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Research Article

NOVEL FORMATION THREE COMPLEXES OF CEFIXIME-COPPER USING ACETATE–ACETIC ACID BUFFER AND DETERMINATION OF CEFIXIME IN PURE AND PHARMACEUTICAL DOSAGE FORMS

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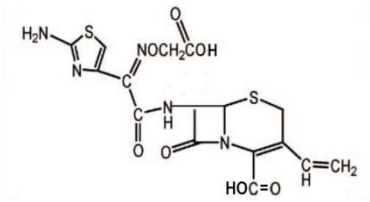
ABSTRACT

Novel formation three complexes of cefixime-copper using acetate–acetic acid buffer at pH4.8 and determination of cefixime in pure and pharmaceutical dosage forms in methanol (96%, v) has been developed. Optimal temperature and time for coupling were established at 50±1°C with time 25min. The formation complexes, CEFI:Cu(II) gives, in the first time, three forms; the first one is [(CEFI)$_2$(Cu).2HCl], the second is [(CEFI)(Cu).HCl] and the third [(CEFI)(Cu)$_2$.2HCl]. The reaction between CEFI and Cu(II) occurred at stoichiometric ratio of 2:1, 1:1 and 1:2, respectively. The maximum absorbance of the complexes occurred at λ= 510, 500 and 450 nm. The molar absorptivity is 1.52 x 10^3, 1.06 x $10^3$and 3.42 x 10^3 L.mol^{-1}.cm^{-1}, respectively. All reaction conditions have been optimized to obtain the complexes. Under optimum conditions Beer's law was obeyed at concentrations ranging from 0.4535 to 18.138 µg.mL^{-1}with correlation coefficients ≥0.9980 in all cases with RSD generally less than 4.8%. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.036 µg.mL^{-1} and 0.109 µg.mL^{-1}, respectively. The proposed method was novel, simple, accurate and successfully applied to the determination of CEFI in pharmaceuticals with average recovery of 96.59 to 102.16%, the results obtained agree well with the contents stated on the labels.

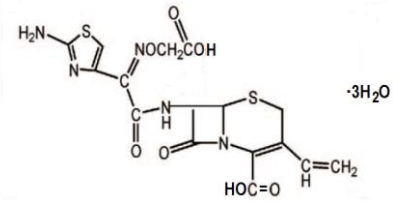
Keywords: Cefixime (CEFI), Complexes [(CEFI)$_2$(Cu).2HCl], [(CEFI)(Cu).HCl]and [(CEFI)(Cu)$_2$.2HCl], Methanol, Acetate–acetic acid buffer, Pharmaceuticals, Spectrophotomety.

INTRODUCTION

Cefixime (CEFI) (6*R*, 7R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-(*Z*)-[*o*-(carboxymethyl)- oxime] trihydrate is third-generation cephalosporin antibiotic**[1]**, see Scheme 1.



Cefixime



Cefixime trihydrate

Scheme 1: Chemical structure of cefixime and cefixime trihydrate.

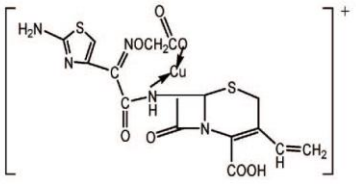
Cefixime (H_2cefixi) reacts with transition metal(II) ions (as Mn, Co, Ni and Cd) to give [Mn(cefixi).(H_2O)$_2$], [Co(cefixi).(H_2O)$_2$], [Ni(cefixi).(H_2O)$_2$] and [Cd(cefixi).(H_2O)$_2$] complexes. Cefixime (H_2cefixi) reacts with $FeCl_3$.6H_2O to give [Fe(cefixi).Cl(H_2O)] complex. The mentioned complexes were characterized by physicochemical and spectroscopic methods, and an octahedral geometry is suggested for their structure**[2]**.

An accurate, precise and ecofriendly spectrophotometric method is presented for the determination of Cefixime based on the formation of a yellow colour product with ninhydrin in the presence of bicarbonate with an absorption maximum at 438 nm. The reaction proceeds quantitatively at 97 ± 1°C in 15 min. The calibration curve is linear over the range of 45-65 µg.mL^{-1} with a regression coefficient (r) of 0.9987 (n = 5). The calculated molar absorptivity and Sandell sensitivity values are 4.1536 x 10^3 L.mol^{-1}.cm^{-1} and 0.0072 µg/cm^2, respectively. The limits of detection (LOD) and quantification (LOQ) calculated as per ICH guidelines are 1.13 and 3.40 µg.mL^{-1}, respectively**[3]**.

Spectrophotometric method is based on the formation of yellow to yellowish brown complex between palladium (II) chloride and five cephalosporins (as cefixime) in the presence of sodium lauryl sulphate (SLS) as surfactant. The reaction conditions were studied and optimized. For each drug, the composition of this complex as well as its stability constant were also investigated. The proposed method was used for the determination of the above-mentioned drugs in their commercial preparations**[4]**.

A new, simple, sensitive and accurate spectrophotometric method has been developed for the assay of metoprolol tartrate (MPT), which is based on the complexation of drug with copper(II), [(Cu)$_2$(MPT)$_2$Cl$_2$] at pH 6.0, using Britton-Robinson buffer solution, to produce a blue adduct. The latter has a maximum absorbance at 675 nm and obeys Beer's law within the concentration range 8.5-70 µg.mL^{-1} of MPT**[5]**.

A new and novel UV spectrophotometric method has been developed for the determination Cu(II) in synthetic mixture and water samples. The method is based on complex formation of Cu(II) with cefixime, [(Cefixime)(Cu)]$^+$, immediately in 1, 4-dioxan-distilled water medium at room temperature, see Scheme 2. The complex showed maximum absorption wavelength at 336 nm**[6]**.



