SPECTROPHOTOMETRIC ANN ATOMIC ABSORPTION DETERMINATION CEFIXIME BY CLOUD POINT EXTRACTION IN PURE FORM

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Abstract

A simple, rapid, accurate, sensitive and ecofriendly method has been developed for the quantitative determination of Cefixime (CFX) in pure form and pharmaceutical preparations by using a combination of cloud point extraction with UV-Visible absorption spectrophotometric method. Analytical applications of complexation with metal ions by reacting Cefixime (CFX) with Iron (III) to form chelate complexes under limited experimental conditions. The method based to dissolved CFX in 0.1 M NaOH, 10% (v/v) Triton X-114 and mixed with (1000 µg mL^{-1}) Iron (III). The formation of CFX- Fe (III) complex was formatted at pH 11 and wavelength at 439 nm. The complex of CFX- Fe (III) obey Beer's Law in the range 10-160µg/ml. LOD and LOQ values for the complex were 1.5865 µg/ml and 5.2887 µg/ml respectively. Method was validated and successfully applied to drug formulations like syrup infusion The results of analysis have been validated statistically and by recovery studies and were found satisfactory.

Keywords: ***antibiotic , Beers Law , Iron ion, absorption***

التقدير الطيفي و الأمتصاص الذري للسفكسيم بواسطة الاستخلاص بنقطة الغيمة في المادة النقية

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المستخلص

تم تطوير طريقة طيفية بسيطة وحساسة وسريعة وصديقة للبيئة لتقدير دواء السيفيكسيم في المادة النقية والمستحضرات الصيدلانية باستخدام طريقة الاستخلاص بنقطة الغيمة. تعتمد الطريقة على تكوين معقدات مع بعض ايونات العناصر كالحديد, تم اذابة السيفيكسيم في 0.1 M هيدروكسيد الصوديوم ,10 % X−114 ترايتون , وتمزج مع (1000 ميكروغرام مل $^{-1}$) الحديد (III)عندالرقم الهيدروجيني 11 والطول الموجي، 439 نم مع ايون الحديد (III). معقدات السيفيكسيم مع الحديد تطيع قانون البير في نطاق 10−160 ميكروغرام / مل. وكانت قيم حد الكشف وحد الكشف الكمي 1.58655 ميكروغرام / مل و 5.2887 ميكروغرام / مل تم التحقق من صحة الطريقة وتطبيقها بنجاح على تركيبات الأدوية مثل شراب. وقد تم التحقق من صحة نتائج التحليل إحصائيا ودراسات كانت مرضية.

الكلمات المفتاحية :مضادات حيوية ، الامتصاصية، عنصر الحديد الثلاثي، قانون البير.

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INTODUCTION

that are produced by microorganisms and by chemical synthesis ,Antibiotics are drugs preparations which contain some chemical substances. These substances at very low concentrations are known to totally destroy or partially inhibit microorganisms, Antibiotics are the chemotherapeutic agents that kill or inhibit the growth of microorganisms. Antibiotics have wide spread application in the treatment of bacterial disease (3) Cefixime is the only oral third generation cephalosporin with a broad spectrum of antimicrobial effect on Haemophilus influenzae, Moraxella catarrhalis, Neisseria gonorrheae, Escherichia coli and Klebsiella resistant to ampicillin, other oral cephalosporins and trimethoprim-sulfamethoxazole. This characteristic of cefixime permits its use in urinary and respiratory tractinfections (6) Cefixime (CFX)((6*R*,7*R*)-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), is a compound with potent mucolytic activity, for which it is used as an expectorant and bronchosecretolytic in therapeutics (13) The structures of drugs are shown in (Figure.1)

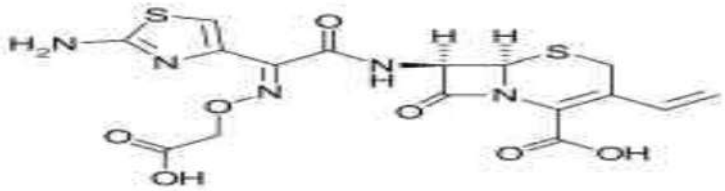

Figure 1. The structure of Cefixime [14]

It is used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. One of the major problems with this drug is its very poor solubility in biological fluids that results into poor bioavailability after oral administration. It shows erratic dissolution problem in gastric and intestinal fluid due to its poor water solubility. Rate of absorption and/or extent of bioavailability for such insoluble drugs are controlled by rate of dissolution in gastrointestinal fluids (2) which describes a liquid chromatographic method for its assay in bulk form. In order to assure the quantity of cefixime in dosage forms, several methods have been reported which include liquid chromat- ography-mass spectrometry (10) ,high performance liquid chromatography (7-10) ,high performance thin layer chromatography (11-12) derivative spectrophotometry (16), voltammetry (7) , and capillary electrophoresis (12). The cloud point procedure (CPE) is based on the following phenomenon: an aqueous solution of some surfactant be comes turbid and separation to two isotropic phases if some condition such as temperature or pressure is changed or if an appropriate substance is added to the solution (14) This study was aimed to develop simple, economical, rapid, precise,accurate and ecofriendly method for determination of single drug by using Cloud Point Extraction

MATERIALS AND METHODS

Instrumentation and apparatus

1- UV-Visible spectrophotometer SHIMADZU, Double beam UV-Vis, model -1800 made (Japan)

2- Hotplate Stirrer (Model L-81 Labinco bv).

3-Electric Balance (Sartorius, 4digitals, made in Germany).

4-OVEN (Memmert , maximum temperature 250,made in western Germany)

5-Water Bath **(**A thermostat water Bath, model Unitemp**)**

6-Centrifuge (Triup International corp ,TRIU 800 Centrifuge ,made in Korea**).**

7-pH-meter (model BP 3001).

