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Simultaneous Determination Of Amoxicillin And Clavulanate Potassium By Derivative Spectrophotometric Method

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Abstract : A first-derivative spectrophotometric method is described for simultaneous determination of Amoxicillin (AMX) and Clavulanate potassium (CLV). All measurements are made at the zero-crossing wavelengths at 215.0 nm for AMX and 240.0 nm for CLV. Calibration graphs were linear over the range 2.0-90.0 mg/l of AMX and 10.0-90.0 mg/l of CLV. The detection limites were achieved 0.25 and 1.5 mg/L for AMX and CLV respectively. The proposed methods were suitably applied to assay of pharmaceutical preparations.
Keywords: Derivative spectrophotometry, Amoxicillin, Clavulanate potassium.

1. Introdution

Amoxicillin and Clavulanate potassium tablets for oral suspension is an oral antibacterial combination consisting of the semisynthetic antibiotic amoxicillin and the p-lactamase inhibitor, clavulanate potassium (the potassium salt of clavulanic acid). Amoxicillin is an analog of ampicillin, derived from the basic penicillin nucleus, 6-aminopenicillanic acid [1].

Clavulanic acid is produced by the fermentation of Streptumyces. It is a plactam structurally related to the penicillins and possesses the ability to inactivate a wide variety of p-lactamases by blocking the active sites of these enzymes. Clavulanic acid is particularly active against the clinically important plasmid mediated p-lactamases frequently responsible for transferred drug resistance to penicillins and cephalosporins. The clavulanate potassium molecular formula is $CsHsKNO_5$ and the molecular weight is 237.25. Chemically clavulanate potassium is potassium (Z)-(2R,SR)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-l-azabicyclo[3.2.0]-heptane-2-carboxylate[2].

Amoxicillin and clavulanate are both well absorbed from the gastrointestinal tract, reaching peak serum levels 60–90 min and 40–120 min, respectively, after separate oral administration. Combining the two drugs does not affect their pharmacokinetics.

A single dose of 250/125 mg of amoxicillin/clavulanate produces a mean peak concentration of 4.2 mg/L for amoxicillin and 2.6 mg/L for clavulanic acid [3].

Derivative spectrophotometry opens up possibilities for increasing selectivity of the analytical methods without the need for prior separation and masking agents procedure [4]. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve. Most of the spectrophotometers are equipped with suitable software. On the other hand, the high solubilization capacity of surfactants and micellar systems permits modification or development of analytical procedures and improves the sensitivity and selectivity of the analytical methods [5].

In the proposed method, a simple, rapid, sensitive and selective method has been developed for simultaneous determination of amoxicillin and clavulanate potassium in mixtures by first derivative spectrophotometry using a zero-crossing technique.

2. Experimental

2.1. Reagents

All chemical and solvents were of analytical reagent grade and were used without further purification. A standard stock solution of AMX (1000.0 mg/l) was prepared by dissolving 0.1g of amoxicillin (Merck) in water and diluting with distilled water to 100.0 ml in volumetric flask. A standard stock solution of CLV (1000.0 mg/l) was prepared by dissolving 0.1g of clavulanate potassium (Merck) in water and diluting with distilled water to 100.0 ml in volumetric flask.

Titrazol buffers (Merck) were used at different pHs for this study.

2.2. Pharmaceutical Products

A commercial of co amoxiclv by product of Kowsar company IRAN that each capsule has 625 mg.

2.3. Apparatus

All spectral measurements and treatment of data were carried out in 1cm quartz cells using a Perkin-Elmer Lambada 25 double beam spectrophotometer. Measurements of pH were made using a Jenway Model 3510 pH-meter equipped with a glass-saturated calomel combined electrode.

2.4. Procedure

Different volumes of stock solutions of AMX and CLV (1000.0 mg/l) were used
simultaneously and placed in a 10 ml calibrated flask and diluted with distilled water to gave final different concentration of AMX and CLV.

First derivative spectrums of sample solutions were recorded against its blank in the wavelength rang of 200-300 nm with interval ($\Delta\lambda$=2nm) using scan speed of 960 nm/min and spectral slit width 2 nm. The first derivative analytical signals were at zero-crossing wavelength of 215 nm and 262 nm respectively, for AMX and CLV determination.