Scheme 2: Chemical structure of Cu(II)-cefixime complex, [(Cefixime)(Cu)]$^+$, immediately in 1, 4-dioxan-distilled water medium at room temperature.

Cephalosporins (as cefixime) were determined spectrophotometrically in the pure form and in pharmaceutical

formulations by using ferrihydroxamate method. Using cefotaxime sodium as model drug with ester functional group, it was shown that proposed method gives equally accurate and precise results even in the presence of ester functional group**[7]**.

A sensitive, accurate and rapid flow injection analysis (FIA) method for the determination of cefotaxime, cefuroxime, ceftriaxone, cefaclor, cefixime, ceftizoxime, and cephalexin is proposed. The results have been compared with those obtained using the official methods. Excellent agreement between the results of the proposed method and the official methods was obtained**[8]**.

Two spectrophotometric methods have been developed for the determination of cefixime in pure and in its pharmaceutical formulations at λ_{max}=290 and 720 nm. These methods obey Beer's law in the concentration range of 1 to 15 µg.mL^{-1} and 5 to 25 µg.mL^{-1} respectively. The methods are statistically evaluated for accuracy and precision**[9]**.

Three simple and sensitive spectrophotometric (at λ_{max}=630 nm), difference spectroscopic (at λ_{max}=268 nm), and liquid chromatographic (LC) methods are described for the determination of cefixime. The conditions were optimized, and Beer's law was obeyed for cefixime at 1 to 16 µg.mL^{-1} and 10 to 50 µg.mL^{-1}, respectively. The third method, high-performance LC, was developed for the determination of cefixime using 50 mM potassium dihydrogen phosphate (pH 3.0) methanol (78 + 22, v/v) as the mobile phase and measuring the response at λ_{max}= 286 nm. The calibration curve was obtained for cefixime at 5 to 250 µg.mL^{-1}. The results obtained in the analysis of dosage forms agreed well with the contents stated on the labels**[10]**.

A simple, accurate and precise colorimetric method for the analysis of pharmaceutical formulation containing both ofloxacin and cefixime was developed. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies**[11]**.

A simple, precise and accurate kinetic spectrophotometric method for determination of cefradine anhydrous, cefaclor monohydrate, cefadroxil monohydrate, cefalexin anhydrous and cefixime in bulk and in pharmaceutical formulations has been developed. The method has been successfully applied to the determination of the studied drugs in commercial pharmaceutical formulations**[12]**.

A simple, accurate and precise spectrophotometric method has been proposed for the determination of eleven cephalosporins (as cefixime) in bulk drug and in pharmaceutical formulations. The method was successfully applied for analysis of the studied drugs in their pharmaceutical formulations and the recovery percentages ranged from 96.6 to103.5%**[13]**.

An accurate and precise colorimetric method is presented for the determination of ofloxacin and cefixime in same pharmaceutical formulation. Cefixime forms a greenish colored product with Fehling solution and the absorbance of greenish colored species formed was measured at 490 nm against reagent blank and Beer's law was obeyed in the concentration range of 5-40 µg.mL^{-1}. The amount of cefixime and ofloxacin present in the sample was computed from calibration curve**[14]**.

A simple, sensitive, rapid, accurate, precise and economic dual wavelength spectrophotometric method was developed for the simultaneous determination of cefixime trihydrate (CEFI) and ofloxacin (OFLO) in combined tablet dosage form. The method was based on determination of ofloxacin at 350 nm using its absorptivity value and cefixime at 264 nm after deduction of absorbance due to ofloxacin. The two drugs follow Beer-Lambert's law over the concentration range of 2-14 µg.mL^{-1}. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found**[15]**.

A new simple, accurate and cost-effective spectrophotometric method has been developed for the analysis of some cephalosporins (as cefixime) in bulk samples and pharmaceutical dosage forms. Beer's law was obeyed at concentrations ranging from 5 to 60 µg.mL^{-1} with correlation coefficients >0.9980 in all cases. Overall recoveries were of the order of 95–103% with errors generally less than 6%. The method was successfully applied to the determination of the cephalosporins in dosage forms**[16]**.

Spectroscopic analytical study for the determination of cefixime in pure and its Syrian pharmaceutical formations through complexation with Cu(II) in acetate buffer at pH = 7.8 has been developed. The method is based on the formation pink colour complex between cefixime and Cu(II). The maximum absorbance of the coloured complex occurred at λ = 546 nm and the molar absorptivity is 3.28 × 10^3 L mol^{-1} cm^{-1}. The reaction conditions have been optimized to obtain the complex. Under optimum conditions the absorbance of complex was found to increase linearly with increase in concentrations of cefixime, which corroborated with the correlation coefficient values (1:1). The linear range of the calibration curve was 0.453-9.069 mg mL^{-1} with correlation coefficients = 0.9975 in all cases. Overall recoveries were of the order of 98.00-101.50 %. The limit of detection and limit of quantification was found to be 0.075 mg mL^{-1}and 0.22 mg mL^{-1}, respectively. The proposed method was simple, economic, accurate and successfully applied to the determination of cefixime in Syrian pharmaceuticals, the results obtained agree well with the contents stated on the labels**[17]**.