8- Atomic absorption spectrophotometry (AAS)**,** Company using GBS-933 Flame Plus Atomic Absorption Spectrophotometer

Table 1. Standard conditions for Atomic Absorption

Element	*λ(nm)*	maximum current *(mA)*	*SBW (nm)*	*Flame gases*	*SENS check(a)*
Fe	**248.3**	**30**	**0.2**	**Air-Acetylene**	**6**

Drug and Materials

The chemicals used for this work are of high purity and used as received. distilled water was used in the preparation of all solutions and for final rinsing of glass wares. A pure grade of Cefixime was obtained from Drug

Industries and Medical Appliance (SID) Samarra/ Iraq. A stock solution of (2.205×10^{-3} M) for the drug Cefix was prepared by dissolving 0.1g in minimum amount of water and diluted to mark with water in a 100 ml volumetric flask. 0.1 M of NaOH (BDH, UK) . A stock solution (1000 µg mL^{-1}) of Iron Ion (III) (95.5%, Sigma, USA) were prepared by dissolving 2.9g of Iron Ion in 1000 mL volumetric class . Triton X-114 (purity >99.9%), from AMRESCO LLC (Solon, USA). A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL with water in a 100 mL volumetric flask.

Preparation of buffer solutions

Table 1. The Preparation of bicarbonate buffer solutions

Buffer : pH 11.0	
100 mL (0.05)M $NaHCO_3$ +mls of 0.1 M NaOH	
pH	mls of 0.1 M NaOH added
11.0	45.4 mL

Table 2. The Preparation of hydrogen ortho phosphate buffer solutions

Buffer : pH 11.0	
100 mL (0.05)M Na_2HPO_4 +mls of 0.1 M NaOH	
pH	mls of 0.1 M NaOH added
11.0	8.2mL

Recommended CPE Procedure for cefix drug: Aliquots 10 mL of a solution containing known amount of Cefixime drug was mixed with Fe^{+3}ion Then pH was adjusted by using 0.1M NaOH and 10% (v/v) Triton x-114. The mixture was shaken for 1 min and left to stand in a thermo-stated bath at 50 ^oC, for 20 min. Separation of the phases was achieved by centrifugation at 4000 rpm for 10 min, with stirring at 5°C in ice bath the remaining of micellar phase was dissolved by ethanol, the measurements of absorbance of the complex were followed by UV-visible spectrophotometer with used 1.0 cm quartz cell at λ max equal to 439 nm for CFX- Fe (III) complex against blank

Preparation of pharmaceutical samples

Two types of pharmaceuticals for CFX namely capsules and syrup . The powder of five capsules were mixed, homogenized, and the content of one capsule (0.5339g) which equivalent to 533.9mg of active drug was dissolved in sufficient amount of water with continuous shaking and filtered. The filtrate solution was transferred into a 100 mL volumetric flask and diluted to mark with water.solution contains 4000 µg mL^{-1} of CFX from which 1000 µg mL^{-1} was prepared by dilution. 25 mL containing different concentrations of the prepared sample solution were transferred to centrifugal tubes and each solution followed the recommended CPE procedure for cefix and the content of drug was measured spectrophotometrically at λ max of 439nm. the pharmaceuticals for syrup As each (5ml) from drug contains (100mg) Cefixime . Solution is prepared by taking (5mL) from syrup and dissolved in ethanol then solution is filtered and dilute in(100mL) volumetric flask by distilled water, so that it gives (1000µg mL^{-1}) from Cefix . The same procedure is applied for syrup , CPE procedure for Cefix and the content of drug was measured spectrophotometrically at λ max of 439 nm

2.5. Statistical Analysis

Excel 2010 (Microsoft Office R) was employed to carry out all statistical calculations

RESULTS AND DISCUSSION

Absorption spectra

In an attempt to ascertain the occurrence in the reaction system, an absorption maximum at 439 nm (Figure 2) which was adopted of CPE for the drug . The absorption spectrum of the complex product formed was also recorded against the corresponding metal blank between 200 to 1100 nm before obtaining optimum conditions according to the recommended CPE procedure using a SHIMADZU, Double beam UV-Vis, model UV-1800 with 1.0 cm quartz cell. It was observed that the absorption maximum of the product complex of Cefix in 1.0 mL of 10% TX-114 occurred 439nm, giving the molar absorptivities of 1.9×10^{2} $L.mol^{-1}.cm^{-1}$ for Cefix drug with Iron. Thus the wavelength maximum at 439nm for the Cefix complex product was used throughout this study for ppm amounts.

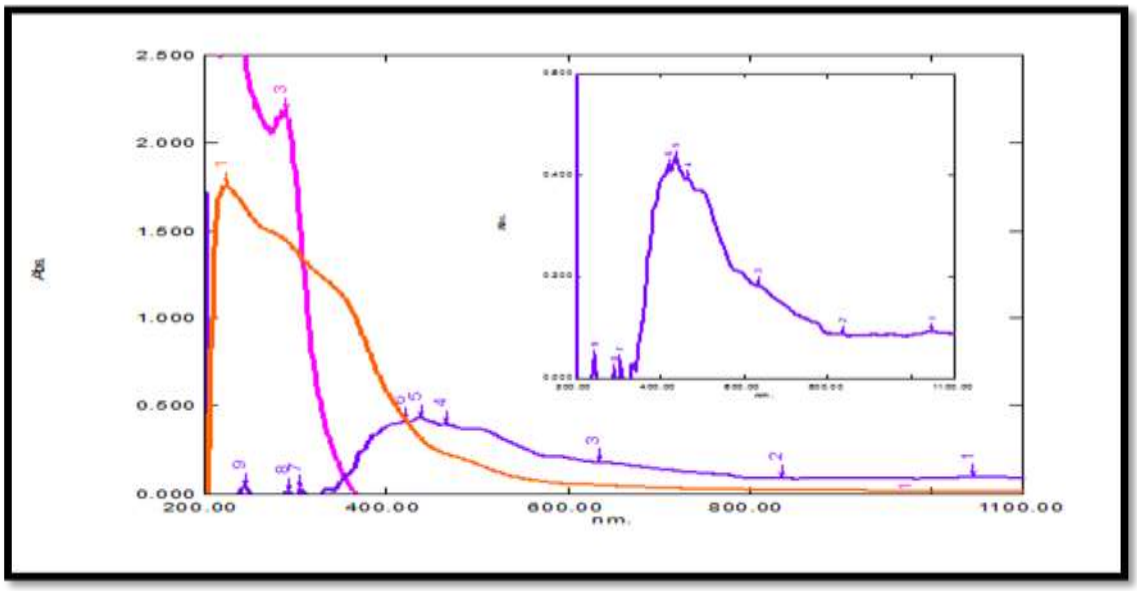

Figure 2. The absorption spectrum of the CFX - Fe(III) complex

Optimization of CPE

A group of experiments has been conducted to study the effect of several variables that affect the extraction efficiency of the CPE and maximize the sensitivity of the detection system for drug under study using a classical optimization. The variables such as the concentration of metal ion, best of pH, best of buffer, best of volume buffer, Triton X-114 amount, equilibration temperature and incubation time.

