Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, No. 2, pp. 187–190, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

Short Communication

Spectrophotometric determination of amoxycillin and dicloxacillin in binary mixtures and in capsules

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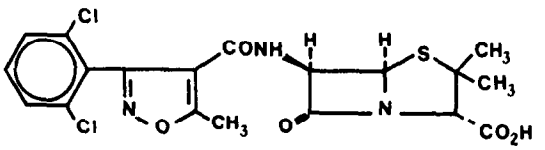
Keywords: *UV-spectroscopy; first-order derivative; amoxycillin; dicloxacillin.*

Introduction

Penicillins in combinations provide a broader spectrum of antibacterial activity and may be advantageously prescribed in cases of β-lactamase-producing strains. Synergistic activity of some penicillin combinations was reported for gram-negative bacteria [1–4]. Synergy between amoxycillin (**I**) and dicloxacillin (**II**) has been demonstrated against clinical isolates of some β-lactamase-producing and non-producing strains [5, 6]. Pharmaceutical preparations containing both the mentioned β-lactam antibiotics are now normally found on the drug market.

There have been various different analytical procedures proposed for the determination of amoxycillin, including spectrophotometric measurement of the copper chelate [7] or the penicillenic acid formed by reaction with mineral acids and copper(II) [8], spectrofluorometric (EX: 366 nm, EM: 430 nm) determination after reaction with formaldehyde and high-performance liquid chromatography (HPLC) [9], which is also the method of choice for dicloxacillin [10]. The British Pharmacopoeia 1988 [11] recommends a titrimetric procedure for amoxycillin in bulk form using standard mercury(II) nitrate and potentiometric detection of the equivalent complex, and a colorimetric one using immidazole mercury reagent at 325 nm in the case of capsules containing the drug. No official procedure has yet been approved for dicloxacillin, although the method described for amoxycillin seems to be applicable. In combination of the two penicillins, the determination of the component amoxycillin by adopting the BP 1988 procedure cannot be achieved. No analytical method has yet been investigated for simultaneous determination of both penicillins in combination.

The present communication describes a spectrophotometric method for simultaneous determination of amoxycillin and dicloxacillin in pharmaceutical preparations. The method is sensitive and selective for each drug and has the clear advantage of being direct, simple and rapid.



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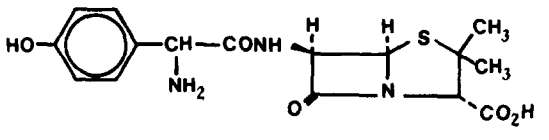

I

Experimental

Apparatus

A UV–vis DMS 90 double-beam spectrophotometer with matched 1 cm quartz cuvettes was attached to a Hewlett–Packard 7015B *X–Y* chart recorder.

Materials

Amoxycillin trihydrate. The drug was used as supplied (Gist-Brocades, Amsterdam, The

Netherlands). Purity was assessed by the BP 1988 method [11] and found to be 99.1% (moisture content 12.6%).

Dicloxacillin monohydrate. The drug was utilized without further treatment, the claimed purity was 99.6% (moisture content 3.9%) (Gruppo Lepetit S.p.A., Milan, Italy).

Water was freshly doubly-distilled.

Standard and sample solutions

The stock solutions (0.1%, m/v) of amoxycillin trihydrate or dicloxacillin monohydrate were prepared in water; the solutions were kept below 5°C in a refrigerator for no longer than 5 days.

The working solutions (0.002–0.005%, m/v) of each drug were prepared by making the required dilutions in water.

The sample solutions were prepared by weighing the contents of at least 10 capsules and mixing homogeneously. Aliquots of the powder equivalent to 20–50 mg of both amoxycillin trihydrate and dicloxacillin monohydrate were transferred into 100-ml calibrated flasks, dissolved in about 70 ml water by mechanical shaking for about 2–3 min and the volume completed with water. The contents of each flask were filtered into dry containers using dry filter paper and a further 10-times dilution of the clear filtrates was made to reach the final concentrations of 20–50 μg ml^{-1}, i.e. 0.002–0.005% (m/v).