UV-Vis spectroscopic method for the analysis of cefixime (CEFI) in pure form and pharmaceutical formations through complexation with Cu(II) using acetate-NaOH buffer in mixture water:methanol has been developed. Optimal temperature and time for coupling were established at 25±5^{o}C with time ranging from 0 to10min. The formation complex, CEFI:Cu(II) gives maximum absorbance of the yellow color occurred at λ= 410 nm and the molar absorptivity is 5.12 x 10^3 L.mol^{-1}.cm^{-1}. The reaction between CEFI and Cu(II) occurred at a stoichiometric ratio of 1:1. All reaction conditions have been optimized to obtain the complex. Under optimum conditions Beer's law was obeyed at concentrations ranging from 0.2267 to 22.671 µg.mL^{-1}with correlation coefficients ≥0.9995 in all cases with RSD generally less than 4.0%. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.030 µg.mL^{-1} and 0.091 µg.mL^{-1}, respectively. The proposed method was simple, economic, accurate and successfully applied to the determination of CEFI in pharmaceuticals with average recovery of 98.00 to 103.00%, the results obtained agree well with the contents stated on the labels**[18]**.

The spectrophotometric (UV and Vis)**[1-20]**, chromatographic (HPLC, GC and TLC)**[9, 21-23]** and electrochemical (polarography, voltammetry and other)**[24-30]** methods most prevalent to identify different drugs in pure and pharmaceutical dosage forms.

In the present work, Novel formation three complexes of cefixime-copper using acetate–acetic acid buffer at pH4.8 and determination of cefixime in pure and pharmaceutical dosage forms in methanol has been applied.

MATERIALS AND METHODS

Instruments and apparatus

Spectrophotometric measurements was made in a Biotech E.M**.** UV-Visible spectrophotometer with 1.00 cm quartz cells. The pH measurement was performed with EUTECH COPERSCAN-500. A ultrasonic processor model POWERSONIC 405 was used to sonicate the sample solutions. The solution was kept in a thermostat at 50^{o}C. The diluter pipette model DIP-1 (Shimadzu), having 100 µL sample syringe and five continuously adjustable pipettes covering a volume range from 20 to 5000 µL (model PIPTMAN P, GILSON), were used for preparation of the experimental solutions. Electronic balance (Sartorius-2474; d=0.01 mg) was used for weighing the samples.

Reagents

Cefixime tree hydrate (99.0%) was of pure from Parabolic Drugs-INDIA, the purity 88.6% as cefixime, which was determined by HPLC method**[10]**. Copper perchlorate hexahydrate was of pure from Fluka (Switzerland). Sodium acetate, acetic acid and all other reagents were of analytical grade, and alcohols were of extra pure from Merck (Germany).

Buffer solution: 0.118 M $NaCH_3COO$ + 0.082 M CH_3COOH was prepared in distilled water.

A ***stock solutions of cefixime:*** An accurately weighed 0.12795 g standard sample of Cefixime tree hydrate was dissolved in methanol, transferred into a 25 mL standard flask and diluted to the mark with methanol to obtain 0.01 $mol.L^{-1}$cefixime (waiting time more than 24 h). $2.5x10^{-3}mol.L^{-1}$ (1.1336 $mg.mL^{-1}$) and $2.5x10^{-4}$ $mol.L^{-1}$ (113.36 $\mu g.mL^{-1}$) were prepared in methanol, stock solutions of cefixime (a) and (b) respectively.

A stock solution of Cu(II) (c): $2.5x10^{-2}mol.L^{-1}$ and ***(d)*** $2.5x10^{-5}$ $mol.L^{-1}$ were prepared in methanol.

Sample preparation

A commercial formulations (tablet and capsule) were used for the analysis of cefixime by using spectrophotometric analysis. The following commercial formulations were subjected to the analytical procedures:

(1) ***Supraxime*** (tablet or powder for syrup), Asia pharmaceutical industries- Aleppo – SYRIA, each tablet contains: 400 mg or 200 mg cefixime, and powder (25 g in bottle) for syrup contain 1200 mg/bottle (48 mg CEFI in 1 g powder).

(2) ***Cifime*****,** Delta for medicaments -Aleppo–SYRIA, Each capsule contains: 400 mg cefixime and each tablet contains: 200 mg cefixime.

(3) ***Cefixime-ElSaad***, ElSaad pharma - Aleppo–SYRIA, Each tablet contains: 400 mg or 200 mg cefixime.

(4) ***Bioxime*** (tablet), Shifa pharmaceutical industries –Aleppo–SYRIA, each tablet contains: 400 mg cefixime.

(5) ***Cefix*** (capsule)**,** Alpha, Aleppo pharmaceutical industries - Aleppo–SYRIA, Each capsule contains: 400 mg cefixime**.**

Stock solution of pharmaceutical formulations

Crushed three tablets (or the contents of three capsules) of each studied pharmaceutical formulations, mix well and weigh equivalent tenth the weight of one tablet (or content one capsule) or 1041.67 mg from powder for syrup (contain 50 mg CEFI), solve it in 40 ml methanol by using ultrasonic, filtered over a 50 mL flask and diluting to 50 mL with methanol. The stock solutions content: 800, 400 and 1000; 800 and 400; 800 and 400; 800; and 800 $\mu g.mL^{-1}$ of cefixime for mentioned pharmaceuticals respectively.

Working solutions of pharmaceuticals

The solutions were prepared daily by diluting 0.050 mL from stock solution of pharmaceutical formulations in 20 mL methanol (content 1.0 mL buffer solution) and 2.00 mL from stock solution (c) of Cu(II) and diluting to 25 mL with methanol (working solutions content: 1.600, 0.800 and 2.000; 1.600 and 0.800; 1.600 and 0.800; 1.600; and 1.600 $\mu g.mL^{-1}$cefixime for mentioned pharmaceuticals respectively).