Effect of metal ions concentration

The effect of Iron ion concentrations upon the absorbance values of the extracted complex using (1000µg/mL) of drug solution. The optimum concentration of the metal ions that gave maximum absorbance was 80µg/ml of the optimum concentration of Fe(III) ion were for complex The absorbance is measured and the absorbance results are shows in Figure 3

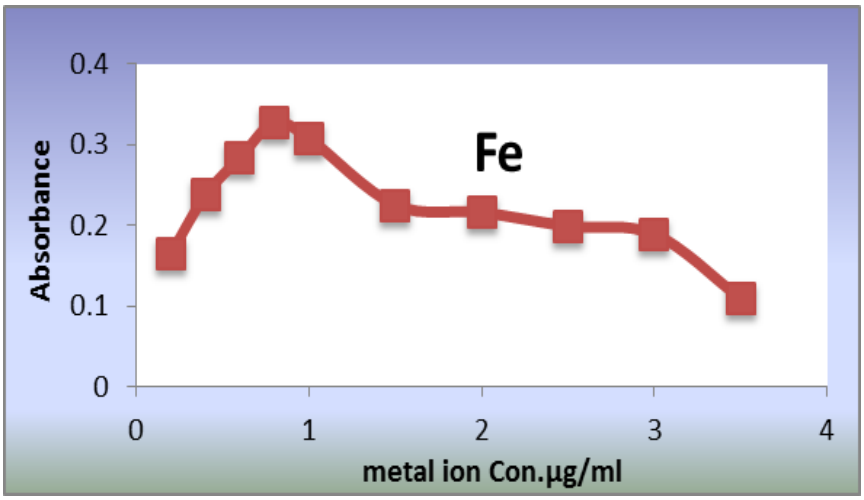

Figure 3. Effect of Optimum concentration Fe(III) ion conc. on absorbance of drug-metal complex

Effect of Ph

The pH plays a unique role on metal-ligand formation and subsequent extraction, and is proved to be a main parameter for CPE[17], to find the best basic function of the ion extraction process different value of pH 1-14.

The results are shown in Figure 4, the best separation was achieved at pH =11 for Fe(III) show the value of absorbance intensity for the complex drug- Fe against the value of pH , Plotting of the absorbance values the value of pH is shown in figure 4

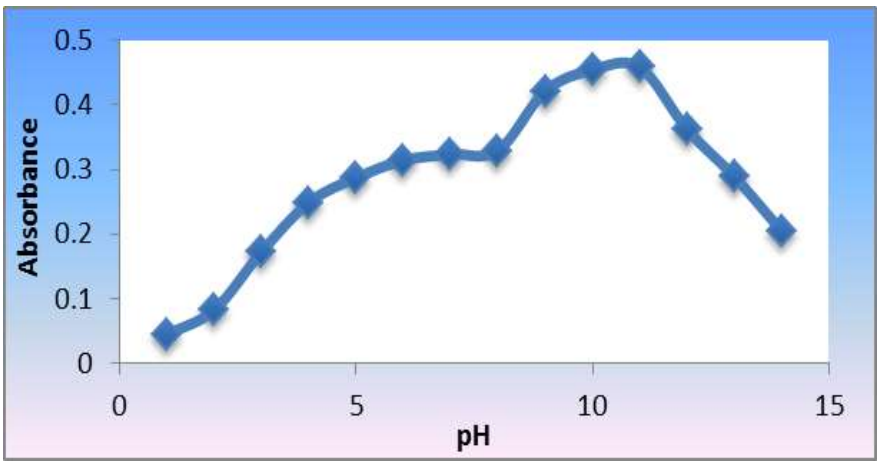

Figure 4. pH effect on the absorbance of drug- Fe(III) complex

Effect of buffer solutions

The best values of buffer pH 11 recorded for the highest absorbance values were ,The absorbance is measured the absorbance results are shown in table (3).for complex (Fe+ Cefixime)

Table 3. buffer pH 11

Preparation buffer pH 11	Absorbance
Sodium *bicarbonate buffer solutions*	**0.563**
Sodium hydrogen ortho phosphate	**0.268**

Effect of volumes buffer solutions

Figure (5) show the value of absorbance intensity for the complex drug- Fe against the value of buffer solution, the best values of sodium bicarbonate buffer solutions recorded for the highest absorbance values

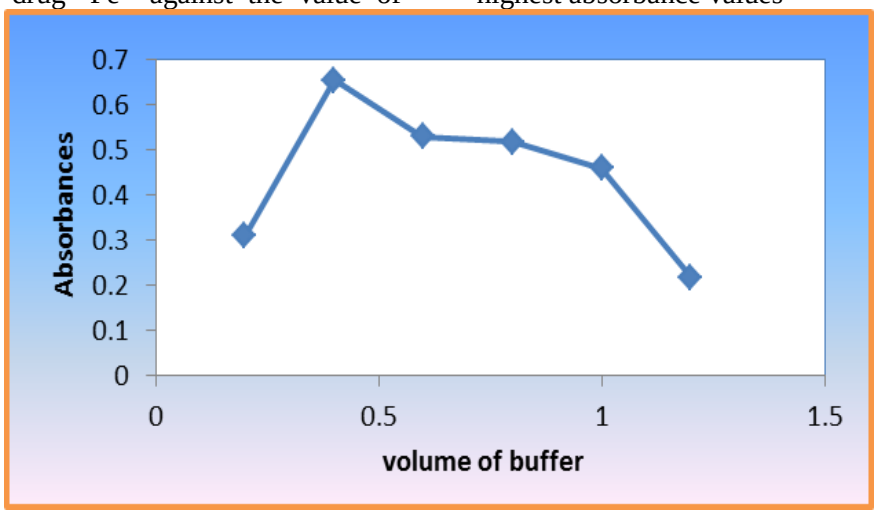

Figure 5. buffer of pH effect on the absorbance of drug- Fe(III) complex

3.2.5. Effect type of surfactant with complex

The type of surfactant plays very substantial role in cloud point extraction process where each surface owns spectral properties depend on practical basis of Micelles .Aliquots of 10mL of a solution contains [1mL Cefixime , 0.8mL Fe , 0.4 mL buffer pH 11] for Iron metal in 10mL volumetric flask and use different surfactant for complex [Tween 20, Tween80, CTAP, SDS, Triton X-100, Triton X-114] at 50^0C for 20 min incubation time then it centrifugeted at 4000 rpm for 10min , separated the surfactant- rich phase and dissolved in 1mL ethanol then measured by UV-Vis at λ**max** = 439nm for complex results shown in Figure 6 It was observed that Triton ×- 114 which have maximum absorbance at 439 nm is Plotting the absorbance values of the cloud point versus the type of surfactant

