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# Different spectrophotometric methods applied for the analysis of binary mixture of flucloxacillin and amoxicillin: A comparative study

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ABSTRACT

Three different spectrophotometric methods were applied for the quantitative analysis of flucloxacillin and amoxicillin in their binary mixture, namely, ratio subtraction, absorbance subtraction and amplitude modulation. A comparative study was done listing the advantages and the disadvantages of each method. All the methods were validated according to the ICH guidelines and the obtained accuracy, precision and repeatability were found to be within the acceptable limits. The selectivity of the proposed methods was tested using laboratory prepared mixtures and assessed by applying the standard addition technique. So, they can be used for the routine analysis of flucloxacillin and amoxicillin in their binary mixtures.



## 1. Introduction

Flucloxacillin (FLX), (6R)-6-[3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-carboxamido] penicillanic acid Fig. 1(a), is a bactericidal agent used primarily for the treatment of infections due to staphylococci resistant to benzyl penicillin [1–3]. There are several methods available in the literature for the quantification of FLX including spectrophotometry [4–8], high-performance liquid chromatography [9–13], polarography [14] and potentiometry [15].

Amoxicillin (AMX), (6R)-6-[α-D-(4-hydroxyphenyl) glycylamino] penicillanic acid Fig. 1(b), is one of the most frequently used β-lactam antibiotics in the world. It is employed to treat humans and animals [1–3]. Various analytical methods have been reported for the determination of AMX based on spectrophotometry [4,16–24], capillary electrophoresis [25,26], high-performance liquid chromatography [27–29] and electrochemical techniques [30–33].

Few methods were reported for determination of both components including high-performance liquid chromatography [34–37], spectrophotometry [38] and chemometric assisted techniques [39].

In the present work, a comparative study was done between three methods namely ratio subtraction [40,41] for the determination of FLX in the presence of AMX, absorbance subtraction [42–44] and amplitude modulation [42–44] for the determination of FLX and AMX listing the advantages and the disadvantages of these methods.

## 2. Experimental

### 2.1. Materials and reagents

A. Flucloxacillin (99.73%) and amoxicillin (99.84%); kindly supplied by EIPICO pharmaceutical Company, Egypt;
B. Flumox® Capsules dosage form; labeled to contain 250 mg of each of FLX and AMX batch number 1405340, manufactured by EIPICO Pharmaceuticals Company;
C. Sodium hydroxide; El-NASR Pharmaceutical Chemicals Co., Egypt;
D. Distilled water.

### 2.2. Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1800 PC connected to a compatible IBM and an HP1020 laser jet printer. The bundled software, UV-Probe personal spectroscopy software version 2.43 (SHIMADZU) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min with 0.2 nm interval.

### 2.3. Standard solutions

#### 2.3.1. Preparation of AMX working standard solution

AMX standard working solutions; 100 μg/mL in 0.1 N sodium hydroxide.

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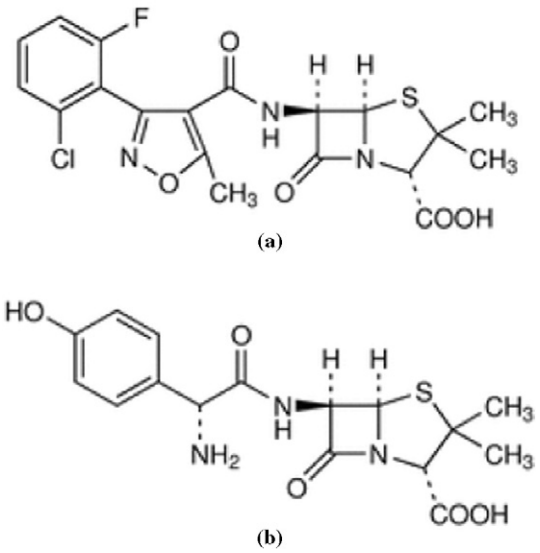


**Fig. 1.** Chemical structure of (a) flucloxacillin and (b) amoxicillin.

*2.3.2. Preparation of FLX working standard solution*

FLX standard working solutions; 100 μg/mL in 0.1 N sodium hydroxide.

## 3. Procedure

*3.1. Linearity and construction of calibration curves*

*3.1.1. Ratio subtraction method*

Different aliquots of FLX standard solution (100 μg/mL) ranging from 50–400 μg were transferred to 10-mL volumetric flasks and completed to volume with 0.1 N sodium hydroxide. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using 0.1 N sodium hydroxide as a blank. The absorbance was measured at 223 nm.