Working standard addition solutions of pharmaceuticals

The solutions were prepared as the follows: same mentioned volumes of stock solutions of pharmaceuticals in 20 mL methanol (content 1.0 mL buffer solution) and 2.00 mL stock solution (c) of Cu(II) with 0.200, 0.400, 0.600, 0.800 and 1.000 mL from stock solution (b) of cefixime and diluting to 25 mL with methanol.

Procedure: A 10 mL volume of a solution containing an appropriate concentrations of cefixime and Cu(II) in methanol with buffer solution (or working solutions of pharmaceuticals or working standard addition solutions of pharmaceuticals) at temperature 50 ± 2°C within 25 min at λ= 450 nm be ready for measurement.

RESULTS AND DISCUSSION

The different experimental parameters affecting the formed of cefixime:Cu(II) complexes were extensively studied in order to determine the optimal conditions for the formation complexes and determination of cefixime.

Spectrophotometric results

UV-Vis spectra by using acetate-acetic acid buffer as blank were studied. The Cu(II) solutions and cefixime solutions do not absorb in range 400-600 nm, while the cefixime:Cu(II) complexes solutions in buffer have absorption at occurred at λ= 510, 500 and 450 nm, and the molar absorptivity is $1.52x10^3$, $1.06x10^3$and $3.42x10^3$ $L.mol^{-1}.cm^{-1}$, respectively, (Figure 1).

Composition of cefixime:Cu(II) complexes

The stoichiometric ratio between CEFI and Cu(II) of investigated macrolides in methanol using acetate-acetic acid buffer at pH4.8 and formation of cefixime:Cu(II) complexes were employed to determine by mole-ratio method.

Molar ratio method

The stoichiometry of cefixime:Cu(II) complexes by molar ratio method according to following equation: A_{max}= f([Cu(II)]/[cefixime]), confirms that the ratio of complex cefixime:Cu(II) is equal to 2:1, 1:1 and 1:2 (We suggest to them the following formulas: $[(CEFI)_2(Cu).2HCl]$, [(CEFI)(Cu).HCl] and $[(CEFI)(Cu)_2.2HCl]$, respectively); see Scheme 3 and Figures (2, 3). Where the concentration of cefixime is constant 1×10^{-4} M and the concentrations of Cu(II) is change from 0 to$3x10^{-4}$ M. Figure 2 also showed that, UV-Vis spectra have one isosbestic point at λ=310 nm.

The effect of temperature

The effect of temperature on the produced adduct was studied. It was found that heating at 50±1°C was better than another temperature.

The effect of time

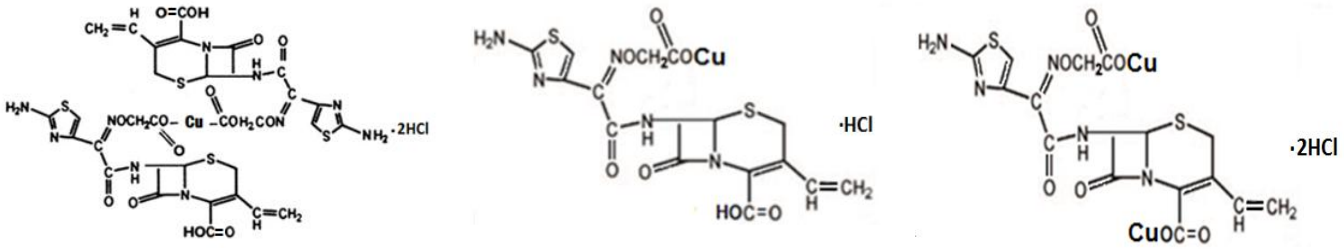
The effect of time on formation of complex was studied. It was found that better time was 25 min.

The effect of acetate-acetic acid buffer in methanol (96%, v)

The better buffer was continue sodium acetate (0.118 M) and acitic acid (0.082 M) in water.

Calibration curve

The calibration curves for cefixime in pure form through formation complex {$[(CEFI)(Cu)_2.2HCl]$} showed excellent linearity over concentration ranges of 0.4535-18.138 $\mu g.mL^{-1}$, see Figure 4. The spectra characteristics of the cefixime:Cu(II) complexes solutions as ε, λ_{max}, Beer's law, the equation (y=0.007520x+0.000643; y=absorbance, x=concentration of cefixime in $\mu g.mL^{-1}$, 0.000643 = intercept and 0.007520 = slope) and the correlation coefficient (R^2=0.9984) are summarized in Table-1.



$[(CEFI)_2(Cu).2HCl]$ **[(CEFI)(Cu).HCl]** **$[(CEFI)(Cu)_2.2HCl]$**

Scheme 3: Chemical structure of three complexes of cefixime-copper using acetate–acetic acid buffer at pH4.8 in methanol (96%, v).

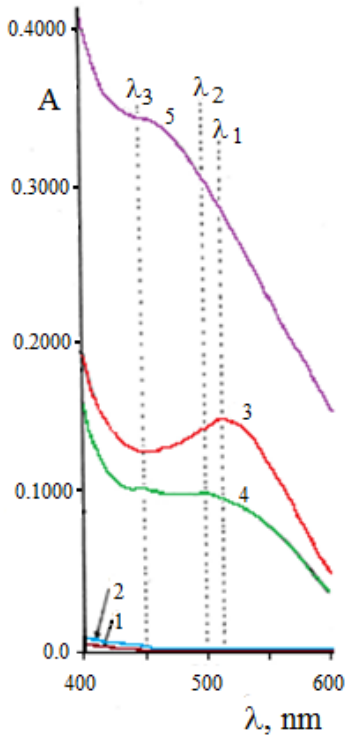


Fig. 1: UV-Vis spectra of:

1- 1x10^{-4} M Cu(II); 2- 1x10^{-4} M Cefixime (CEFI); 3- 1x10^{-4} M ***complex$_1$*** [(CEFI)$_2$(Cu).2HCl] at λmax$_1$=510 nm, {1x10^{-4} M CEFI with 0.5x10^{-4} M Cu(II)}; 4- 1x10^{-4} M ***complex$_2$*** [(CEFI)(Cu).HCl] at λmax$_2$=500nm, {1x10^{-4} M CEFI with 1x10^{-4} M Cu(II)}; 5- 1x10^{-4} M ***complex$_3$*** [(CEFI)(Cu)$_2$.2HCl] at λmax$_3$=450 nm, {1x10^{-4} M CEFI with 2x10^{-4} M Cu(II)}; {using acetate-acetic acid buffer at pH4.8 in methanol (96%, v); ℓ =1cm}.

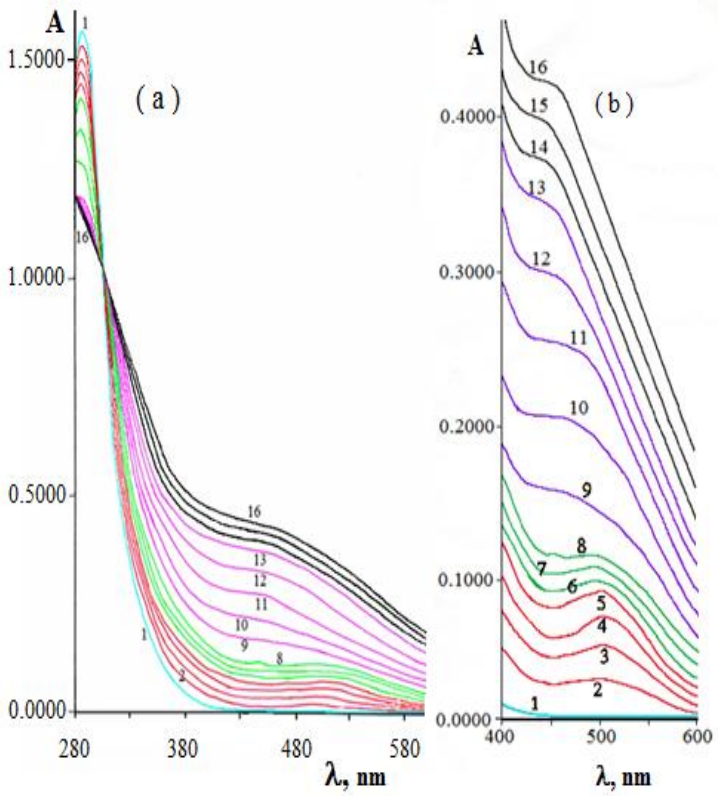


Fig. 2: UV-Vis spectra of 1.00x10^{-4} M cefixime with Cu(II) at concentrations as the follows:

1- 0; 2- 0.125x10^{-4}; 3- 0.250x10^{-4}; 4- 0.375x10^{-4}; 5- 0.500x10^{-4}; 6- 0.667x10^{-4}; 7- 0.833x10^{-4}; 8- 1.000x10^{-4}; 9- 1.200x10^{-4}; 10- 1.400x10^{-4}; 11- 1.600x10^{-4}; 12- 1.800x10^{-4}; 13- 2.000x10^{-4}; 14- 2.333x10^{-4}; 15- 2.667x10^{-4} and 16- 3.000x10^{-4}M. {using acetate-acetic acid buffer at pH4.8 in methanol (96%, v); ℓ =1cm}.

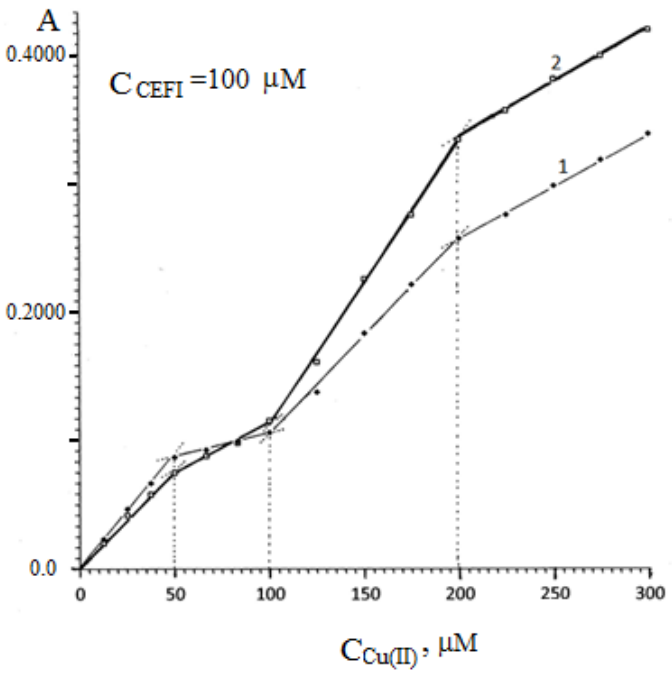


Fig. 3: Molar ratio method to calculate coupling ratio for cefixime:Cu(II) complexes:

[(CEFI)$_2$(Cu).2HCl], [(CEFI)(Cu).HCl] and [(CEFI)(Cu)$_2$.2HCl] using acetate-acetic acid buffer at pH4.8 in methanol (96%, v) as blank, CCEFI=1x10^{-4}M (100 µ M),

1- λmax= 510 nm; 2- λmax= 450 nm, (ℓ =1 cm).