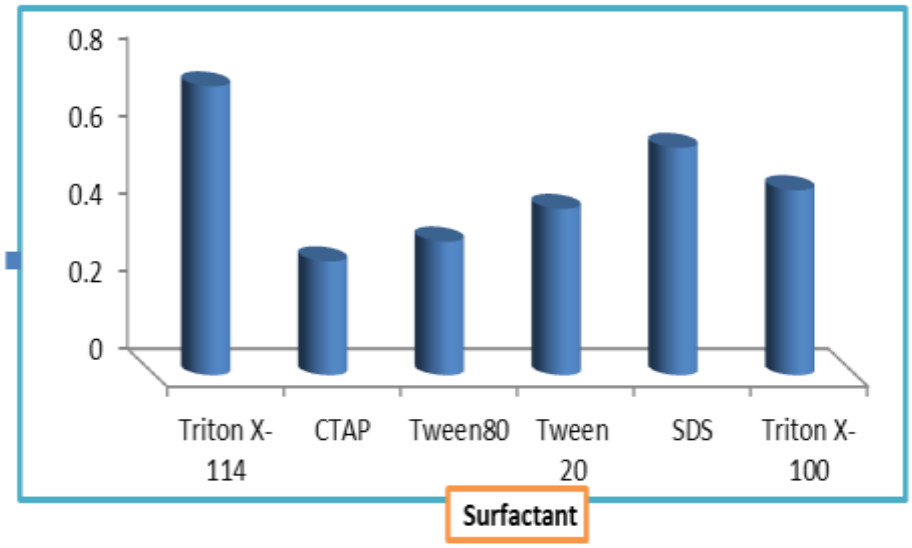

Figure 6 . Type of Surfactant for for Fe(III)

Effect of Triton X-114 Amount

Most studies confirm that the amount of an nonionic surfactant type TX-114 as an extracting medium plays an important role for maximizing the extraction efficiency by minimizing the phase volume ratio (Vs/Va) and therefore improving the pre-concentration factor of the CPE procedure ' Therefore, the amount of TX-114 was investigated by varying the volume of 10% TX-114 between (0.2-2.0 mL) . The results are presented in Figure 7. It was noticed that the absorbance values of Cefix drug continued to increase dramatically and reached maximum at 1.0 mL of 10% TX-114 (i.e. 1. 0% TX-114 in 10 mL solution) for Fe metal . These values were selected as optimal amount and used in the proposed method for the detection of Cefix, Plotting the absorbance values of the cloud point versus the volume of Triton X-114 is shown in Figure 7

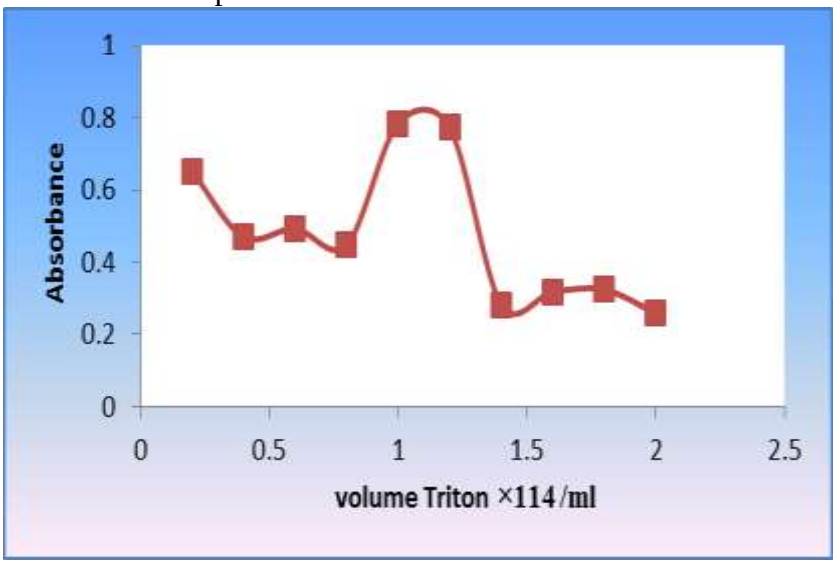

Figure 7. Effect of the TX-114 amount on absorbance of Complex product [Conditions: For complex

Effect of Equilibration Temperature

the efficient phase separation, which reflects certainly the magnitude of extraction efficiency of each target analyte. Figure 8 shows the variation on the absorption signal via varying the temperature between 35 to 80^{o}C at 20 min for incubation time for drug

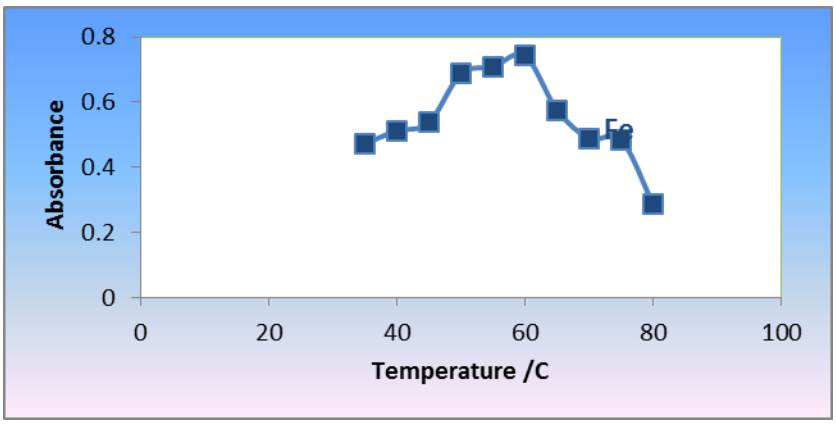

Figure 8. Absorbance Versus Temperature for (Fe-CEF)complex

The results show that the highest absorbance and extraction efficiency of the drug at temperature at 60°C for Cefixime with Fe(III)for 10 min complex then decreases in absorbance at higher temperature due to decomposition of product which reduces the extraction efficiency. This temperature is fixed in subsequent experiments.

