λ_{max} of ampicillin and amoxycillin are the same, that of cloxacillin being only slightly different; thus there is a possibility of interference. In the present paper, a spectrophotometric method is reported for the determination of ampicillin in the presence of cloxacillin.

Experimental

Reagents

Standard ampicillin trihydrate and cloxacillin sodium were obtained from Beecham Research Laboratories, UK. Phosphate buffers of pH 2–8 were prepared according to the British Pharmacopoeia [13].

Reaction of hydrolysed ampicillin with formaldehyde in phosphate buffer (pH 2.5)

One hundred milligrams of ampicillin trihydrate in water was hydrolysed with 20 ml of 2 M sodium hydroxide. After 20 min, 10 ml of 2 M sulphuric acid and 100 ml of formaldehyde–phosphate buffer (pH 2.5) (1.5:98.5, v/v) were added. The resulting mixture was heated on a water-bath for about 1 h. The mixture was extracted with chloroform (3 × 30 ml) and the solvent was removed *in vacuo*; the residue was washed with water and dried over magnesium sulphate to give yellow crystals. Recrystallization with chloroform–petroleum ether (60:40, v/v) gave pure samples of melting-point 199–202°C. The UV absorption spectrum of a methanolic solution of the solid showed maxima at 344 nm and about 370 nm when diluted with 2 M sulphuric acid. The IR spectrum of the product showed peaks: 1580 cm^{-1} (C=C, aromatic, conjugated double void); 1618 cm^{-1} (C=N); and 1655 cm^{-1} (amide band). Proton NMR showed the following characteristics: δ 2.34 (singlet, 3H, CH_3); 7.35 (multiplet, 4H); 8.2 (doublet of doublet, 2H); 11.5 (broad peak, N—H, D_2O exchangeable).

Determination of ampicillin

Effects of temperature and time on absorbance. The effect of reaction temperature on

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absorbance was studied by carrying out the reaction above with 0.02 mg ml^{-1} of ampicillin at different temperatures (29–100°C). The absorbance was determined at 373 nm after a 1 in 2 dilution of the reaction mixture with 2 M sulphuric acid.

The heating time for the reaction mixtures was also varied between 20 and 100 min.

Calibration graph. Aliquots of a stock solution of ampicillin (1 mg ml^{-1}) were hydrolysed with either 2 ml of 2 M sodium hydroxide or 5 ml of 2 M sulphuric acid. One millilitre of 2 M sulphuric acid was added to the mixtures hydrolysed with sodium hydroxide after 20 min. Fifty millilitres of formaldehyde–phosphate buffer (pH 2.5) (1.5:98.5, v/v) was added to each aliquot and heated at 100°C for 1 h. (Solution A). Another set of aliquots corresponding to the first set were treated in the same way but without hydrolysis with either 2 M sodium hydroxide or 2 M sulphuric acid (Solution B). One in 2 dilutions of Solutions A and B were made with 2 M sulphuric acid and the absorbance of Solution A was measured at 373 nm using Solution B as reference.

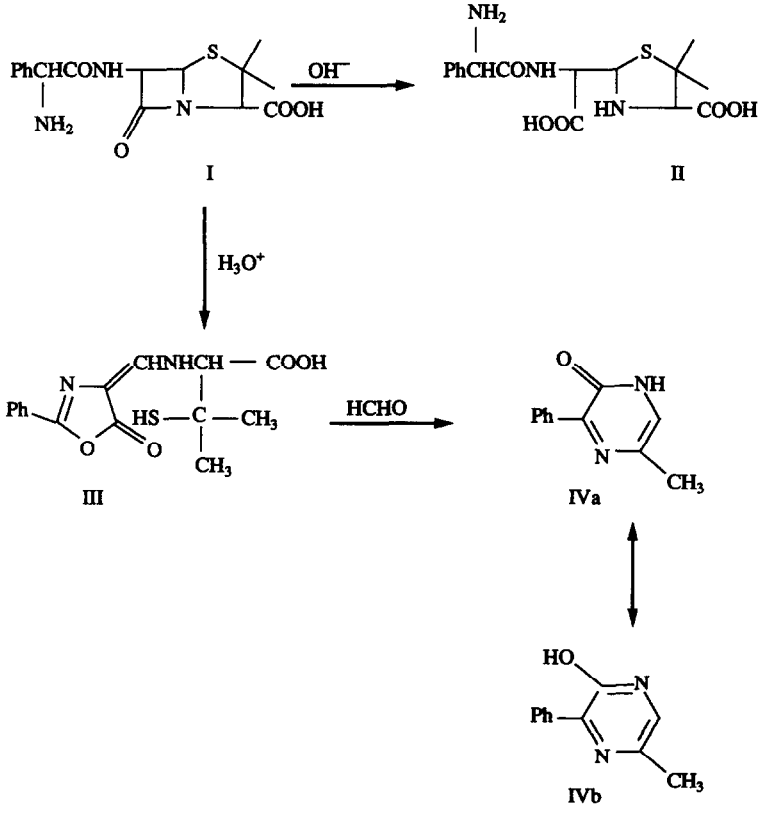
Assay of dosage forms. The assay of dosage forms (capsules, injection and syrup) was carried out with an aliquot (5 ml) equivalent to a 1 mg ml^{-1} ampicillin solution as described above.