Analytical results

Spectrophotometric determination of cefixime through complexation with Cu(II) using acetate-acetic acid buffer in optimal conditions using calibration curve was applied. The results, which summarized in Table 2 showed that, the determined concentration of cefixime was rectilinear over the range of 0.4535 to 18.138 µg.mL^{-1} with relative standard deviation (RSD) was not than 4.8%. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.036 µg.mL^{-1} and 0.109 µg.mL^{-1}, respectively. The proposed method was validated statistically and through recovery studies. The method was successfully applied for the determination of cefixime in pure and dosage form with percent recoveries from 96.59 to 102.16%. The results obtained from the proposed method have been compared with the official HPLC method**[10]** and good agreement was found between them.

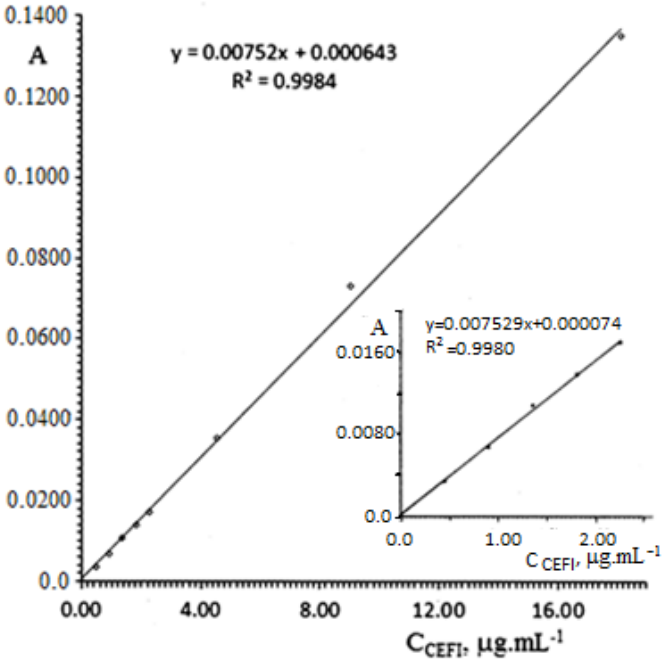


Fig. 4: Calibration curve for determination cefixime through complexation with Cu(II) using acetate-acetic acid buffer at pH4.8 in methanol (96%, v) according to optimal conditions for formation complex [(CEFI)(Cu)$_2$.2HCl]. (ℓ = 1 cm, $\lambda_{max,3}$=450 nm).

Table 1: The optimum parameters established for novel formation three complexes of cefixime-copper using acetate–acetic acid buffer at pH4.8 and determination of cefixime in pure and pharmaceutical dosage forms in methanol.

Parameters	Operating modes
Time of maximum color intensity	25 min
Temperature of solution	50±1°C
Solvent	methanol
$\lambda_{max,\,1}$ of complex [(CEFI)$_2$(Cu).2HCl]	510 nm
$\lambda_{max,\,2}$ of complex [(CEFI)(Cu).HCl]	500 nm
$\lambda_{max,\,3}$ of complex [(CEFI)(Cu)$_2$.2HCl]	450 nm
Molar absorptivity (ε)	1.52x10^3 L.mol^{-1}.cm^{-1}
Molar absorptivity (ε)	1.06x10^3 L.mol^{-1}.cm^{-1}
Molar absorptivity (ε)	3.42x10^3 L.mol^{-1}.cm^{-1}
Working λ_{max}	$\lambda_{max,\,3}$=450 nm
Buffer solution	0.118 M $NaCH_3COO$+0.082 M CH_3COOH
$\lambda_{isosbestic}$	310 nm
ℓ	1 cm
Spectra range	400 – 600 nm
Concentration of Cu(II)	More than 3-5 times of $C_{cefixime}$
Beer's Law Limit, µg.mL^{-1}	0.4535 – 18.138
LOD(3.3SD), µg.mL^{-1}	0.036
LOQ (10SD), µg.mL^{-1}	0.109
Regression equation:	
Slope	0.007520
Intercept	0.000643
Correlation coefficient (R^2)	0.9984
RSD%	4.8

Table 2: Spectrophotometric determination of cefixime in pure form through formation of complex [(CEFI)(Cu)$_2$.2HCl] using acetate-acetic acid buffer at pH4.8 in methanol according to optimal conditions.

x_i, µg.mL^{-1} (taken)	$^*\overline{x}$, µg.mL^{-1} (found)	SD, µg.mL^{-1}	$\frac{SD}{\sqrt{n}}$, µg.mL^{-1}	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, µg.mL^{-1}	RSD %
0.4535	0.440	0.021	0.0093	0.440± 0.026	4.8
0.9069	0.867	0.039	0.017	0.867± 0.048	4.5
1.3604	1.33	0.057	0.025	1.33± 0.070	4.2
1.8138	1.88	0.073	0.032	1.88± 0.090	4.1
2.2673	2.27	0.091	0.041	2.68± 0.113	4.0
4.5345	4.83	0.17	0.074	4.83± 0.20	3.6
9.0690	8.77	0.27	0.122	8.77± 0.34	3.1
18.137	18.17	0.56	0.249	18.17± 0.69	3.1