Effect of the Incubation Time . Amount of 10mL solution is prepared containing , for complex [1mL Cefixime ,0.8mL Fe ,0.4mL buffer pH 11 and 1mL10%(v/v)Triton X-114] then it is completed to the mark by distilled water, are mixed and the temperature is 60^0C for Fe and the incubation time varies from (5-50) min to form cloud point extraction then is measured by UV-Vis at **λmax** = 439 nm for complex Figure 9

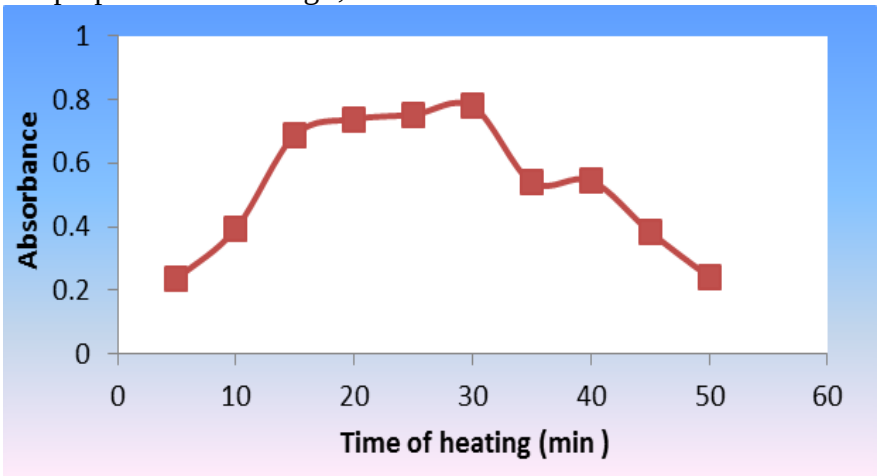

Figure 9. Absorbance Versus Time for (Fe -CEF)complex

This time represents the amount of heat accumulated in the solution that allows Micelles lose water molecules in order to give small size hydrophobic with high viscosity easily entrap the product in it. It is clear that the optimum incubation time is (30)min for Fe and Maximum absorbance for all extracted Fe (III) complex were observed after (30) min

Order of Additions

The effect of order for additions of the metal on the absorbance of each analyte by the general CPE was tested. Figure 10 shows that the best order of addition is the number 1 for target analytes due to giving a highest absorption signal among the others. The absorbance is measured and the absorbance results are shown in table 4

Table 4. Data of absorbance to order additions

No	Order Additions	Absorbance at λmax =439 for Fe(III)
1	*D+ M+B+T*	0.767
2	*M+D+B+T*	0.684
3	*D+B+M+T*	0.607
4	*M+B+D+T*	0.732

D= drug [Cefixime] , M= metal [Fe^{+3}] ,B= Buffer, T=Triton X-114

Plotting of the absorbance values versus the order additions is shown in Figure 10

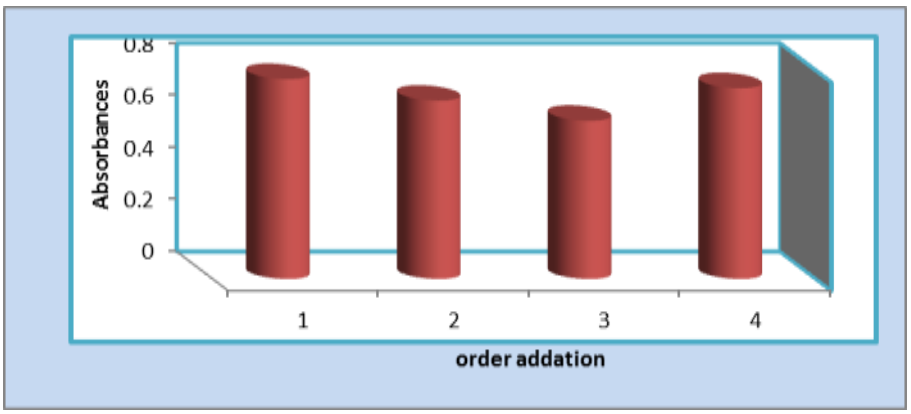

Figure 10 . Effect of Order Additions for (Fe-CEF) complex.

It is noted that the best addition is the first order of complex (Fe^{+3}-CEF) because if it's another order gets lost in the intensity of color and this order fixed in subsequent experiment

Effect of organic solvents

Different organic solvents are examined to evaluate their effects on the intensity of the resulting complex and Plotting of the absorbance values versus the solvent is shown in Figure 11

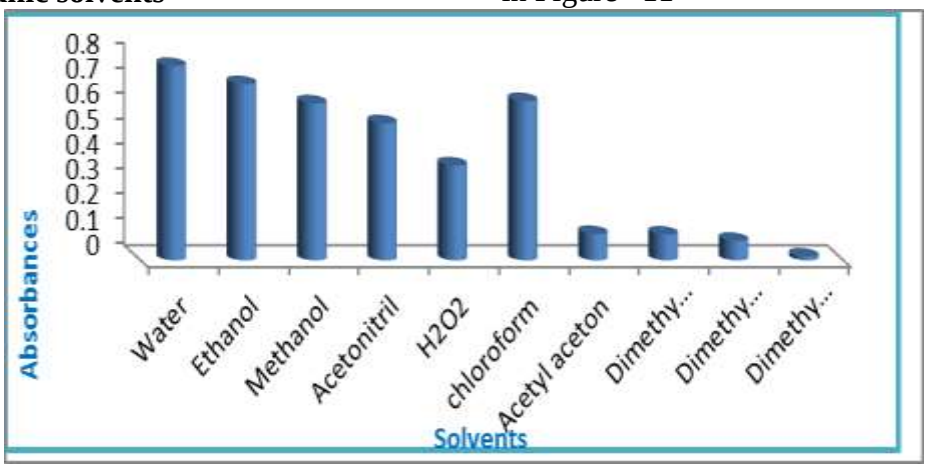

Figure 11. Effect of solvents for(Fe –CEF) complex

It has been shown that water is the optimum solvent, economically, sensitivity method, available, to provide and nontoxic. This solvent is fixed in subsequent experiment