UV-measurements

The D_1-curves of the working aqueous solutions containing varying amounts of each drug were scanned in the range 350–200 nm against water as a blank. The values at 234 nm for amoxycillin and at 225 nm for dicloxacillin were determined and the concentration vs their d_1A-values were plotted, in order to obtain the calibration curve. To determine the contents of amoxycillin and dicloxacillin in capsules, the d_1A values of the prepared aqueous extracts at 234 and 225 nm for each drug were measured against water. The amount of each drug was calculated as follows:

Results and Discussion

The British Pharmacopoeia procedure [11] for the determination of amoxycillin in capsules was applied to specimen capsules from local sources. The products from the reaction of amoxycillin and dicloxacillin in the formulated dosage, with the imidazole–mercury reagent, exhibit overlapping maxima at wavelengths specified for drug determination.

The UV spectrum (200–350 nm) of aqueous solutions of amoxycillin shows absorption maxima at about 227 and 274 nm. On the other hand, aqueous solutions of dicloxacillin exhibit typical maxima at about 205, 275–280 nm. The absorption spectra and their first derivatives for aqueous solutions of amoxycillin, dicloxacillin and their equimixture are shown in Fig. 1. Spectroscopic data for aqueous solutions of each drug are given in Table 1. Accurate absorption measurements of each drug in binary mixtures appears to be quite impossible because of the band overlap. However, the band overlap might be solved by Vierordt's simultaneous equation method [12], although this may be influenced by differences between the sample and reference, or by the matrix in the pharmaceutical formulations, leading to erroneous results [13]. Preliminary separation of amoxycillin and dicloxacillin by dissolving in 0.1 M hydrochloric acid {only amoxycillin goes into solution leaving dicloxacillin, followed by adopting the iron(III) hydroxamate method [14]} is recommended by the manufacturer for the determination of each of the separated penicillins [15]. Derivative spectrophotometry is useful for dealing with such problems [16–18]. The first-derivative, D_1, of the dicloxacillin spectrum shows a characteristic trough at 225 nm, where d_1A, i.e. $dA/d\lambda$, for amoxycillin is zero, so dicloxacillin can be specifically measured at that wavelength. Amoxycillin has its typical trough at 234 nm, but dicloxacillin has also some $dA/d\lambda$ reading. The absorption due to dicloxacillin at 234 nm in mixtures with amoxycillin can be easily treated in the calculation by considering the relative D_1 response at this wavelength to that of the same drug at

$$\text{Amoxycillin (mg \%)} = \frac{(d_1A_{234}\ \text{amox} - 0.448\ d_1A_{225}\ \text{diclox}) \times 1000}{15.02}\ \text{(as trihydrate)},$$

$$\text{Dicloxacillin (mg \%)} = \frac{(d_1A_{225}\ \text{diclox}) \times 1000}{23.36}\ \text{(as monohydrate)}.$$