* n=5, t= 2.776

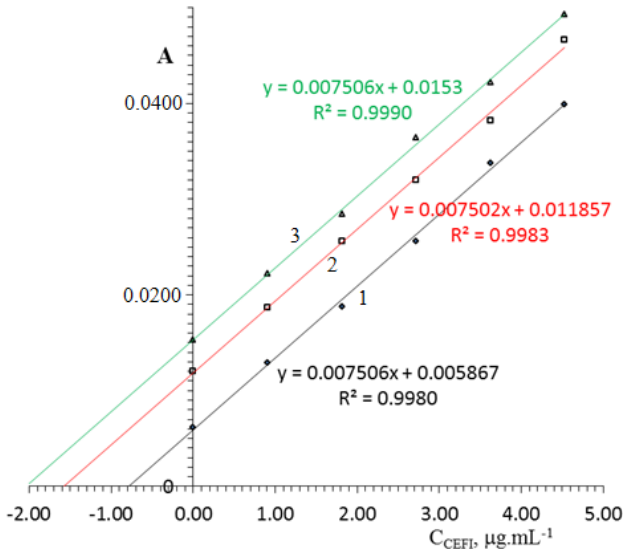


Fig. 5: The standard addition curve for determination of cefixime in *Supraxime* - Asia pharmaceutical industries, Aleppo–SYRIA using spectrophotometric method through formation of complex [(CEFI)(Cu)$_2$.2HCl] with acetate - acetic acid buffer at pH4.8 in methanol according to optimal conditions: 1- 200 mg/tab., 2- 400 mg/tab., 3- 1200 mg/25g powder or mg/bottle (for syrup).

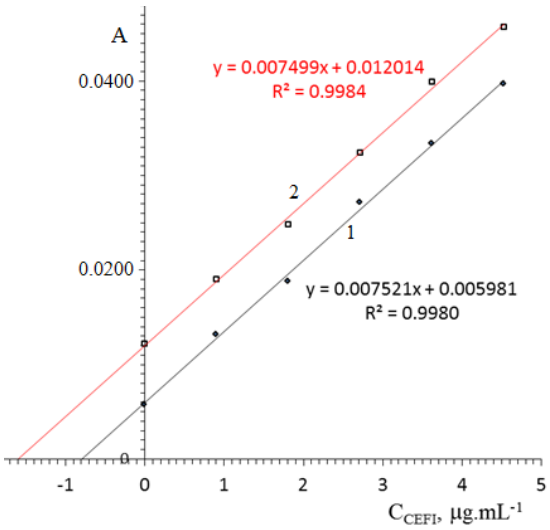


Fig. 6: The standard addition curve for determination of cefixime in *Cifime*, Ctd.tab., Delta for medicaments, Aleppo–SYRIA, using spectrophotometric method through formation of complex [(CEFI)(Cu)$_2$.2HCl] with acetate - acetic acid buffer at pH4.8 in methanol according to optimal conditions: 1- 200 mg/tab., 2- 400 mg/tab.

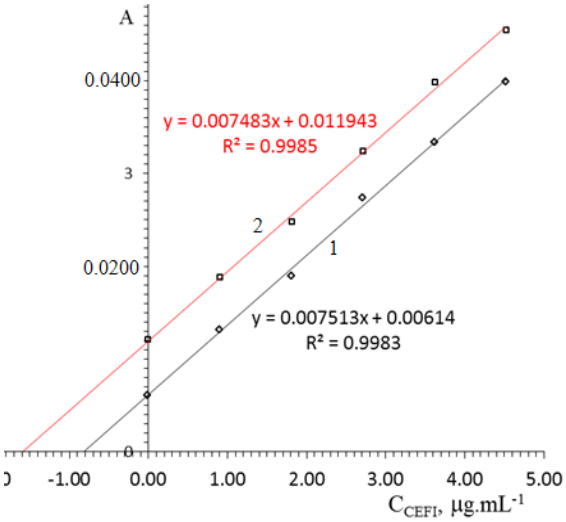


Fig.7: The standard addition curve for determination of cefixime in *Cefixime-ElSaad,* Al-Saad pharmaceutical industries, Aleppo–SYRIA, using spectrophotometric method through formation of complex [(CEFI)(Cu)$_2$.2HCl] with acetate - acetic acid buffer at pH4.8 in methanol according to optimal conditions: 1- 200 mg/tab., 2- 400 mg/tab.

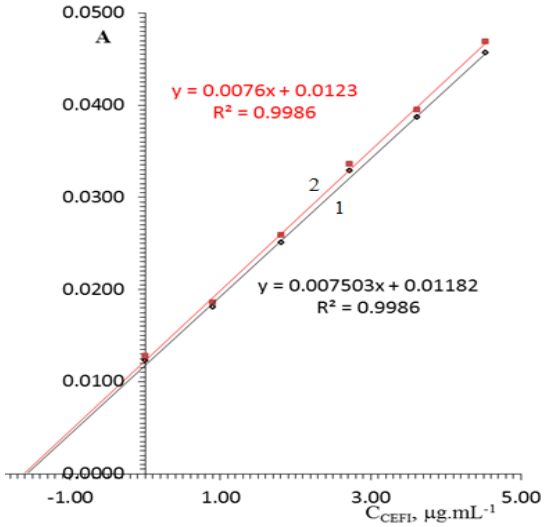


Fig. 8: The standard addition curve for determination of cefixime in *Bioxime*, 400 mg/tab. Shifa pharmaceutical industries, Aleppo – SYRIA (1) and *Cefix*, 400 mg/caps., Alpha, Aleppo pharmaceutical industries, Aleppo – SYRIA (2) using spectrophotometric method through formation of complex [(CEFI)(Cu)$_2$.2HCl] with acetate - acetic acid buffer at pH4.8 in methanol according to optimal conditions.

