Simultaneous Determination of Cephalexin and Cefixime by First and Second Derivative Ultraviolet Spectrophotometry

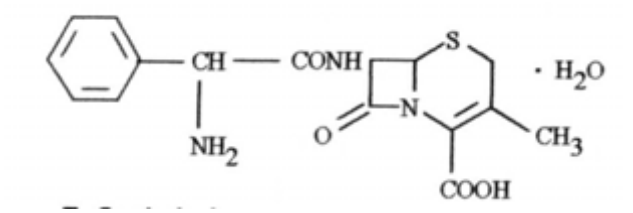
Maha A. Mohammmed **Elham.N.Mezaal**
Kawther .A.Sadiq
Department of chemistry, colleg of Education-Ibn Alhetham
University of Baghdad

Abstract

A new spectrophotometric method for individual and simultaneous determination of cefixime and cephalexin depending on the first and second derivative mode techniques. The first and second derivative spectra of these compounds permitted individual and simultaneous determination of cefixime and cephalexin in concentration interval of (4– 24μg.ml^{-1}) by measuring the amplitude of peak-to-base line, pea to peak at certain wavelengths and the area under peak at selected spectrum intervals. The methods showed reasonable precision and accuracy and have been applied to determine cefixime and cephalexin in two different pharmaceutical preparations.

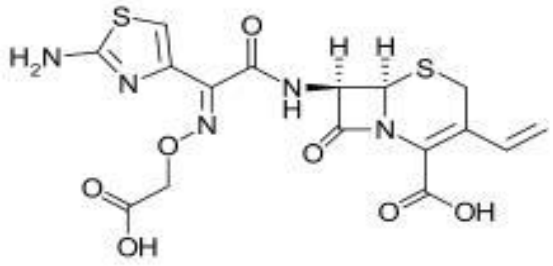
Introduction

Derivative spectrophotometry (DS) is one of the advanced modern spectrophotometric techniques. It is based on so called derivative spectra [1] which are generated from parent zero order ones. The derivatisation [2] of zero-order spectrum can lead to separation of overlapped signals, elimination of background caused by presence of other compounds in a sample. The mentioned properties can allow quantification of one or few analytics without initial separation or purification. Nowadays, this technique becomes very useful, additional tool which helps to resolve various analytical problems. It has found application in many fields of analysis, especially in pharmaceutical, clinical and biochemical as well as in inorganic or organic analysis. Cephalexin, a semisynthetic derivative of cephalosporin, is known to have antibacterial action against gram-positive and gram-negative bacteria. Cephalexin is a potent cephalosporin and exhibits a broad spectrum of antibiotic activity, weak bond ability to blood protein, no metabolites, low toxicity and to be rapidly absorbed following oral administration to give a high serum levels and urine concentration. The drug, therefore, is widely used for clinical chemotherapy [3,4].Chemical structure of Cephalexin(CEP) Molecular formula $C_{16}H_{17}N_3O_4.H_2O$ Molecular weight 365.4 g/mol Chemical structure Figure(1).



Figure(1) Chemical formula of Cephalexin

Cefixime (CFX) Molecular formula $C_{16}H_{15}N_5O_7S_2$ Molecular weight 453.452 g/mol ((6R, 7R)-7-[(Z)-2-(2-amino-4-thiazolyl)-2-(carboxy-methoxyimino) acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo-[4,2,0]-oct-2-ene-2-carboxylic acid). (Figure 2) are third-generation oral cephalosporin antibacterial.



Figure(2): Chemical formula of Cefixime

It has a broad antibacterial spectrum against various gram-positive bacteria and gram-negative bacteria, including Haemophilia influenza [5] and is used as an antibacterial and especially against gram negative, gram positive and anaerobic bacteria pathogens including β- lactamase producing strains. It consists of high affinity for penicillin binding proteins with deceitful site of activity. It acts by inhibition of bacterial cell-wall synthesis. It is clinically used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and urinary-tract infections [6]. Literature survey revealed the estimation methods of cefixime tri hydrate or with other drugs by HPLC [7, 8]. Calorimetric method [9]. Flow injection analysis [10] and HPTLC [11]. Ofloxacin [12] (OFL) a fluorinated carboxyaquinolone [13] The aim of the presented paper is to review the recent applications and achievements of derivative spectrophotometry in chemical analysis [14].