2.5. Analysis of Pharmaceutical Formulations

For preparation of sample, 20 capsule were accurately weighed and powdered in a mortar. A mass corresponding to a tablet was dissolved in 0.1 M HCl in 100 ml calibrated flask. After 30 min of mechanically shaking, the solution was filtrated in a 100 ml calibrated flask through Whatman no: 40 filter paper. The residue was washed three times with 10 ml solvent then the volume was completed to 250 ml with water.

3. Results And Discussion

3.1 Spectrophotometric Measurements

In Fig.1 the zero-order spectra of 50.0 mg/l AMX , 40.0 mg/l of CLV in the wavelength range of 200-300 nm are shown. AMX exhibits an absorbance maximum at 210 nm and for CLV at 240 nm. As can be seen, there is a clear overlapping of two spectra, which prevents the simultaneous determination of the two compounds by

direct UV-Vis absorbance measurement. The pH of the solutions of AMX and CLV varied. The results were obtained showed that the λ_{max} and sensitivity is not dependent on the pH range between 1.0 to 10.0.

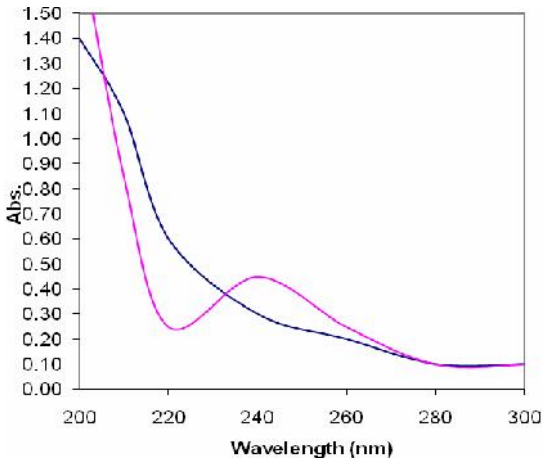

Fig.1: Absorption (zero-order) spectra of (A) 50.0 mg/l of AMX; (B) 40.0 mg/l of CLV

A suitable technique for overcoming the problem is derivative spectrophotometry with the zero-crossing method being the most common procedure for preparation of analytical calibration graphs. In the zero-crossing derivative method, the measurements selected are those which exhibit the best linear response, give a zero or near zero intercept on the ordinate of the calibration graphs and it is necessary that zero-crossing wavelength do not change by varying concentration of related species. Zero-crossing method can not be used for determination of species which their spectra are shifted with change of concentration.

3.2. The Linear Plot (Calibration Curve)

First-order spectra of solutions containing fixed concentration of AMX (10.0 mg/l) and different concentration of CLV (10.0-40.0 mg/L) are shown in Fig 2 and first-order spectra of solutions containing fixed concentration of CLV (10.0 mg/L) and different concentration of AMX (10.0-25.0 mg/L) are shown in Fig 3.

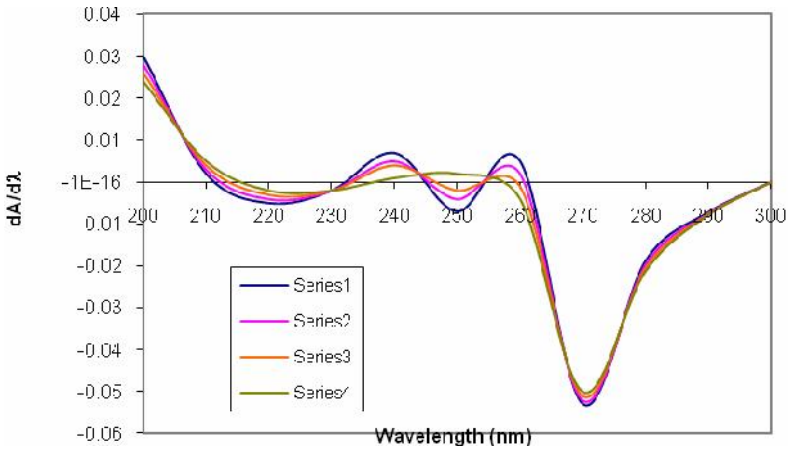

Fig 2: First derivative spectra of solution containing fixed 10.0 mg/L AMX and CLV concentrations (1)10.0 mg/L (2) 20.0 mg/L (3) 30.0mg/L (4) 40.0mg/L.

