

Determination of Cefalexin by Direct (UV- Vis) Spectrophotometer and Indirect(Flame Atomic Absorption) Technique

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Abstract

A new method for the determination of the drug cefalexin in some Pharmaceuticals using (UV-Vis) and indirect Flame Atomic Absorption Spectrophotometer (FAAS) , Fe III should forms a chelating complex with cefalexin (CEX -Fe III) at pH (1-8) and the best pH for the formation of (CEX -Fe III) chelating complex was (2) .The complex extracted with Methanol and Dimethy-Sulphoxide .The mole-ratio method has been used to determine the structure of chelate (CEX - Fe III) and found to be 2:1 LM (Ligand : Metal.) .

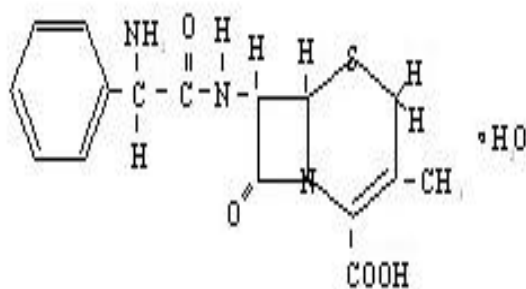
Keywords : Cefalexin , chelating complex.

الخلاصة

طريقة حديثة لتقدير السيفالكسين في بعض العقارات الصيدلانية و ذلك باستخدام تقنيتي الأشعة فوق البنفسجية-المرئية و الطريقة غير المباشرة للامتصاص الذري أللهبي .حيث وجد أن الحديدك يستطيع أن يكون معقد كيليتي مع السيفالكسين في مدى من قيم pH تتراوح ما بين (١ - ٨) و أن افضل قيمة لـ pH تعطي اعلى ممتصية كانت عند (pH = ٢) و المعقد الناتج استخلص باستخدام مزيج من الميثانول و ثنائي مثيل السلفوكسايد و كانت النسبة المولية للتعقيد (CEX-FeIII) هي (٢ : ١) على التوالي.

Introduction

Cephalosporins are β -lactam antibiotics obtained originally From a cephalosporium mold.These antibiotics have the same mechanism of action as the Pencillins, but differ in antibacterial spectrum¹ . Cephalosporins discovered by Bortzu² in 1948. Cefalexin is the First generation of caphalosporins .The chemical names for cefalexin are :-7-(D - α -aminophenyl acetamido)des-acetoxy cephalosporic acid.³ Or 7-(D-2-Amino-2-phenylacetamido)-3-methyl-3-cephem-4- carboxylicacid.⁴ The chemical formula is C₁₆ H₁₇ N₃ O₄ S . H₂O . And the chemical structure for the drug :



Cefalexin monohydrate Molecular weight: 365.4 gm Melting point: 326.8 °C Cefalexin is a white to faint yellow powder slightly soluble in water, insoluble in ethanol , chloroform and ether . *Anacona et. al*⁵ prepared complexes for β -lactam antibiotics with some metals, and they identify it by spectroscopic and physio-chemical methods ,also they studiedthe reaction of these antibiotics with some transitional elements like Mn II , Cu II ,Zn II, and confirmed the structures of products using I .R. and N.M.R. spectra. on the other hand, different solutions of cephalosporins and chloride salts of essential and trace elements prepared in a study using different temperatures (37°Cand 60 °C) in order to accomplish the complex formation in molar ratio of the drug and metal salts 1:1⁶. Also *Abdel Gaber et.al*⁷ studied the complexes of ions ZnII , CuII, Ca II with cephalosporins potentially they calculated the Molar - Ratio for (Liganed- Metal) at 25 °C (1:1).