*3.1.2. Absorbance subtraction method*

Different aliquots of FLX and AMX standard solutions ranging from 50–400 μg were transferred to a two separated sets of 10-mL volumetric flasks and completed to volume with 0.1 N sodium hydroxide. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using 0.1 N sodium hydroxide as a blank. The absorbances of two drugs at 235.6 nm (isoabsorptive point) and AMX only at 291 nm ($\lambda_2$) were recorded, then the absorbance factor of AMX at 235.6 nm and 291 nm [$=A_{235.6}/A_{291}$] was calculated.

*3.1.3. Amplitude modulation method*

Different aliquots of FLX and AMX standard solutions ranging from 50–400 μg were transferred to a two separated sets of 10-mL volumetric flasks and completed to volume with 0.1 N sodium hydroxide. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using 0.1 N sodium hydroxide as a blank, and then divided by the normalized absorption spectrum of AMX solution (1 μg/mL). The amplitude of ratio spectra for FLX and AMX at isoabsorptive point 235.6 nm and 290 were recorded respectively.

*3.2. Application to laboratory prepared mixtures*

Accurate aliquots of FLX and AMX were transferred from their working solutions into a series of 10-mL volumetric flasks to prepare mixtures containing 1:1 ratios of both drugs. The volumes were completed with the solvent. The spectra of the prepared series from 200 to 400 nm were recorded and stored. The concentrations of FLX and AMX were calculated as described under linearity for each of the proposed methods.

*3.3. Application to pharmaceutical preparation*

Content of eight Flumox® capsules were weighed and finely powdered. A portion of powder equivalent to 10 mg of both components was weighed, transferred into 100-mL volumetric flask and dissolved in 75 mL of 0.1 N sodium hydroxide. The solution was shaken vigorously for 15 min then sonicated for 30 min. The volume was completed to 100 mL with 0.1 N sodium hydroxide and filtered. Necessary dilutions of the filtrate were made with 0.1 N sodium hydroxide to obtain different concentrations of FLX and AMX samples as stated under linearity. To assess the accuracy of the proposed methods, standard addition technique was applied.

## 4. Results and discussion

The zero-order absorption spectr of FLX and AMX, show a certain degree of overlapping with isoabsorptive point at 235.6 nm and AMX is more extended in plateau region in which FLX has no absorbance as shown in Fig. 2. This overlap does not permit direct determination of FLX and AMX in their binary mixture. To overcome this problem, many manipulations have been done allowing the determination of

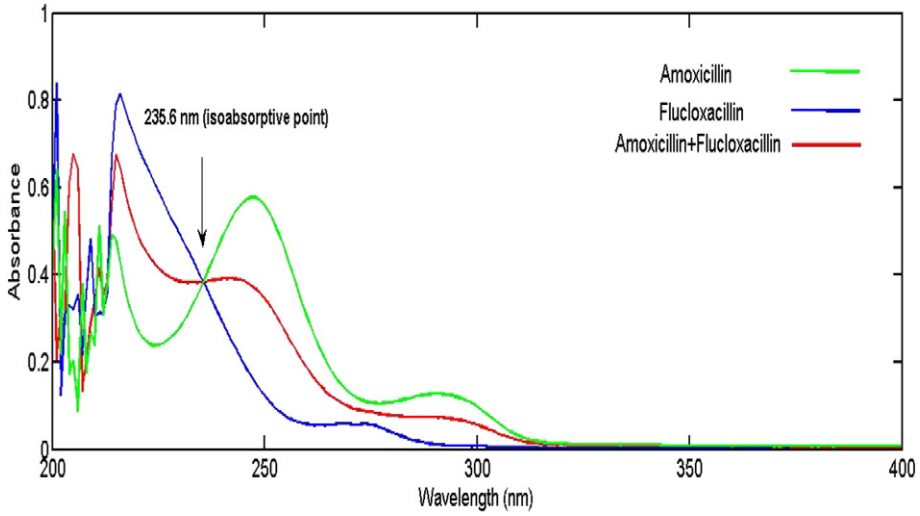


**Fig. 2.** Absorption spectra of FLX (10 μg/mL), AMX (10 μg/mL) and their mixture (5 μg/mL) of each.