Table 3: Regression equations and correlation coefficients for spectrophotometric determination of cefixime in pharmaceuticals through formation of complex [(CEFI)$(Cu)_2$.2HCl] using acetate- acetic acid buffer at pH4.8 in methanol (96%, v) according to optimal conditions, (m' = intercept/slope, µg.mL^{-1})

Pharmaceutical preparations	Regression equations*	Correlation coefficients	m'	Amount of cefixime (m), mg/tab., caps. or mg/bottle (for syrup)
Supraxime-400 mg /tab.	y=0.007502x+0.011857	R^2=0.9983	1.5805	$m_{CEFI/tbl.}$=250m'=395.13
200 mg /tab.	y=0.007506x+0.005867	R^2=0.9980	0.7816	$m_{CEFI/tbl.}$=250m'=195.41
1200 mg/25g powder or mg/bottle (for syrup)	y=0.007506x+0.01530	R^2=0.9982	2.0384	$m_{CEFI/bottle.}$=600m'=1223.02
Cifime- 400 mg /caps.	y=0.007499x+0.012014	R^2=0.9984	1.6021	$m_{CEFI/caps.}$=250m'=400.52
200 mg /tab.	y=0.007521x+0.005981	R^2=0.9980	0.7952	$m_{CEFI/tbl.}$=250m'=198.80
Cefixime-ElSaad - 400 mg/tab.	y=0.007483x+0.011943	R^2=0.9985	1.5960	$m_{CEFI/tbl.}$=250m'=399.00
200 mg/tab.	y=0.007513x+0.006140	R^2=0.9983	0. 8173	$m_{CEFI/tbl.}$=250m'=204.31
Cefix- 400 mg /caps.	y=0.007600x+0.01230	R^2=0.9986	1.6184	$m_{CEFI/caps.}$=250 m'=404.60
Bioxime- 400 mg /tab.	y=0.007503x+0.01182	R^2=0.9986	1.5754	$m_{CEFI/tbl.}$=250m'=393.84

*y= A, x= concentration of cefixime (µg.mL^{-1})= m'.

Table 4: Spectrophotometric determination of cefixime in pharmaceuticals through formation of complex [(CEFI)$(Cu)_2$.2HCl] using acetate- acetic acid buffer at pH4.8 in methanol according to optimal conditions

Commercial name	Contents, mg/tab., or caps. or mg/bottle (for syrup)	$\bar{x}$, mg/tab.or caps., or mg/bottle (for syrup)	RSD %	Recovery %
Supraxime - 400 mg /tab.	400	395.13	4.5	98.78
200 mg /tab.	200	195.41	4.7	97.71
1200 mg/bottle (25 g powder for syrup)	1200	1223.0	4.3	101.92
Cifime- 400 mg /caps.	400	400.52	4.5	100.13
200 mg /tab.	200	198.80	4.8	99.40
Cefixime-ElSaad - 400 mg/tab.	400	399.00	4.5	99.75
200 mg/tab.	200	204.31	4.7	102.16
Cefix, 400 mg/caps.	400	404.15	4.4	101.15
Bioxime, 400 mg/tab.	400	393.84	4.4	98.46

* n=5

APPLICATIONS

Many applications for the determination of cefixime in some pharmaceutical preparations with a spectrophotometric method through complexation with Cu(II) using acetate–acetic acid buffer at pH4.8 in methanol (96%, v) according to optimal conditions were proposed.

Regression equations and correlation coefficients were included in Table 3. Standard addition curves for determination of cefixime in different pharmaceutical preparations were used, see Fig. 5-8. The amount (m) of cefixime in one tablet (or one capsule or one bottle for syrup powder by mg/tab., or mg/caps. and mg/bottle) calculated from the following relationship: m = h. m', where: m' is the amount of cefixime in tablet, capsule, or bottle (powder for syrup) calculated from the standard additions curve according to the following regression equation: y=a.x+b; when y=0; m'=x= b/a= intercept/slope (µg.mL^{-1}), h conversion factor is equal to 250 for tablet and capsule pharmaceuticals and 600 for syrup powder. The results of quantitative analysis for cefixime in some pharmaceutical preparations were calculated using the standard additions method were summarized in Tables 4. The proposed method was simple, economic, accurate and successfully applied to the determination of CEFI in pharmaceuticals with average recovery of 96.59 to 102.16%, the results obtained agree well with the contents stated on the labels. The results obtained by this method were validated by HPLC**[10]**.

CONCLUSION

Novel formation three complexes of cefixime-copper using acetate–acetic acid buffer at pH4.8 and determination of cefixime in pure and pharmaceutical dosage forms in methanol has been developed. The formation complexes, CEFI with Cu(II) gives, in the first time, three forms; the first one is [$(CEFI)_2$(Cu).2HCl], the second is [(CEFI)(Cu).HCl] and the third [(CEFI)$(Cu)_2$.2HCl]. The reaction between CEFI and Cu(II) occurred at stoichiometric ratio of 2:1, 1:1 and 1:2, respectively. The maximum absorbance of the complexes occurred at λ= 510, 500 and 450 nm. The molar absorptivity is 1.52 x 10^3, 1.06 x $10^3$and 3.42 x 10^3 L.mol^{-1}.cm^{-1}, respectively. All reaction conditions have been optimized to obtain the complexes. Under optimum conditions Beer's law was obeyed at concentrations ranging from 0.4535 to 18.138 µg.mL^{-1}with correlation coefficients ≥0.9980 in all cases with RSD generally less than 4.8%. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.036 µg.mL^{-1} and 0.109 µg.mL^{-1}, respectively. The proposed method was novel, simple, accurate and successfully applied to the determination of CEFI in pharmaceuticals with average recovery of 96.59 to 102.16%, the results obtained agree well with the contents stated on the labels**[10]**.

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