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Lozano and Borrás⁸ studied titrimetrically the complexes of Cefalexin with metals ZnII ,Cd II, they analysis the result by Electrical methods using (Least squares computer. program Super quad). They prepared complexes of cefalexin like Zn (CEX)₂ , 3H₂O and Cd (CEX)(OH) H₂O , these complexes have been identified by I.R and N.M.R Spectra . In addition Abo El-Maali et-al⁹ determined some cephalosporins antibiotic with Cd II or Zn II by using voltimetric method which determined the presence of (ligand - Metal)1:1 like in complexes cefataxime , Cefuroxime and 2:1 like ceftazidime. Moreover, Iqbal and Ahmad¹⁰ prepared complexes of cephalaxin with copper II and ZnII these complexes have been characterized by microanalysis and by magnetic and spectroscopic analysis. The complexes, were found five-coordinate monohydrate, and ML₂ .On complexation with copper and zinc the antimicrobial activity of cephalaxin improved Significantly. These results suggested that metallic elements should be seriously considered during drug design .Also, Alekseev et al.¹¹ studied the complex formation in solutions containing Nickel (Ni⁺⁺) cations, glycine anions (Gly⁻), and β-lactam antibiotics, they found mixed-ligand complexes is formed of [Ni Gly Ampicillin], [Ni Gly Amoxicillin] ,and [Ni Gly Cephalexin]. Alekseev¹², in another study, found that the complex formation of Neodymium III (Nd³⁺) ions with Ampicillin, Amoxicillin and Cephalexin anions (L) in aqueous solution at 20°C and ionic strength of 0.1 (KNO₃) by pH titration was in form of NdL and Nd(OH)L complexes using weak alkaline solution. A new study discussed the spectrophotometric determination of Cephalexin as intact cephalaxin or its degradation product, cephalaxin was determined in the range (1×10⁻⁵-18×10⁻⁵ M) and the limits of detection were 0.3×10⁻⁵ M. These results were compared with reversed- phase HPLC determination, the UV-Vis spectrophotometric method was improved to be selective and reproducible¹³.

Instruments , Materials and Method

A - Instruments

Shimadzu (AA- 670) Flame Atomic Absorption Spectrophotometer .

1. U.V-Visible Spectrophotometer(CARY 100)wave length 200 -1100 nm .
2. PH meter type 60 A .

B - Materials

All the chemical stock solution were prepared from analytical grade BDH,SDI ,India and Germany .

C- Method

Stock solution of Fe III

A solution of 1000 ppm of Fe⁺³ was prepared by dissolving 0.2896 gm of FeCl₃ in small amount of Water and complete the volume to 100 ml by using volumetric flask ,. Then 10 ml of the stock solution was diluted to 100 ml with distilled water to Prepare 100 PPMsolution .

stock solution of cefalexin (CEX)

A solution of 1000 ppm of CEX was prepared by dissolving 0.05 gm of (CEX) in water and complete the volume to 50 ml by using volumetric flask .Then a stock solution of 100 ppm was prepared as above .

Spectral study

Cefalexin spectrum

10 ml of stock solution (CEX) was transferred to a volumetric flask 5 ml and diluted with distilled water then the Absorbance was measured at 200-600 nm wave length using water as blank .

Ferric spectrum

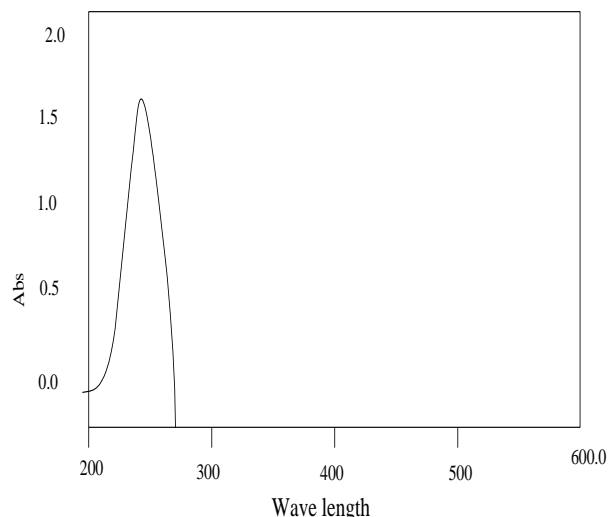
1ml of stock Ferric solution was transferred to a volumetric flask 5ml and diluted with distilled water, then the Absorbance was measured at 200-1100 nm using water as blank.