Effect of interference

The effect of some foreign organic compounds and Inorganic compounds, which often found in environmental, were studied by adding 1mL of (100 µg/mL) Equal amounts organic compounds, Inorganic compounds to 1mL of (100 µg/mL) of complex. The color was developed following the recommended procedure described earlier

Table 5. Effect of interference

100ppm interference	Absorbance at λmax =439 for Fe
With out	0.772
Lactose	0.196
Starch	0.568
Arabic Gum	0.212
Talc	0.315
Glucose	0.248
$Ca_3(PO_4)_2$	0.201
$CaCO_3$	0.011

It was observed that the table 5 were not interfering with the determination at levels found in complex form

After the study of the effect of different physical and chemical conditions on the absorbance intensity of the colored product,

Selected optimum conditions

Table 6 . The optimum conditions for the determination of Cefixime

Optimum	Concentrations	Range selected	Optimum quantities of complex (Cefxi-Fe)
ƛ max(nm)	----	190-1100	439
Effect of volume of metal ion required	1000 ppm	0.2 -3.5 mL	0.8mL
Effect of PH	0.1M(NaoH)or 0.1M(HCl)	1-14	11
Buffer pH	----	----	Sodium bicarbonate buffer solutions
Effect of volume of Buffer	----	0.2-1.6mL	0.6mL
Effect of volume of triton x114 required	10%(v/v)	0.2 -2.0mL	1 mL
Effect of time heating	----	5-60 min	30 min
Cefixime solution required	1000 ppm	10 -160 ppm	100ppm

the optimum conditions for the proposed procedure were summarized in (Table 6) and were used in all subsequent experiments

Preparation of calibration curve in CPE Amount of 10ml solution is prepared containing increasing concentration of drug Cefixime by taking (10-160) µg mL^{-1} Cefixime ,0.8mL Fe ,0.4mL buffer pH 11 and 1mL 10%(v/v)Triton X-114] then it is completed to the mark by distilled water, are mixed ,heated at optimum temperature in the thermostat water bath at optimum incubation time, to form cloud point then aqueous phase is separated by centrifugation at 4000 rpm for 30min ,1mL ethanol is added to the surfactant-rich phase to dissolve it then is measured by UV-Vis at λ**max** = 439 nm for complex , triplicate manner The absorbance measurements are illustrated in table 7

Table 7 .The absorbance measurements of standard solutions of complex (CFX-Fe)

RSD%	Found	Recovery%
3.1126	9.8732	98
1.3157	19.3098	96
1.7169	27.478	91
0.3322	40.2957	100
0.4441	52.8309	105
1.2053	59.7323	99
0.2012	67.9014	97
0.4491	80.859	101
0.2980	92.4081	102
0.3614	101	101
0.5158	116.915	97
0.0989	140.295	100
0.0874	158.88	99

The calibration curve was . Plotting the mean absorbance values of the cloud point versus the concentration (ppm) of (CFX- Iron) as shown in Figure 12

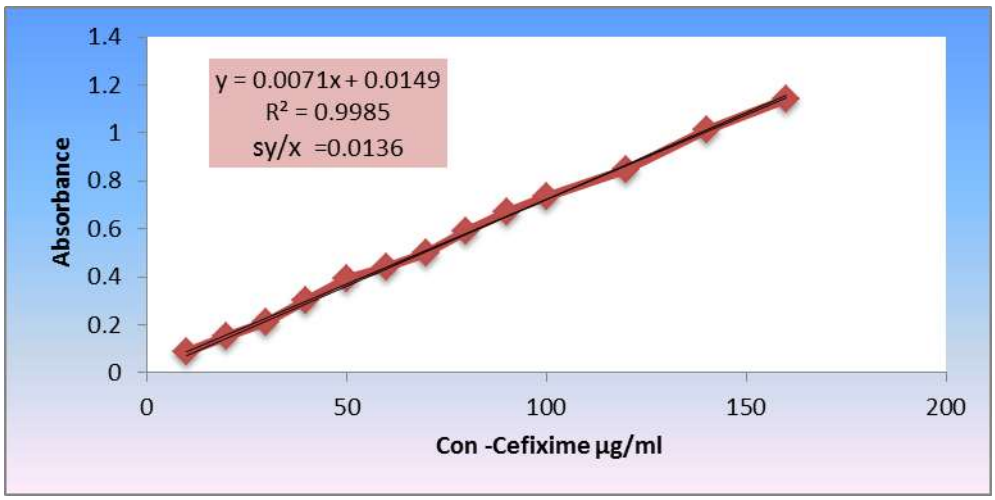

Figure 12. (Cefixime + Fe) calibration curve

Stoichiometric determination of color complex :

Continuous variation method (Job`s method): A series of (1, 2, 3, 4, 5, 6, 7, 8, 9) ml of (1×10^{-4}) mol $L^{-1}$of the solution that contain Cefixime was pipette into each of 10ml volumetric flask then(9,8,7,6,5,4,3,2,1) ml of (1×10^{-4}) mol $L^{-1}$of metal and Cefixime the absorbance of the solution was measured by UV-Vis spectrophotometer at λ_{max} 439nm the stoichiometric ratio between Cefixime with metal 1:1 results are shown in the Table 8

Table 8 .The continuous variation method of Cefixime with (Iron) complex

V D mL	V M mL	VD / VT	Absorbance at λ= for Color 439 compound
1	9	0.1	0.067
2	8	0.2	0.159
3	7	0.3	0.290
4	6	0.4	0.357
5	5	0.5	0.519
6	4	0.6	0.479
7	3	0.7	0.278
8	2	0.8	0.140
9	1	0.9	0.032

Plotting the value of absorbance versus the VD / VT is shown in Figure 13

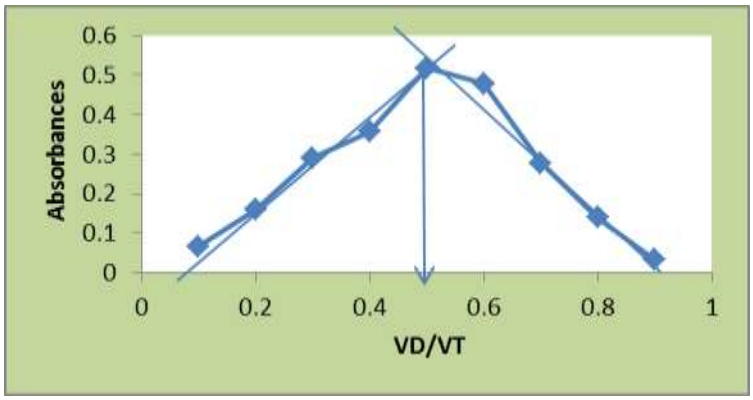

Figure 13 . Continuous variation method

V_D: values of the compound (Cefixime)

V_M: The values of the metal (Iron).

