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Comparative Study of RP-HPLC and UV Spectrophotometric Techniques for the Simultaneous Determination of Amoxicillin and Cloxacillin in Capsules

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ABSTRACT

Reversed-phase HPLC and UV spectrophotometric techniques using water as solvent have been developed and validated for the simultaneous determination of amoxicillin and cloxacillin in capsules. For both techniques, the linearity range of 60.0–140.0 μg/mL was studied. The spectrophotometric data show that non-derivative techniques, such as absorbance ratio and compensation, and ratio spectra first-order derivative could be successfully used for the co-assay of amoxicillin and cloxacillin. Based on the statistical comparison of spectrophotometric and chromatographic data, the interchangeability between HPLC and UV spectrophotometric techniques has been suggested for the routine analysis.

Key words: Absorbance ratio, amoxicillin, capsules, cloxacillin, compensation, HPLC, UV derivative spectrophotometry

DOI: 10.4103/0975-1483.63168

INTRODUCTION

Amoxicillin, formerly amoxycillin [Figure 1 a], is a moderate-spectrum beta-lactam antibiotic used to treat infections caused by penicillin-sensitive gram-positive bacteria as well as some gram-negative bacteria.[1] Amoxicillin is resistant to inactivation by gastric acid. It is usually the drug of choice because it is more rapidly and more completely absorbed than other beta-lactam antibiotics when orally administered. To overcome its sensitivity to destruction by beta-lactamases, amoxicillin has been co-administered with clavulanic acid, a potent betalactamase inhibitor[2] in pharmaceutical preparations.

Cloxacillin is a semisynthetic penicillin used as the sodium salt [Figure 1 b] to treat staphylococcal infections due to penicillinase-positive organisms.[1] Unlike amoxicillin, this antibiotic is incompletely absorbed from the gastrointestinal tract, and absorption is reduced by the presence of food in the stomach. To produce a wider spectrum of activity,

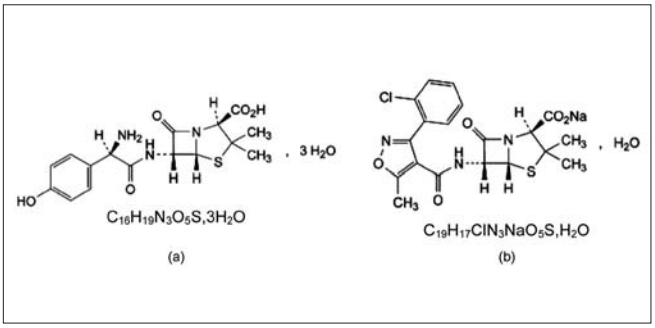

Figure 1: Chemical structures of (a) Amoxicillin trihydrate and (b) Cloxacillin sodium

cloxacillin may be co-formulated with other antibacterials, in particular with amoxicillin (ratio 1:1, w/w) in capsules.

In the literature, the high performance liquid chromatography (HPLC) technique has been reviewed as a valuable tool for the analysis of antibiotics in formulated and unformulated samples.[3] As a result, this technique has been widely used for the simultaneous determination of penicillins such as amoxicillin and cloxacillin in pharmaceuticals, biological fluids, and tissues.[4–8]

On the other hand, amoxicillin was also spectrophotometrically analyzed without prior separation using UV derivative techniques in the combination with clavulanic acid[9-12] or in antibiotic pharmaceutical mixtures.[13-16]

Albeit HPLC is often an official method for the analysis of antibiotics, the need for other simple, reproducible and accurate analytical methods still exists. Thus, this study was carried out to compare HPLC and UV spectrophotometric techniques using water only as solvent for the simultaneous determination of amoxicillin and cloxacillin in their combined capsules.

EXPERIMENTAL DETAILS

Apparatuses and conditions

A UNICAM UV 300 double beam spectrophotometer (Thermo Spectronic, USA) with a fixed slit width (1.5 nm) connected to an IBM computer loaded with Thermo Spectronic VISION32 software and 1 cm quartz cell were used for the registration and treatment of absorption spectra. For all solutions, zero-order spectra were recorded over the range from 200.0 to 300.0 nm against a blank (water) at Intelliscan mode to enhance the signal-to-noise ratio of absorbance peaks without extended scan duration with a $\Delta\lambda$ = 0.1 nm (i.e. 30-120 nm/min). To get the best signal-to-noise ratio and resolution, spectra and their corresponding derivative ones were further smoothed by using Savitzky – Golay filter (order 3, number of coefficients 125).

For the compensation technique, at any wavelength λ, the absorbance (A) of a mixture of two species X and Y (which do not interact with each other) is governed by the law of absorbance additivities, $A_m=A_X+A_y$. If the absorption of Y is subtracted from A_m, the absorption characteristics of the mixture gradually approach that of X as C_Y increases. Finally, the absorption curve of mixture coincides with the absorption curve of X at the end-point, for which C_Y used as subtrahend is exactly the concentration of Y in the mixture. For a pure substance, the absorbance ratio at two wavelengths is constant over a certain range of concentration (i.e. independent of concentration and whether another absorbing component is present). Thus, the identification of Y is based on this ratio i.e. the absorbance ratio of the mixture is equal to that of pure Y meaning the concentration of Y in the sample solution is equal to that of pure Y.

For the absorbance ratio technique, the principle is based on the linear relationship between the absorbancy ratio value of a binary mixture and the relative concentration of such a mixture. The quantification analysis of AMO and CLO in a binary mixture is performed using the following equations:

$$C_1 = \frac{Q - b_1}{a_1} \frac{A_{iso}}{a_{iso}} \times 10^3$$

$$C_2 = \frac{Q - b_2}{a_2} \frac{A_{iso}}{a_{iso}} \times 10^3$$

where $Q=A/A_{iso}$

C_1 and C_2: concentrations of AMO and CLA, respectively

A_{iso}: absorbance at isosbestic point

a_{iso}: absorptivity at isosbestic point $\left(=\frac{A_{iso}}{C_1+C_2}\right)$

a_1: slope of regression equation (Q versus $\frac{C_1}{C_1+C_2}$)

a_2: slope of regression equation (Q versus $\frac{C_2}{C_1+C_2}$)

b_1 and b_2: intercept values of these regression equations

A: absorbance of mixture solution measured at the mixture's maximum wavelength

10^3: conversion factor of concentration unit from mg/mL to μg/mL.

For the UV derivative techniques, first-order derivative and ratio spectra first-order derivative spectra were evaluated for possible simultaneous determination of AMO and CLO.

High performance liquid chromatogram (HPLC) analysis was performed on an Agilent 1100 Series Diode-Array-Detector chromatograph (Agilent Technologies, USA) at ambient temperature. An Apollo C_{18} (150 × 4.6 nm, 5μm) was used. All solutions were filtered through a 0.45 μm membrane filter before injection into the chromatograph.