Formation complex with Fe III

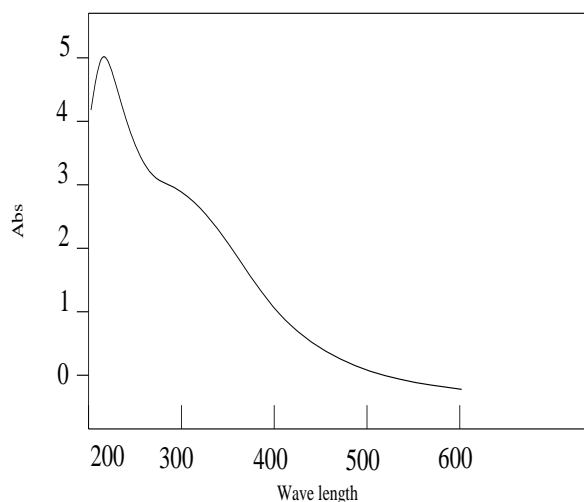
(2-5) ml from stock solution of (CEX) 1000 PPM was transferred to a volumetric flasks 5ml and 1ml of Fe III 1000 PPM was added .The chelating complexes can be extracted by using 2ml mixture of Methanol + Dimethyl sulphoxide (0.5 :1.5) .The formation of complex was studied at room temperature The reaction was heated at different temperature with different pH .

Results and discussion

Drug spectra : measuring (CEX) spectra as in figure (1) we see one Absorption peak at λ_{max} (264)nm using water as blank . Metal spectra : figure (2) for Fe III spectra show Absorption peak at λ_{max} (208) nm using water as blank.Chelating complex spectra : CEX- FeIII.Figure(3) for chelating complex show two new peaks,the first at λ_{max} (340) nm and the second at (358) nm which indicate the Formation of complex between drug and FeIII using organic solvent as blank. Table (1) show different compounds and the wave length at λ_{max} for each drug and FeIII.



Fig(1) The UV absorption spectrum for cefalexin



Figure(2): The UV absorption spectrum for Fe III solution

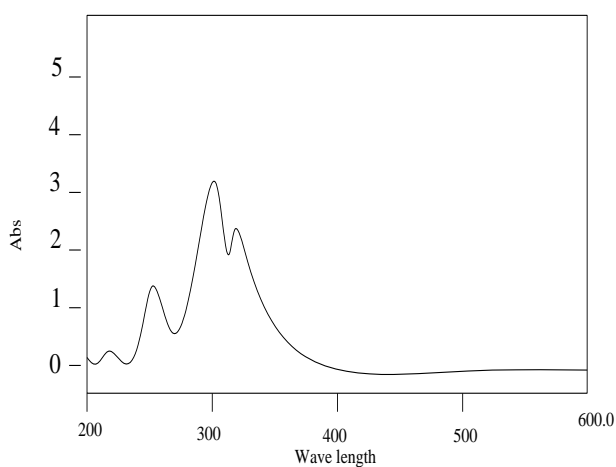


Figure (3) :The UV absorption spectrum for the complex CEX-FeIII

Table(1) Color and λ max for the drug FeIII and complex

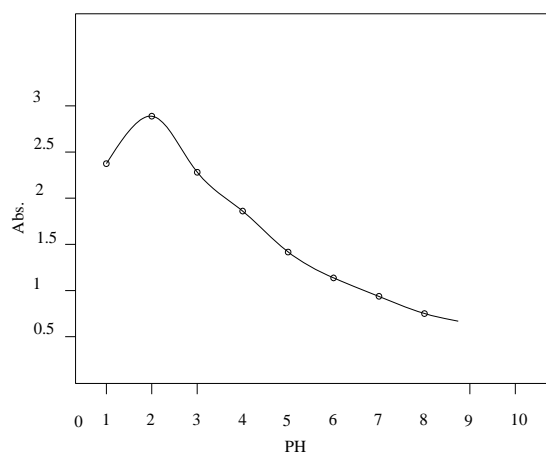
Compound	Max(nm) λ	Color
(CEX)	264	White
Fe III	208	Orange
Complex	341+358	Yellow