Determination of cloxacillin

Cloxacillin in the ampicillin–cloxacillin dosage forms was determined by a modification of the method of Davidson and Stenlake [12], as follows.

To aliquots of a stock solution of cloxacillin (1 mg ml^{-1}) was added 25 ml of phosphate buffer (pH 6) and the solution was diluted to 50 ml with water. The absorbance of each solution was measured at 275 nm using a 1 in 2 dilution of phosphate buffer (pH 6) as the blank. The experiment was repeated as above but with the addition of 1 ml of ampicillin solution (4 mg ml^{-1}) to each flask before dilution to volume.

Assay of dosage forms. Dosage forms were analysed by using 5–24 ml of the equivalent of a 1 mg ml^{-1} cloxacillin solution as described above.



Scheme 1
Schematic representation of the reaction of hydrolysed ampicillin with formaldehyde–phosphate buffer (pH 2.5).

Results and Discussion

Reaction of hydrolysed ampicillin with formaldehyde in phosphate buffer

Ayim [14] reported that hydrolysed ampicillin and cephalexin can be converted into a compound with maximum absorbance at about 400 nm by reaction with formaldehyde in an acidic medium. This study has shown that both penicillenic and penicilloic acids (the acid- and base-hydrolysed products, respectively) react quantitatively with formaldehyde in an acidic medium to give a pyrazinone derivative. It was further observed that formation of the derivative could form a basis for the spectrophotometric determination of ampicillin in the presence of cloxacillin.

The reaction involves hydrolysis of the β-lactam ring to either penicillenic or penicilloic acid, followed by a rearrangement in the presence of formaldehyde to give 5-methyl-3-phenylpyrazinone (IV) as represented in Scheme 1.

The pyrazinone derivative was observed to have different wavelengths of maximum absorbance in acidic and basic media. In an acidic medium, protonation leads to bathochromic shift. The λ_{max} is 340 nm in a basic medium but 373 nm in an acidic medium (Fig. 1). Because cloxacillin has a λ_{max} about 340 nm, the λ_{max} of 373 nm in acidic medium was selected. Penicillenic acid has a λ_{max} between 320 and 340 nm which is not affected by the addition of acid or alkali; however, it does react with formaldehyde, as does penicilloic acid. Thus the presence of these breakdown products (penicillenic and penicilloic acids) in samples could interfere with the determination of ampicillin. To eliminate the contribution of the breakdown products to the absorbance, the difference spectrophotometric approach was employed. From Fig. 2 the difference spectrophotometric spectrum gave lower values of absorbance; this difference suggests the presence of the breakdown products.

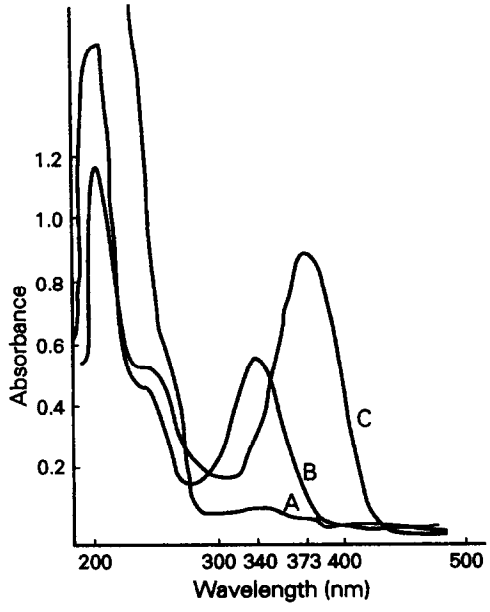


Figure 1
UV-spectrum of cloxacillin (A). UV-spectra of pyrazinone derivative (IV) in alkali (B) and acid (C).