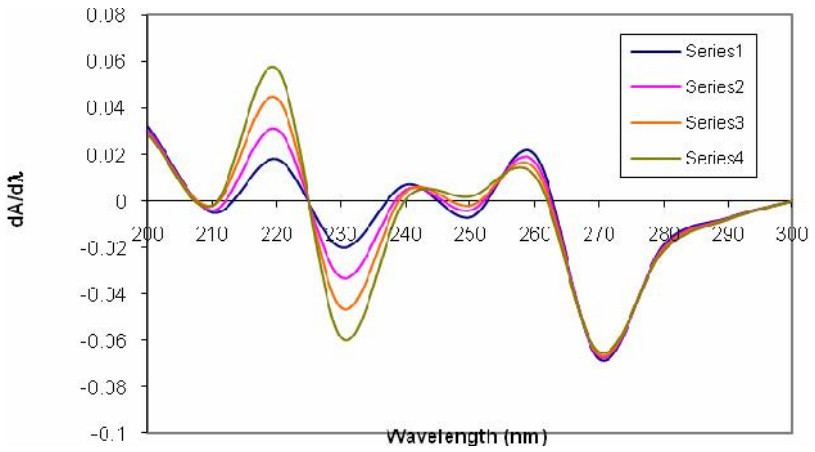

Fig 3: First derivative of solution containing fixed 10.0 mg/L AMX and CLV concentrations of (1)10.0mg/L (2) 15.0mg/L (3) 20.0 mg/L (4) 25.0mg/L.

As shown in Fig 2 and 3 has been seen that zero-crossing wavelengths do not change by varying concentration of the related species.

Two calibration graphs were constructed at the zero-crossing wavelengths (215 nm for AMX and 240 nm for CLV) for the simultaneous determination of AMX and CLV. In table 1 figures of merit of first derivative zero-crossing method for simultaneous determination of AMX and CLV were shown.

The linear range of AMX and CLV are 2.0-90.0 and 10.0-90.0 mg/L, respectively and detection limit were obtained 0.25 and 1.5 mg/L for AMX and CLV, respectively. The relative standard deviation achieved were 3.5% for 20.0 mg/l of AMX and 4.5% for 30.0 mg/l of CLV.

Table.1. calibration data for the determination of fexofenadine and montelukast sodium

Sample	Regression equation	λ (nm)	r	linear range (mg/l)	DL[a] (mg/l)	RSD
AMX	$dA/d\lambda=0.0009C_{AMX}-0.0008$	215	0.9980	2.0-90.0	0.25	3.5[b]
CLV	$dA/d\lambda=0.0007C_{CLV}-0.0024$	262	0.9973	10.0-90.0	1.5	4.5[c]

[a] detection limit (DL)=3sb/m
[b] concentartion of AMX=20.0 mg/L
[c] concentartion of CLV=30.0 mg/L

3.3. Application

In order to confirm the usefulness of the proposed derivative spectrophotometric methods, it has been applied to the simultaneous determination of Amoxicillin (AMX) and Clavulanate potassium (CLV) in different samples commercial pharmaceutical where excellent agreement between reported and obtained results was achieved (Table 2).

Table 2: Simultaneous determination of Paracetamol and Caffeine in real samples

Sample NO. Co amoxiclv[a]	Reported		obtained [b,c]		RSD%	
	AMX	CLV	AMX	CLV	AMX	CLV
1	500.0	125.0	510.0±1.50	120.0±2.5	3.5	4.2
2	500.0	125.0	485.0±1.85	111.5±0.14	3.8	3.5

[a] Purchased by kowsar company IRAN
[b] Mean ± standard deviation (mg) for four determination
[c] After dilution and determination by the proposed method

3.4. Conclusions

The proposed second-derivative method is suitable for simultaneous determination of AMX and CLV without requiring a separation procedure. It is a simple, accurate and precise method which can be used, for rapid and reliable study of AMX and CLV simultaneously in pharmaceuticals Products and can be used in the routine analysis of these compounds.

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