V_T: Total (V M+V D)

Mole – ratio method

Aliquots of 10 mL solution containing (1×10^{-4}) $molL^{-1}$ of (1mL) Cefixime and increasing concentrations (1×10^{-4}) mol L^{-1} of (0.2,0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8) mL of (Fe) Iron (2×10^{-6}--2×10^{-5})mol L^{-1} metal . The absorbance of the solutions were measured by UV-Vis spectrophotometer versus blank at λ

$_{max}$= 439nm the stoichiometric ratio between 1:1 results are shown in the Table 9

Table 9 . The Mole - ratio method of the cefixime with iron

CL	CL / CM	Absorbance at λ= for Color 439nm compound
2×10^{-6}	0.2	0.098
4×10^{-6}	0.4	0.149
6×10^{-6}	0.6	0.192
8×10^{-6}	0.8	0.231
1×10^{-5}	1.0	0.248
1.2×10^{-5}	1.2	0.241
1.4×10^{-5}	1.4	0.235
1.6×10^{-5}	1.6	0.229
1.8×10^{-5}	1.8	0.218

Plotting the value of absorbance versus the C_L / C_M is shown in Figure 14

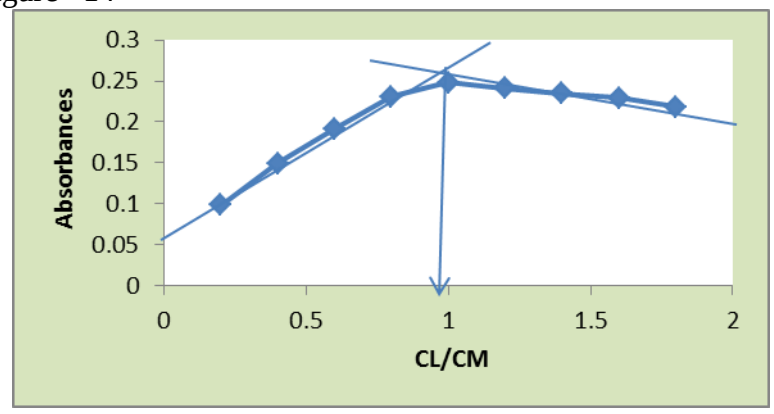

Figure 14 . Mole - Ratio of Cefixime and Iron complex

C_L: concentration of the metal (Iron)

C_M: concentration of the compound (Cefixime)

Applications of the cloud point extraction on pharmaceuticals.

CPE has been applied on pharmaceutical Cefixime, the manufacture company [Novartis] that contains (500mg) from Cefixime .The results are good and of high reliability in the analysis of samples in the pharmaceutical preparation. The results are summarized in the table (10) for Cefixi

Table 10 . Data for determination cefix with iron in the pharmaceutical preparation Capsule (Cefixime) by CPE

Amount of Cefix / µg mL^{-1}	Mean absorbance	Relative stander deviation (RSD)	*Found	Recovery %	Average Recovery%	Erel%	Average Erel%
30	0.215	1.2305	28.18	93.9	97.6	-0.6	-6.8
60	0.430	0.2325	58.46	97.4		-2.5	
90	0.665	0.2604	91.56	101.7		1.7	

Table 11 . Data for determination in the pharmaceutical preparation syrup (cefixime) by CPE

Amount of Cefix / µg mL^{-1}	Mean absorbance	Relative stander deviation (RSD)	*Found	Recovery %	Average Recovery%	Erel%	Average Erel%
30	0.210	0.9523	27.47	91.5	94.0	-8.4	-5.1
60	0.394	0.2538	53.39	88.9		-11.0	
90	0.680	0.3890	93.67	104.0		4.0	

Stability constant of reaction product

The conditional or apparent stability constant of the 1:1 (Drug and metal) product was evaluated and described as shown Complete founding the stability constant [K] colored product Formed imputation of (metal :drug) as followed: A series of solution were prepared containing three different concentration of metal and Cefixime (1:1) and the concentration (1×10^{-4})

$molL^{-1}$ for (Iron with Cefixime) when Formed imputation under this Condition easily to Hydrolysis and the Intensity Absorption was very low Another series of solution was prepared containing three deferent concentration of metal and Cefixime but with abundance of the metal (the best concentration) The complex was prepared with no decomposition express of the intensity absorption A_m Where: K; stability constant C; the concentration of the product complex .and it equivalence the concentration of Cefixime are shown in Table 12

Table 12 . Stability constant of the complex (Fe+ Cefixime) formed

Vol of Cefixime	Absorbance at λ 439nm A_s	A_m	α	K ($l.mol^{-2}$.)	K (Average) ($l.mol^{-2}$)
0.3	0.280	0.311	0.0996	9.076×10^{7}	
0.5	0.510	0.593	0.1399	4.394×10^{5}	3.0×10^{4}
0.7	0.734	0.809	0.0927	1.068×10^{6}	

Atomic absorption spectrophotometry (AAS)

Determination of drug CEF-Fe(III) by using Flame Atomic Absorption Spectrophotometer To be sure about the result obtained by UV-VIS, we used another technical method, Flame Atomic Absorption Spectrophotometer (FAAS), by indirect measurement the absorbance of Fe(III) in the complex to detect the cefixime concentration as in figure (16). The complex CEF-Fe(III**)** was prepared by using optimum condition of pH, temperature, proper solvent etc. (the same conditions mentioned previously in U.V spectrophotometer) except changing the concentration of metal ion, it was found the best concentration of Fe (III) to give maximum absorbance 35 µg /mL, of organic layer is enough to get higher absorbance for complex as in Figure (15) .Also we measured the concentration of cefixime in these pharmaceutical preparations using calibration curve of indirect (FAAS), we got the same result which obtained by U.V method