Experimental

Apparatus

UV-visible spectrophotometer (Shimadzu 1800) with UV-Probe Version 1.10 (Japan) connected to computer was used for the drugs estimation. Quartz cuvettes (1.00 cm) were matched and used for all absorbance measurements.

Chemicals

Pure gift samples of (CEP) and (CFX) Double distilled water were provided by the state company of drug industries and medical appliances (IRAQ_Samara) CEP

(250mg) capsule and Saudi CFX (400mg) capsule from local market. All drugs were used as working standards without further purification

Preparation of stock and working standard solutions:

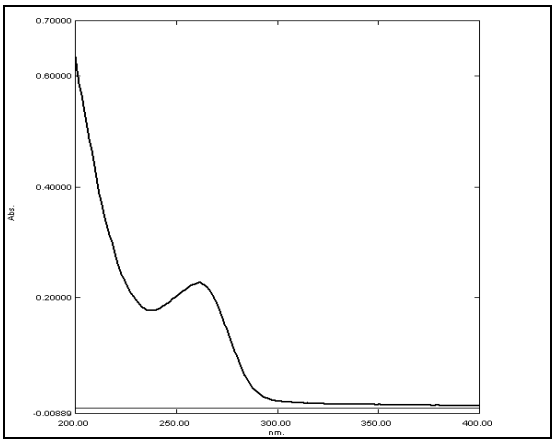
The stock solutions of CEP and CFX (100 µg/mL) were prepared by dissolving 10 mg of drugs in 100 mL water using volumetric flask. The working standard solutions of the respective drugs were prepared by several dilutions using water

Procedure

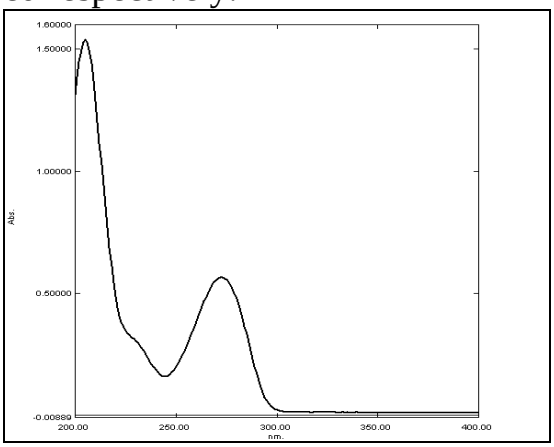
1- Individual determination of CFX and CEP:

In 5 mL calibrated flasks, transfer aliquots of CFX or CEP solution expected to contain (4-24) µg/mL and (2-24) µg/mL respectively and dilute to the mark with double distilled water. The absorption spectra were recoded and show absorption maxima at 261nm for CEP figure(3) and 205 and 272nm for CFX figure(4).

Determination were made by measuring the first and second derivative values and area under peaks of their spectra at certain given wavelengths and wavelength regions. The concentration of CEP and CFX could be determined respectively.