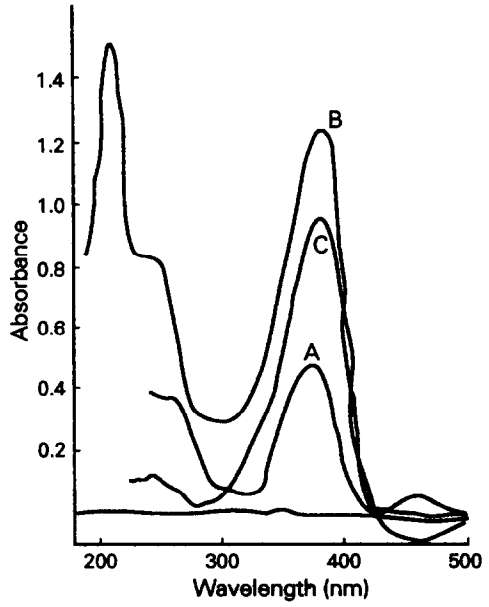


Figure 2
UV-spectra of unhydrolysed ampicillin (A) and the pyrazinone derivative (IV) in acid (B). C is the difference absorption spectrum of B relative to A.

Determination of ampicillin

The effect of temperature on reaction time showed that between room temperature (29°C) and 80°C, there was a sharp change in the rate of reaction with increase in temperature; with further increase in temperature a plateau was reached. An optimal reaction temperature of 100°C was therefore selected (Fig. 3). The effect of heating time on the formation of the product showed a gradual increase in the absorbance up to 60 min; between 60 and 100 min the absorbance remained the same (Fig. 4). Hence 60 min was selected as optimal.

The calibration graph of the absorbance of the pyrazinone derivative (IV) against concentration of ampicillin showed a good linearity and reproducibility over the range 5–35 μg

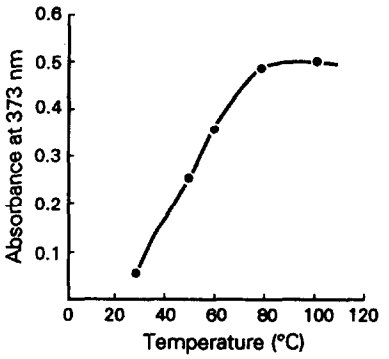


Figure 3
The effect of heating temperature on the absorbance of the ampicillin reaction mixture after heating for 1 h.

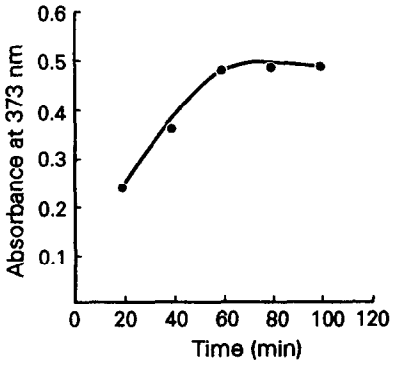


Figure 4
The effect of heating time at 100°C on the absorbance of the ampicillin reaction mixture.

ml^{-1}. The correlation coefficient was found to be 0.9999 via the penicilloic acid intermediate and was 0.9997 through the penicillenic acid intermediate.

Since the method was developed for the assay of ampicillin in combined preparations with cloxacillin, it was necessary to study the effect of the presence of cloxacillin on the absorbance of the ampicillin reaction mixture. From Fig. 1, whereas the λ_{max} of the reaction mixture at 340 nm overlaps with that of cloxacillin, the λ_{max} at 373 nm in an acidic medium does not. Moreover, the absorbance values of different concentrations of ampicillin determined at 373 nm alone were found to be similar to those determined in the presence of cloxacillin. This indicates that cloxacillin does not interfere with the absorption of the pyrazinone derivative (IV) at 373 nm.

Assay of ampicillin in dosage forms. Results of the analysed samples are presented in Table 1. The percentage contents of ampicillin were found to be within the limits specified by the British Pharmacopoeia and the United States Pharmacopeia [15] for all samples except F, which was analysed after the expiry date.