Optimum condition for complex formation

The experimental work showed that the reaction was not proceed at room – temperature so heating was necessary, media must be acidic, for these reasons we study the effect of pH, reaction time , extraction time, concentration of ion Fe III , temperature effect ,suitable solvent for extraction and number of extraction .

pH Effect:

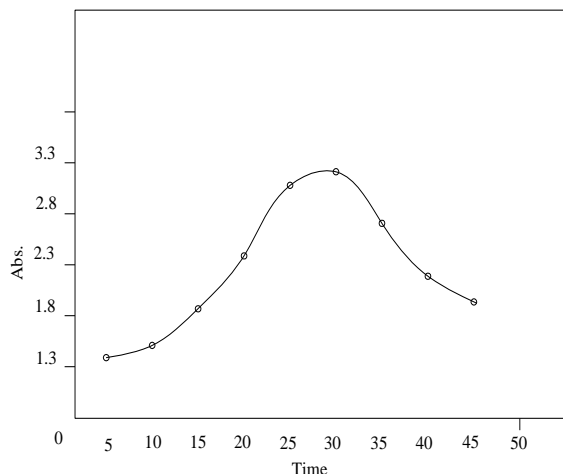
Figure (4) show the Absorbance of (CEX –Fe III) using different pH (1-8) the optimum pH for the complixation was (pH:2).



Figure(4) : Effect of pH on the absorbanc of CEX-FeIII by using UV spectrum

Reaction time

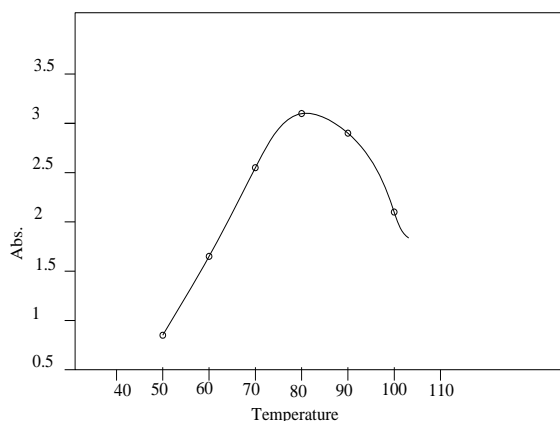
It was found that higher Absorbance of extracted complex was happened After 25 minutes, after that the Absorbance decrease due to dissociation part of complex With increase in heating time . Figure (5) , show that 25 minutes is the best time for reaction to give maximum Absorption for (CEX-Fe III) at 80°C and pH 2.



Figure(5): Effect of reaction time on formation of complex CEX-FeIII at pH 2 by using UV Spectrum

Temperature effect:

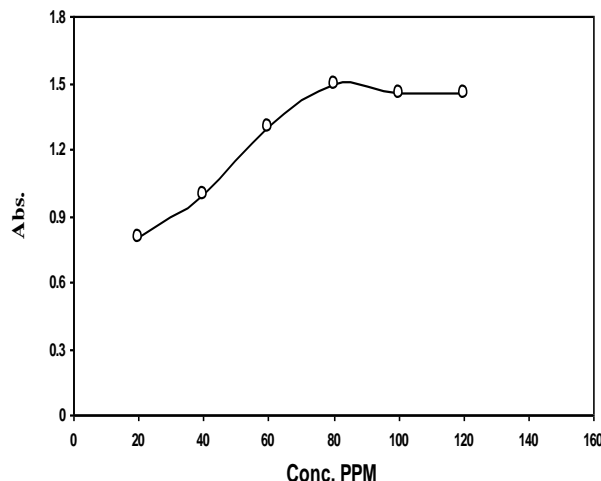
the reaction of drug with metal was proceed slowly it may exceed hours to increase the reaction velocity we proceed the reaction at different temperature (50-100 °C), it was found that 80 °C is the optimum temperature which gave the higher absorption as in figure (6) .