Figure(3) Absorption spectra of 12 µg/ mL Cephalexine



Figure(3) Absorption spectra of 12 µg/mL Cefixime

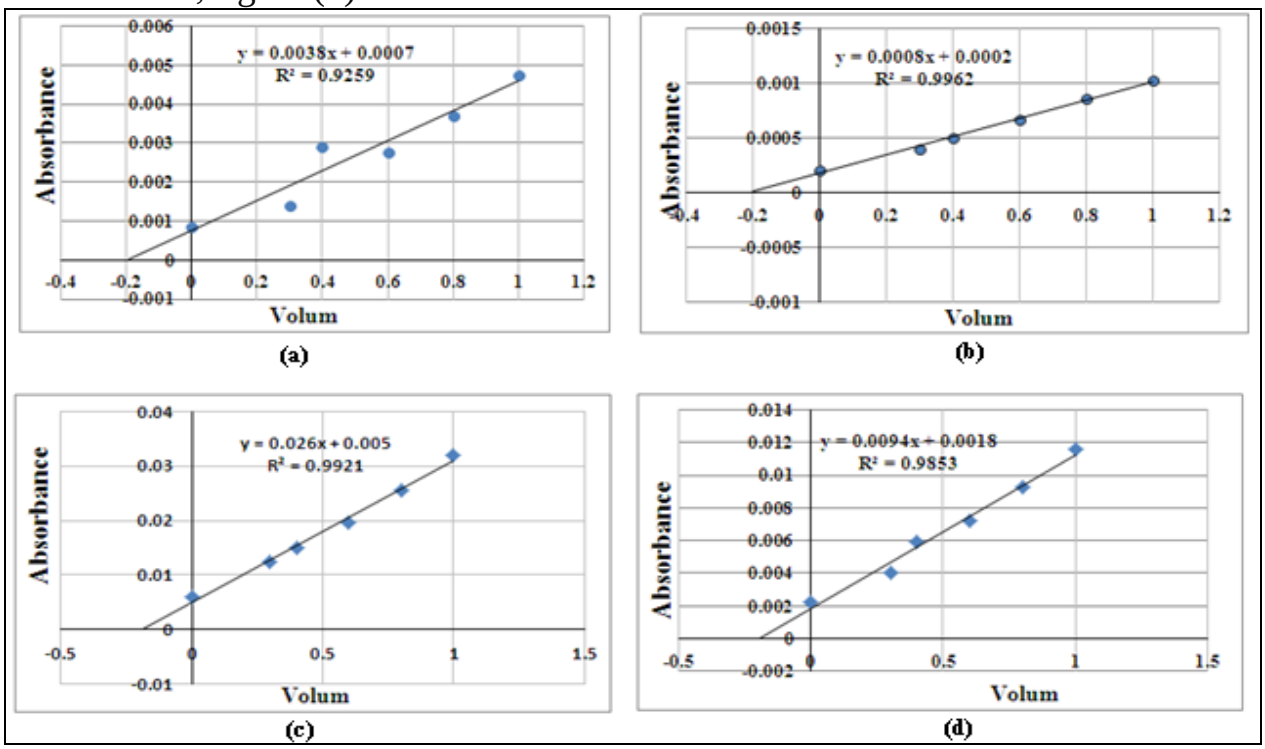
Simultaneous determination of CFX and CEP

(i) The content of series of 5 mL calibration flasks containing (4and 12 µg/mL) for (CFX) with different concentrations (4, 8, 12, 16, 20 and 24 µg/mL) of (CEP) were diluted with double distilled water. The absorption spectra were recorded against blank (prepared by the same manner as test solution but without CFX and CEP). The derivative values of their first and second spectra the concentration of CFX were measured and determined.

(ii) The content of series of 5 mL calibration flasks containing (4 and 12 µg/mL) for (CEP) with different concentrations (4, 8, 12 ,16, 20 and 24 µg/mL) of (CIP) were diluted with double distilled water. The absorption spectra were recorded against blank (prepared by the same manner as test solution but without CEP and CFX). The derivative values of their first and second spectra were measured and the concentration of CEP was determined.

3- Standard addition method

It was carried out by preparing several 10 mL aliquot solutions containing the same amount (40 µg) of CFX or CEP) drug (Capsule), and different amounts of standard (0,30, 40,60,80,100) µg, similarly for (CEP). The first and second derivative were recorded, figure (5).



Figure(5) Determination of CEP in several amount of standard (a,b) and Determination of CFX in several concentration of standard (c,d) by standard additions method.

4- Effect of interference

This research includes, a study about the effect of different interferences on the first and second derivative of spectra for CFX and CEP which are found in pharmaceutical material

A stock solution of Glucose, Lactose and Starch were prepared by dissolving (0.05g)of each additives in 5 mL volumetric flask to get a solution of 10000 µg/mL. In 5 mL calibrated flask containing (8,16) µg/mL of CFX and (12,16) µg/mL of CEP,0.5ml aliquots were transferred of to each additive, from which the absorption spectra were recoded Table (1).

Table No. (1) : Percent recovery for (8,16) µg.mL^{-1}of CEP and (12,16)µg.mL^{-1}of CFX in the presence of 1000 µg.mL^{-1}of excipients.