Table 1
Assay results for dosage forms

Sample	% Ampicillin content	% Cloxacillin content
A	97.4	108.7
B	97.1	110.0
C	91.9	270.0
D	96.3	99.4
E	93.1	110.0
F*	78.0	98.0

A, B and C: Ampiclox capsules, injection and syrup (Beecham Research Lab, UK). D: Preparation from standard samples. E: Ampiclox, Hermandad Pharmaceutics (Murcia, Spain). F: Cloxampi, PDC Pharma (Waterloo, Belgium).
*Sample analysed 1 month after expiry date.

Determination of cloxacillin

The spectrum of cloxacillin showed a peak at 340 nm and two shoulders at 275 and 265 nm. The peak at 340 nm was of low intensity; the shoulder at 265 nm showed high intensity but suffered from interference with the spectrum of ampicillin (Fig. 5). The shoulder at 275 nm showed a relatively high intensity with negligible interference with the spectrum of ampicillin. A calibration curve at 275 nm gave a linear

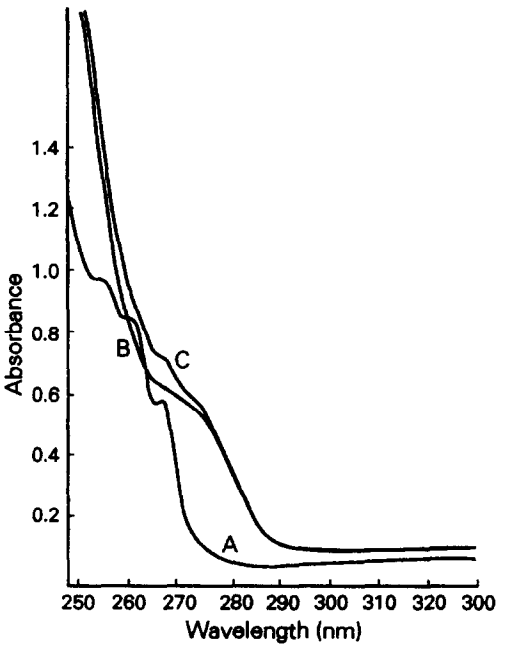


Figure 5
UV-spectra of ampicillin (A), cloxacillin (B) and cloxacillin + ampicillin (C) in phosphate buffer (pH 6).

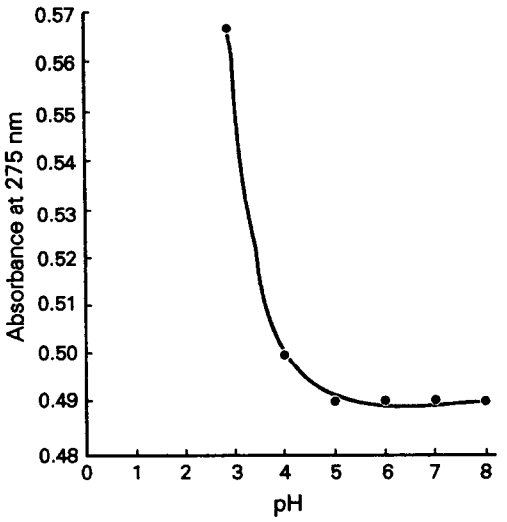


Figure 6
The effect of pH on the absorbance of cloxacillin.

plot in the range 0.11–0.6 mg ml^{-1}. The correlation coefficient was 0.9992.

The effect of pH on the absorbance of cloxacillin at 275 nm showed a sharp fall in absorbance with an increase in pH from 3 to 5; between pH 5 and 8 the absorbance was constant (Fig. 6). pH 6 was therefore selected as optimal.

Assay of cloxacillin in dosage forms. The percentage contents cloxacillin in the analysed samples were found to be within the limits specified in either the British Pharmacopoeia or United States Pharmacopeia except one of the syrup (C) which had an apparent content of over 270%. This was attributed to the presence of such additives as benzoic acid or *p*-hydroxybenzoic acid derivatives which could absorb radiation near 275 nm and thereby interfere with the absorbance of cloxacillin. Extraction of acidified syrup (C) with chloroform prior to analysis resulted in a reduction in the apparent percentage content (180%). It appears that other excipients in the syrup formulation interfere with the λ_{max} of cloxacillin at 275 nm. This is an indication that the method of Davidson and Stenlake [12] is limited in its application; the sensitivity of that method is low (0.11–0.6 mg ml^{-1}) although adequate for assay of some dosage forms.

Acknowledgement — The authors are grateful to Beecham Research Laboratories (UK) for the gift of standard ampicillin and cloxacillin samples, and to Mr T.O. Dawodu for technical assistance.

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[Received for review 8 October 1990; revised manuscript received 18 March 1991]