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Circular dichroism spectroscopy: An efficient approach for the quantitation of ampicillin in presence of cloxacillin

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ABSTRACT

Ampicillin exhibited a negative and a positive cotton effects on the circular dichroism (CD) spectra in the wavelength range of 200–280 nm. Cloxacillin showed a positive cotton band peaking at 228 nm. Three sensitive, precise and accurate CD spectroscopic methods have been developed for the determination of ampicillin and cloxacillin. Method A was used for the determination of ampicillin in presence of cloxacillin by measuring ellipticity at 206 nm. Method B and C were employed to determine ampicillin and cloxacillin based on evaluation of ellipticity at 233 nm and 228 nm, respectively. Methods A, B and C showed linearity in the concentration range of 10–40 μg mL^{-1}, 5–40 μg mL^{-1} ampicillin and 10–80 μg mL^{-1} cloxacillin, respectively. The method A was successfully applied to the determination of ampicillin in commercial dosage forms containing equivalent amount of cloxacillin.

1. Introduction

Ampicillin, [(2S,5R,6R)-6-[[(2R)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3,2,0] heptanes-2-carboxylic acid], is an acid-stable semisynthetic β-lactam antibiotic that inhibits bacterial cell wall synthesis by binding to peptidoglycan synthesizing enzymes (Fig. 1) [1,2]. It has a broad spectrum of antimicrobial activity and it is widely used against gram-positive and gram-negative organisms as well as for enterococcal infections resistant to penicillin G. It is also useful in the prophylaxis and treatment of exacerbations of chronic bronchitis. It is ineffective against penicillin-resistant staphylococci [3]. Ampicillin is a highly recommended therapy in clostridia infections in chicken [4]. However, the dose adjustments are suggested during oral administration in chicken infected with coccidiosis. In aqueous solution ampicillin exists mainly in three different forms [5]: cation ($AmpH_2^+$) in acidic medium, zwitterion at neutral pH and anion in basic medium. The –COOH and $-\overset{+}{N}H_3$ groups of $AmpH_2^+$ are successively deprotonated in the pH ranges 2–4 ($pK_1 = 2.55$) and 6–8 ($pK_2 = 7.05$), respectively [6].

Cloxacillin, chemically designated as monosodium (2S,5R,6R)-6-[o-(2-chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3,2,0]-heptanes-2-carboxylate monohydrate, is a semisynthetic beta-lactamase resistant antibiotic (Fig. 2). It is monobasic penicillin which has dissociation constant (pK_a) of 2.7 [7]. The presence of unstable four-term ring in the beta-lactam moiety makes cloxacillin prone to degradation by heat and/or in presence of alcohols and water [8,9]. It is widely used in non-methicillin resistant *Staphylococcus aureus* infections. There are some side effects of cloxacillin which include gastrointestinal manifestations like vomiting or diarrhea and different hypersensitivity reactions [10,11].

The quality assurance and quality control of active pharmaceutical ingredients and excipients is imperative in order to achieve better remedial effect and a lower toxicity. It is, therefore, necessary to develop simple, sensitive and efficient methods for determination of ampicillin and cloxacillin in pharmaceutical preparations. With increasing regulatory strictness on the control of drugs, variety of analytical methods has been developed to monitor impurities and degradation products in pure and commercial dosage forms [12–14]. It is noteworthy that several attempts have been made for the quantification of ampicillin and cloxacillin either individually or in combination. High performance liquid chromatography has been used most frequently for analyzing ampicillin and cloxacillin in different matrices [15–22]. Capillary electrophoresis [23,24] has played a significant role in the analysis of β lactam antibiotics. Other analytical methods reported in the literature include spectrofluorimetry [25], spectrophotometry [26–31], FT-IR [32], potentiometry (ISE) [33,34], and voltammetry [35].

Circular dichroism spectroscopy is an excellent technique with a high degree of analytical selectivity and hence, it can be used for direct determination of optically active drugs. CD has two major advantages. (i) It is extremely sensitive to absolute configuration as well as to conformational features, which are often completely obscured in ordinary absorption spectrum [36], and (ii) wide range of solvents can be used to study with relatively much smaller sample amounts. The degree of

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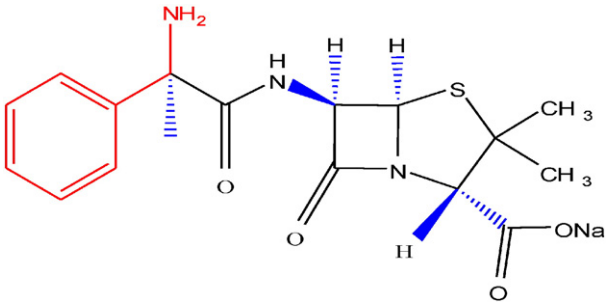

Fig. 1. Chemical structure of ampicillin.

selectivity is sufficient to determine enantiomers in mixtures without any chromatographic separation or any other kind of workup, except a simple extraction into a recommended solvent system. The β-lactam antibiotics such as cefoxitin, cefuroxime and cefotaxime have been determined by CD provided the optically active drugs have cotton effects at wavelength above 250 nm in human serum and/or plasma [37]. The applicability of CD to the direct quantitative determination of β-lactam antibiotics in the voided urine of patients under multiple drug administration has also been demonstrated [38]. The circular dichroism spectra of 15 commercial cephalosporins in common clinical use have been analyzed. On the basis of CD spectra, it was possible to discriminate among the cephalosporin homologs [39]. CD spectra have been characterized for nine penicillins and three cephalosporins dissolved in aqueous pH 5.4 buffer solutions. The discriminations among the penicillin homologs are not possible from the spectral data. The direct determination of penicillin V in laboratory prepared mixtures was accomplished after extraction into the aliquots of the buffer solution [40].