Compound	Excipients	Taken conc.	Found conc. (µg/mL)	%Recovery
Cephalexin	Lactose	6	6.099	99.900
	Starch		5.979	99.580
	Glucose		5.999	99.980
	Lactose	16	15.992	99.890
	Starch		15.998	99.900
	Glucose		16.000	99.999
Cefixime	Lactose	12	11.980	98.990
	Starch		12.090	99.986
	Glucose		11.988	99.969
	Lactose	16	15.997	99.997
	Starch		15.995	99.959
	Glucose		15.999	99.999

Preparation of pharmaceutical formulation

Ten capsules were weighed capsules powder equivalent to 250 mg of (CEP) and six capsules were weighed. The capsules powder equivalent to 400 mg (CFX) respectively are mixed and dissolved in 2 mL double distilled water. The resultant solution was diluted to 100 mL with double distilled water in volumetric flask. The solution was filtrate by using Whitman filter paper no.41to avoid any suspended or undissolved material before analyses**.**

Results and Discussion

Absorption spectra

The absorption spectra of the (CFX) and (CEP) were measured from (200-400 nm.) against double distilled water as blank. Th**e** absorption spectra of CFX and CEP and for their mixture were recorded. Fig (6) (a) shows the absorption spectrum of CEP solution (12µg/mL) with absorption maxima at wavelength 261nm, while spectrum CFX (b) shows the absorption spectrum of solution (12 µg/mL) with two absorption maxima at wavelength 205 and 273nm.The total spectrum of mixture of (12 CFX µg/mL and 12 CEP µg/mL) is shown in curve (c) with (205 and 270 nm) between the absorption maxima of the two components**.**

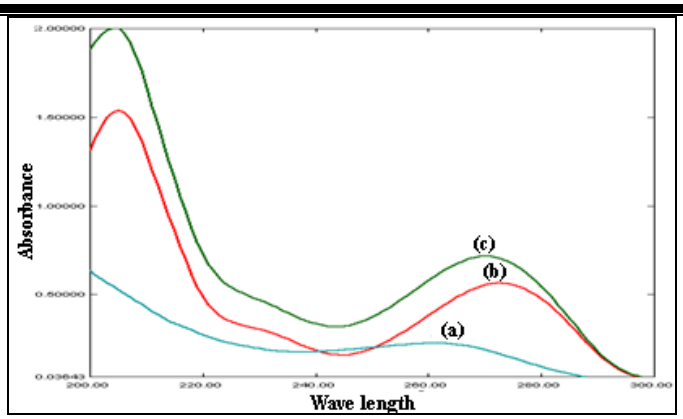


Figure No. (6) Absorption spectra of (a) 12 µg/mL Cefixime , (b) 12 µg/mL Cephalexin e (c) Cefixime and Cephalexin hydrochloride

First and second derivative modes

The first and second order derivative spectra of (CFX) and (CEP) and for their mixture are shown in Figure (7) and Figure (8) respectively. It was obvious that there is a large overlap of the spectra of CFX and CEP using the zero order absorption measurements. Therefore derivative spectrophotometric technique is of a particular utility in determining the concentration of single component in such mixtures with a large spectral overlapping. For this reason, derivative spectrophotometric methods have been applied .Both first and second order modes were tested and the results obtained showed that techniques could be successfully applied when the measurements are carried out under optimum concentration. The present work, graphically (peak-to-base line), technique in addition to peak area were used to deal with derivatives spectra to carry out the measurement. In the first and second derivative modes show a good proportionality to CFX and CEP concentration in their mixtures.

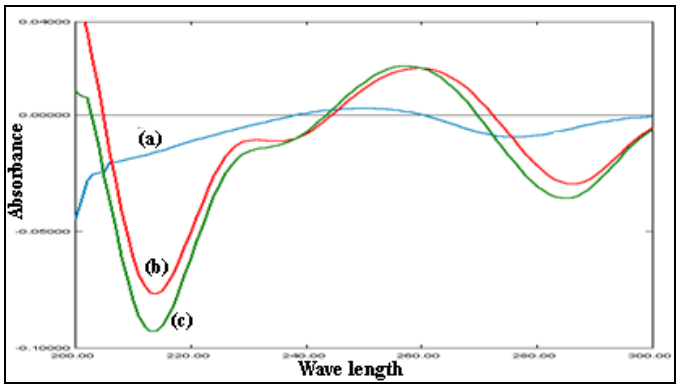


Figure No. (7) first derivatives spectra of (a) 12 µg/mL Cephalexin , (b) 12 µg/mL Cefixime (c) Cefixime and Cephalexin