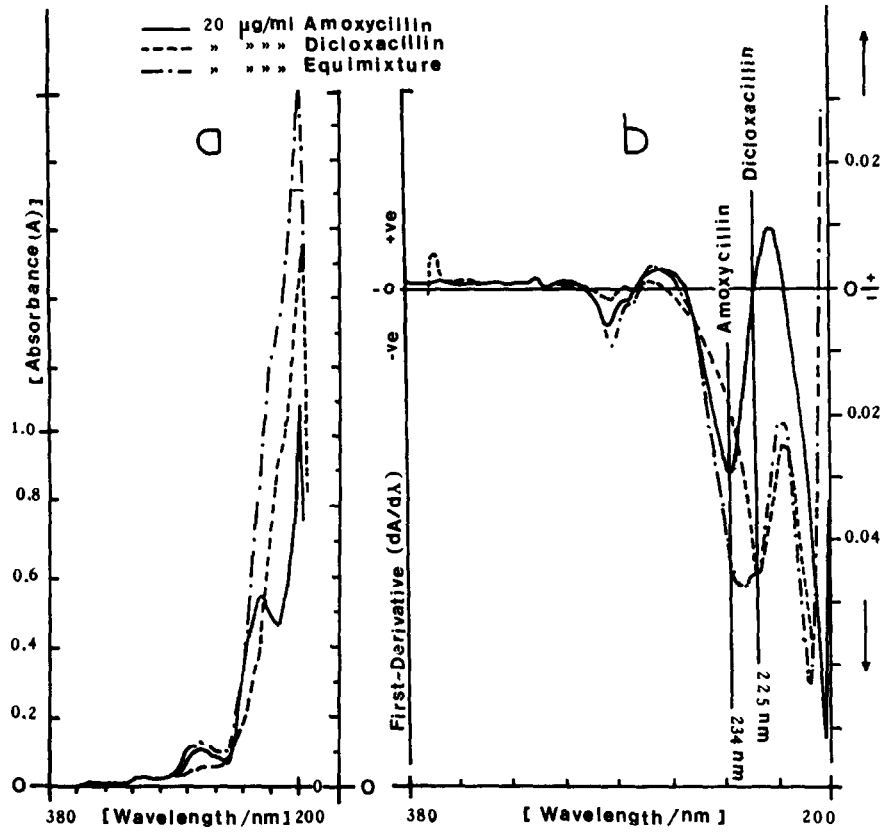

Figure 1
UV-scanning: (a) zero-order, D_0; (b) first-derivative, D_1, of aqueous 20 µg ml^{-1} solutions of amoxycillin trihydrate, dicloxacillin monohydrate and an equimixture.

Table 1
Spectrophotometric data for amoxycillin and dicloxacillin solution in water*

Substance	λ (nm)	ε(1 $mol^{-1}cm^{-1}$)	A(1%, 1 cm)	λ (nm)	$d_1\epsilon$(1 $mol^{-1}cm^{-1}$)	d_1A(1%, 1 cm) [(dA/dλ)1%, 1 cm]
Amoxycillin	205	27425	654.0	225	—	zero
	227	10967	261.5	234	630	15.02
	274	1908	45.5			
Dicloxacillin	205	43056	915.5	225	1099	23.36
	275	1787	38.0	234	493	10.47
	280	1646	35.0			

*The values given will vary according to the instrument used and are only for comparative purposes.

225 nm which equals 0.448 (SD = 0.005, n = 5). Other components of the pharmaceutical preparations studied show no absorption at the selected wavelengths. The recovery by adopting the derivative spectrophotometric procedure was tested by adding known amounts of amoxycillin and dicloxacillin reference materials to the formulated dosage. The results for the assay and recovery of each drug in capsules are given in Table 2. Good percentage mean recoveries were obtained for each drug as indicated by the low SD and relative standard deviations, good correlation coefficients (r) of 0.9998 and 0.9999 were also obtained from the prepared calibration curves of amoxycillin and dicloxacillin, respectively.

Table 2
Assay and recovery of amoxycillin and dicloxacillin in capsules*

	Amoxycillin†	Dicloxacillin
Assay (%)	106.1	107.7
	106.9	106.1
	105.4	105.7
	106.2	106.0
	107.2	106.3
	105.8	105.7
	106.9	106.1
	107.2	106.7
	—	105.9
X (SD)	106.46 (0.68)	106.24 (0.63)
RSD (n)	0.64 (8)	0.59 (9)
Recovery (%)	99.4	100.2
	99.8	100.0
	100.1	100.2
	100.6	99.7
X (SD)	99.89 (0.51)	100.03 (0.24)
RSD (n)	0.51 (4)	0.24 (4)

*Miclox 250® capsules are products of Misr Co. for Pharm. Ind., S.S.A., El-Mataria, Cairo-ET, BN: R/25, each capsule contains 125 mg of amoxycillin trihydrate and 125 mg of dicloxacillin monohydrate.

†d_1A 234 nm/225 nm = 0.448 (SD = 0.005, n = 5) for dicloxacillin.

Acknowledgements — The author would like to thank Dr S.A. Ismaiel, Research Directorate, Misr Co. for Pharm. Ind., S.A.A., El-Mataria, Cairo-ET, for providing reference amoxycillin trihydrate and dicloxacillin monohydrate. Thanks are also due to Mr N.A. Khattab for technical assistance.

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[*Received for review 22 January 1990*]