In this study, CD spectra of ampicillin and cloxacillin dissolved in distilled water have been recorded in the wavelength range 200 to 280 nm. A negative band with maximum at 206 nm was observed in the CD spectrum of ampicillin only. Therefore, analytical methods were proposed for quantitation of (I) ampicillin, (II) cloxacillin, and (III) ampicillin in presence of cloxacillin.

2. Experimental

2.1. Instrumentation

CD spectra were recorded at 25 °C on a JASCO J-815 CD spectrometer (Jasco Corporation; Tokyo, Japan) equipped with a 150 W Xenon lamp, 2.0 mm quartz cuvette and temperature control unit. Instrument

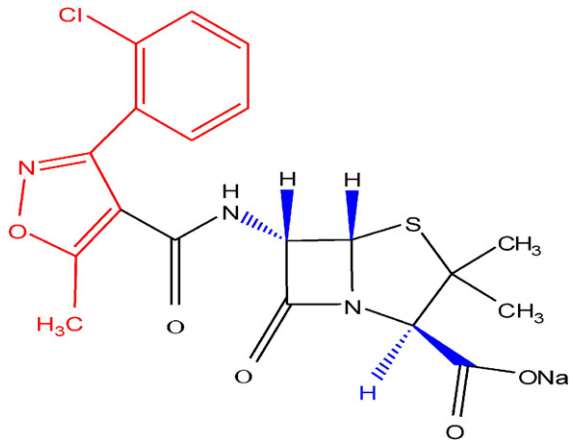

Fig. 2. Chemical structure of cloxacillin.

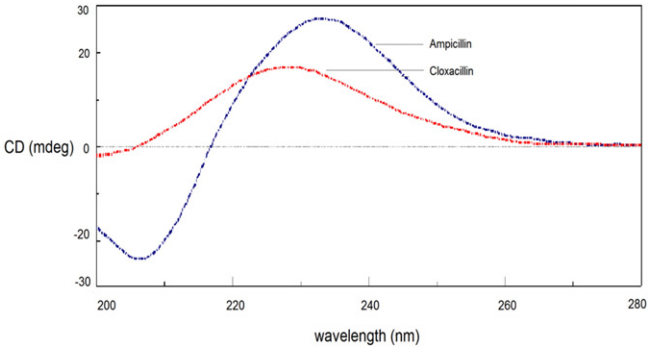

Fig. 3. CD spectra of ampicillin and cloxacillin (dissolved in distilled water). (ampicillin: 100 μg mL^{-1}; cloxacillin: 100 μg mL^{-1})

parameters were as follows: bandwidth of 2 nm, response time of 1 s, standard sensitivity, wavelength range of 400 to 200 nm, scanning rate of 50 nm/min and four scan accumulations (averaged at the end).

2.2. Reagents

Pharmaceutical grade ampicillin was purchased from Sigma (Sigma Chemical Co. USA) while cloxacillin was obtained from Biochem Pharmaceutical Industries Ltd., Mumbai. All the standard chemicals were used without further purification. The three commercial formulations of the drug containing ampicillin and cloxacillin were purchased from different local pharmacies. Roscillin (Ranbaxy Laboratories Ltd., Barotiwala, H.P.) is labeled to contain 500 mg of ampicillin per tablet while megapen (Aristo Pharmaceuticals Ltd., Andheri, Mumbai) and ampoxin (Unichem Laboratories Ltd., Mumbai) are labeled to contain 250 mg of ampicillin and 250 mg of cloxacillin per tablet of each brand. Bioclox (injection) contains 500 mg of cloxacillin per vial.

2.3. Preparation of stock solutions

i. 0.01% ampicillin was prepared in distilled water.
ii. 0.01% cloxacillin was also prepared in distilled water.

Working solutions of variable concentrations were prepared by progressive dilutions of standard stock solutions.

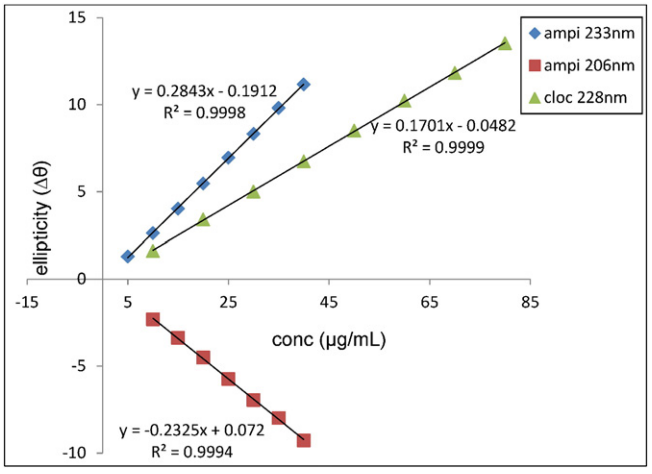

Fig. 4. Calibration plots for determination of ampicillin (A: 206 nm, B: 233 nm) and cloxacillin (C: 228 nm).