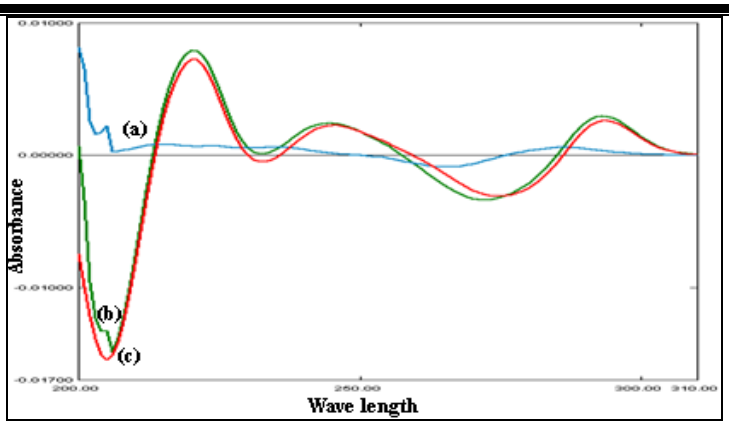
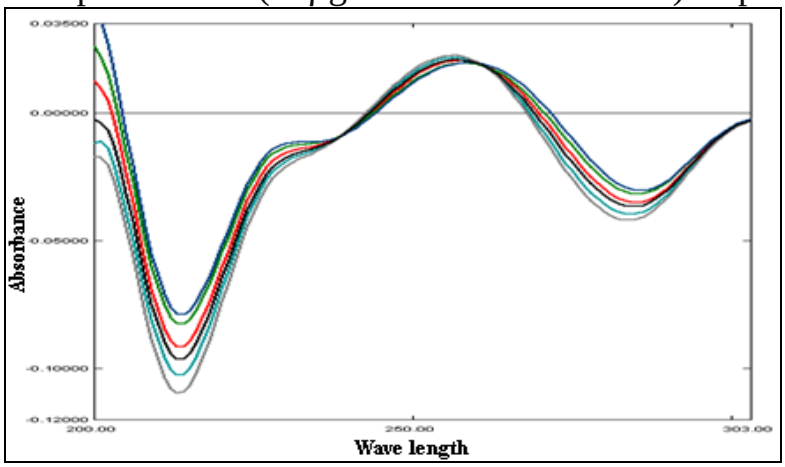


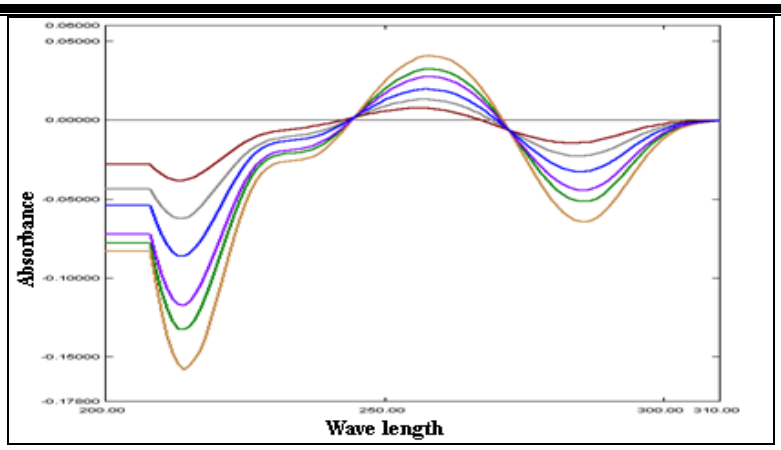
Figure No. (8) second derivatives spectra of(a) 12 µg/mL Cephalexin ,(b) 12 µg/mL Cefixime (c) Cephalexin and Cefixime

To select the derivative order, the first, second, third and fourth derivative spectra of CFX and CEP were also studied. The study of first and second order spectra was simple and gave results of highest accuracy and detection limits. Figure (9)and Figure (10) show sets of first order spectra of mixtures containing different amounts of each of CFX or CEP in the presence of (12µg/mL of CFX and CEP) respectively.