Preparation of calibration curve for CEF

In order to test the linearity of the method and under the optimized conditions established by CPE procedure, Calibration graphs were established by plotting absorbance versus concentration . Figure (15) represent the calibration curve

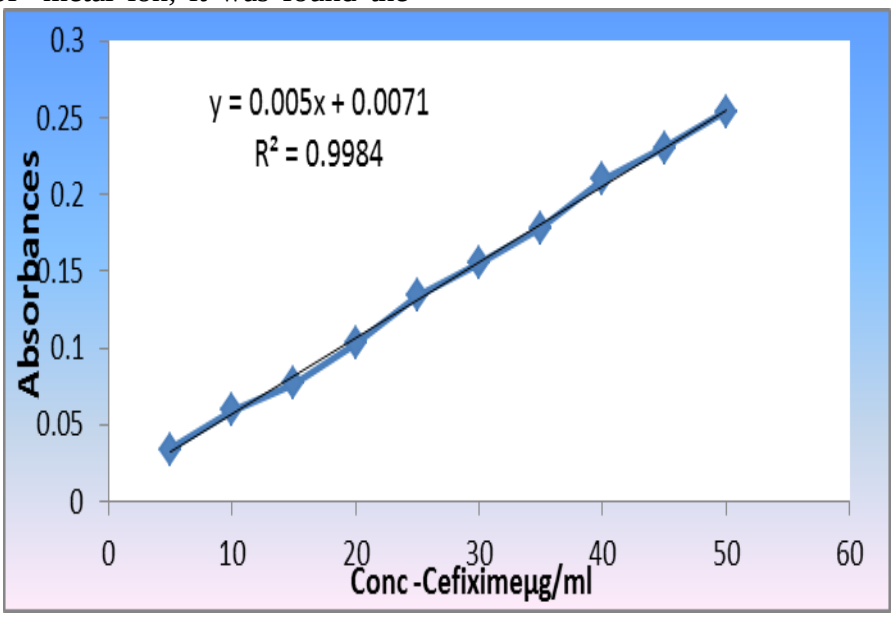

Figure 15 . (Cefixime+ Fe) calibration curve

Table 13 . The absorbance measurements of standard solutions of complex (CFX- Fe)

Found	Recovery%
5.38	107
10.38	103
13.98	93
19.18	95
25.38	101
29.58	98
34.18	97
40.58	101
44.58	99
49.38	98

The calibration curve was . Plotting the mean absorbance values of the cloud point versus the concentration (ppm) of (CFX- Iron) as shown in Figure 16

Optical characteristics Features of the calibration curve

Table 14 .shows the Comparison between the complex methods of the proposed methods

Table 14. Optical characteristic Features of calibration curve

Parameter Concentration rang (µg mL^{-1})	Complex (Cefixime -Fe) by FAAS Method (5- 50 µg mL^{-1})	Complex (cefixime -Fe) by UV-VIS Method (10-160 µg mL^{-1})
Regression equation	y = 0.0050x + 0.0071	y=0.0071x +0.0149
Correlation coefficient(r)	0.9991	0.9992
Correlation coefficient (r^2)	0.9984	0.9985
Variation coefficient (%)	99.84	99.85
Limit of Detection (µg mL^{-1})	1.0770	1.5865
Limit of Quantitation (µg mL^{-1})	3.5901	5.2887
Sandell's sensitivity (µg cm^{-2})	0.2000	0.2320
Molar absorptivity($L.mol^{-1}.cm^{-1}$)	2.2×10^3	1.9×10^2

Applications of the Cloud Point Extraction on Pharmaceuticals

Table 15 . Data for Determination Cefix with Iron in the Pharmaceutical Preparation Capsule (Cefixime) by CPE

Amount of Cefix / µg ml^{-1}	Mean absorbance	*Found	Recovery %	Average Recovery%	Erel%	Average Erel%
20	0.110	20.58	102	103	2.9	3.8
40	0.217	41.98	104		4.95	
50	0.266	51.78	103		3.56	

Effect of metal ions concentration

Figure (16) show the effect of Iron ion concentrations upon the absorbance values of the extracted complexes using (1000 µg/mL) of drug solution . The optimum concentration of the metal ions that gave maximum absorbance was 35µg/mL of Fe(III) as the optimum concentration of Fe(III) ion were 100µg/mL for Cefixime) The absorbance is measured and the absorbance results are shown in table 16 Plotting of the absorbance values versus the concentration of metal ion is shown in figure 16

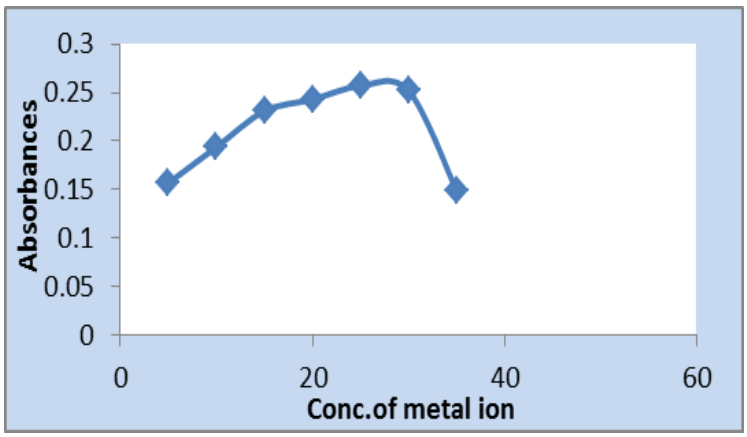

Figure 16. Effect of Optimum concentration . Fe(III) on absorbance of drug-metal complex

Conclusion

CPE preconcentration is an easy, safe and inexpensive methodology for separation and Preconcentration of trace metals in aqueous solutions .The ligand was successfully to formed complex with the some metals ion by cloud point extraction. Is a stable, sensitive and selective complexion successfully to determination Fe (III) in some Pharmaceuticals,the method gives a very low limit of detection and good R.S.D. values and green chemistry

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