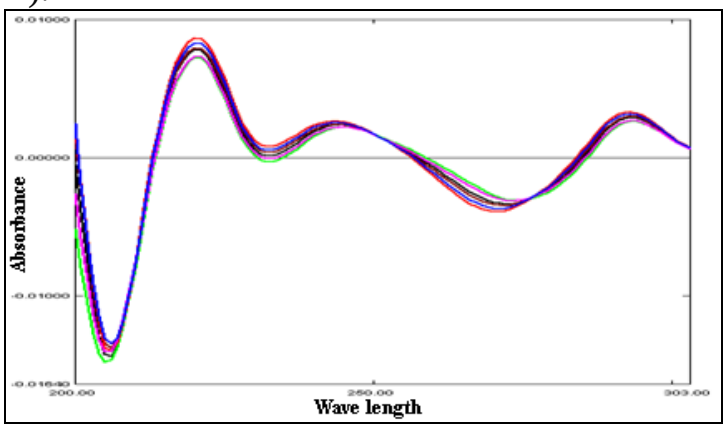
Figure(9): First derivative spectra of mixtures containing(4-24 µg/mL) Cephalexin and 12µg/mL Cefixime

The results in Figure (8) indicated that when the concentration of CFX is kept constant and the concentration of CFX varied, the peak area at the, intervals (272-306 nm) and (245-272nm) were proportional to the concentration of CEP. Moreover, the peak-to-base line at (214) and (286nm) was found to be a function of CEP concentration. The same features were found when inspecting Figure (9) for the determination of CFX. The peak areas in the wavelengths intervals of (275.9-310 nm), and (250-275.9 nm) the peak amplitude measured at peak-to-baseline (250nm) were in proportion to the concentration of CFX (Table 2).

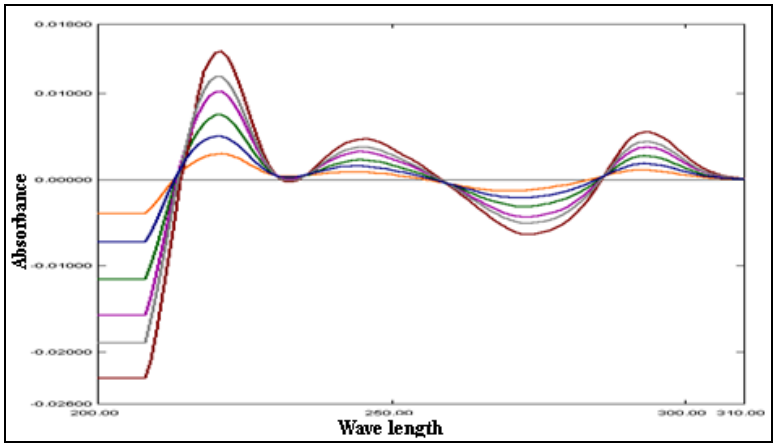


Figure(10): First derivative spectra of mixtures containing(4-24 µg/mL) Cefixime and 12 µg/mL Cephalexin

Figure|(11) and Figure (12) have shown in further sets of second derivative of the same above mixtures. Applying the same mentioned techniques in measuring peak amplitudes (in millimeter) at peak-to-base line of the other compound, and peak areas at selected wavelengths intervals enable the measurement of CEP and CFX respectively (Table 2).



Figure(11):Second derivative spectra of mixtures containing (4-24 µg/mL) Cephalexin and 12 µg/mL Cefixime



Figure(12): Second derivative spectra of mixtures containing (4-24 µg/mL) Cefixime and 12µg/mL Cephalexin

Calibration graph and statistical analysis

The analysis characteristic and most statistical data for each of the proposed methods are given in Table (2). Under the optimum conditions, liner calibration graphs were obtained in the range of (2-24µg/mL**)** with correlation coefficient values in the range (0.9960-0.9991) for deferent techniques.

Table(2) Statistical analysis of the determination of Cephalexin and Cefixime

Compound	Order of derivative	Mode of calculation	(nm)	Regression equation	R	Slop
Cephalexin	First	Peak to base line	250	Y=0.0003x+0.0108	0.9962	0.0003
	First	Peak to base line	276	Y=0.0008x-0.0323	0.9980	0.0008
	First	Peak to Peak	250-276	Y=-0.0006x+0.0431	0.9979	-0.0006
	First	Peak area	260-310	Y=-0.0185x-0.7698	0.9964	-0.0185
	First	Peak area	237-260	Y=0.0042x+0.0466	0.9964	0.0042
	second	Peak to base line	224.3	Y=4E-05X+0.0014	0.9981	4E-05
	second	Peak to base line	285.9	Y=5E-05X-0.0001	0.9990	5E-05
	second	Peak to base line	241.7	Y=3E-05X+0.0018	0.9970	3E-05
	second	Peak to base line	266	Y=-8-05X-0.0015	0.9987	-8E-05
	second	Peak to Peak	266-285.9	Y=0.0001X+0.0014	0.9991	0.0001
	second	Peak area	275.9-310	Y=0.001X+0.0481	0.9968	0.0010
	second	Peak area	250-275.9	Y=-0.0011X-0.0047	0.9996	-0.0011
Cefixime	First	Peak to base line	231	Y=0.001X+0.0027	0.9967	0.0010
	First	Peak to base line	214	Y=-0.006X-0.015	0.9976	-0.006
	First	Peak to base line	286	Y=0.0028X+0.001	0.9965	0.0028
	First	Peak to base line	259	Y=0.0017X-0.0001	0.9979	0.0017
	First	Peak to Peak	231-235	Y=0.0019X+0.004	0.9960	0.0019
	First	Peak to Peak	235-259	Y=0.0027X+0.0009	0.9972	0.0027
	First	Peak to Peak	245-274	Y=0.0004X+0.0002	0.9975	0.0004
	First	Peak to Peak	259-268	Y=0.0042X+0.0031	0.9974	0.0042
	First	Peak area	272-306	Y=-0.0447X+0.0111	0.9981	0.0447
	First	Peak area	245-272	Y=0.0282X+0.0333	0.9980	0.0282
	second	Peak to base line	294	Y=0.0002X+0.0001	0.9980	0.0002
	second	Peak to base line	274	Y=-0.0003X-0.0001	0.9981	-0.0003
	second	Peak to base line	245	Y=0.0002X+7E-05	0.9964	0.0002
	second	Peak to Peak	274-294	Y=0.0004X+0.0003	0.9967	0.0004
	second	Peak to Peak	245-274	Y=0.0004X+0.0002	0.9975	0.0002
	second	Peak area	236-259	Y=0.0024X+0.0012	0.9969	0.0024
	second	Peak area	286-310	Y=0.0026X-0.0022	0.9979	0.0026
	second	Peak area	259-286	Y=-0.0043X-0.0034	0.9982	-0.0043

Accuracy and precision

Under the optimum condition, the accuracy and precision of the proposed method (two different techniques for each of first and second order derivative modes) tested. Table 3 shows the values of relative error percent and relative standard deviation percent for two different levels of concentration of CFX and CEP

Table (3) Precision and accuracy of the methods.

Compound	Method of analysis	Taken (µg/mL)	Found (µg/mL)	RE %	RSD %
Cephalexin	First order peak-to-base lineat 276nm	4	3.950	-1.250	0.043
		12	12.119	0.991	0.994
		20	20.055	0.275	0.013
	Second order peak-to-base line at 285.9nm	4	4.051	1.275	0.093
		12	12.221	1.841	0.405
		20	19.901	-0.495	0.008
Cefixime	First order peak-to-base line at 235 nm	4	4.123	-3.075	0.329
		12	11.979	0.175	0.010
		20	19. 988	0.060	0.083
	Second order peak-to-base line at 245 nm	4	4.101	2.525	0.048
		12	12.112	0.933	0.021
		20	20.104	0.520	0.017

*Average of three determination

Application

Two of proposed methods (namely first derivative peak–to–base line at 276nm and second derivative peak –to- base line at 285.9 nm) were successfully applied for direct determination of CEP in two different drugs. The results obtained are presented in Table (3), and are in quite agreement with the spiked values. On the other hand, CEP has also been successfully determined in two different pharmaceutical preparations by two of proposed methods. The results are shown in Table (3).The standard additions method was used to determine each drug in pharmaceutical Tablets. Accuracy of the proposed method was assisted by determining CFX and CEP solutions using the standard additions method for the above methods and the data obtained for pharmaceutical tablets were listed in Table (4).

Table (4) Results for analysis of Cephalexin and Cefixime in two pharmaceutical formulation sample.

Compound	Method of analysis	Taken (µg/mL)	Found (µg/mL)	(nm)	Regression equation	R	RE%	RSD %
Cphalixcine (Samara) Iraq 250 mg	First	4	4.11	250	Y=-0.0002X-9E-05	0.9995	-2.75	0.608
	Second	4	4.10	285.9	Y=-5E-05X+1E-05	0.9953	-2.50	0.804
Cfixcime Saudi Arabia 400mg	First	4	3.91	259	Y= -0.0016X-0.0004	0.9991	2.25	2.828
	Second	4	3.86	220	Y= -0.0006X-0.0001	0.9990	3.50	0.777

***Average of three determination**

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