Figure(6) Complex absorption value at different temp. by using UV spectrum

Effect of Fe III concentration:

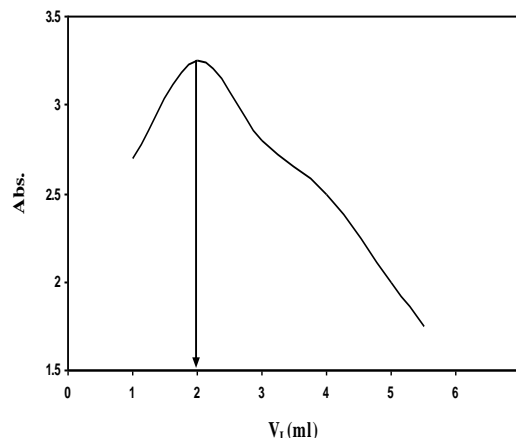
The best concentration for Fe III ion is 80 µg /ml, it gave the maximum Absorbance figure (7) show that effect of concentration PPM on absorbance .



Figure(7): The effect of Fe III Concentration on complex formation by using UV spectrum

Phase Ratio:

it was found 5ml of aqueous layer and 2ml of organic layer is enough to get higher Absorbance for complex as in figure (8), after that it was decrease as we increase the volume of organic layer.



Figure(8): Effect of phase ratio on absorbance of complex CEX-Fe III at pH =2 by using UV spectrum

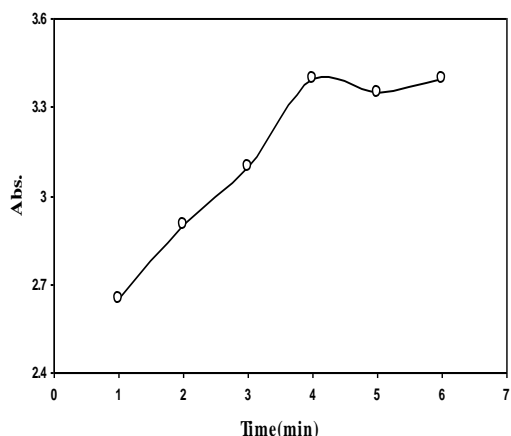
Suitable solvent :

Different organic solvent were used like Benzene, Chloroform ,Acetone Ethanol , Cyclohexanone , Carbon tetrachloride , Methanol , Dimethyl sulphoxide , and Diethyl ether for extraction of complex (CEX-FeIII) , it was found the mixture of Methanol and Dimethyl sulphoxide were the best solvents .

Extraction time_ :

The optimum time for shaking during the extraction of the complex was (4 min.) which give maximum Absorption . figure (9) showed

the maximum Absorption in the extraction was 4 minutes of shaking .



Figure(9) : Effect of shaking time on the extraction process by using UV spectrum

Number of extraction:

extraction process for once is enough to extract the complex ,because the second extraction for the remaining concentration give very small Absorbance less than 0.08 ,so one extraction is enough. This illustrate in table (2)

Table(2): Effect of number of extraction on absorbance of complex

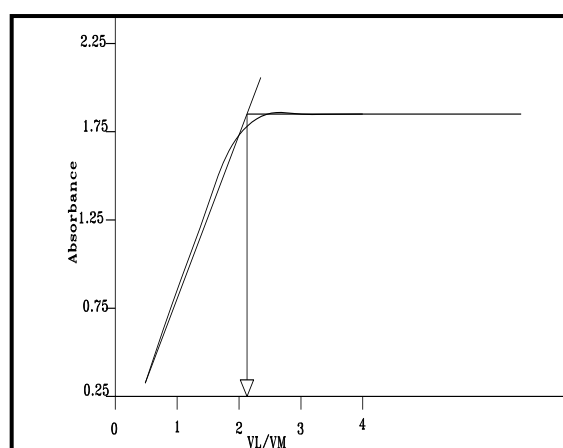
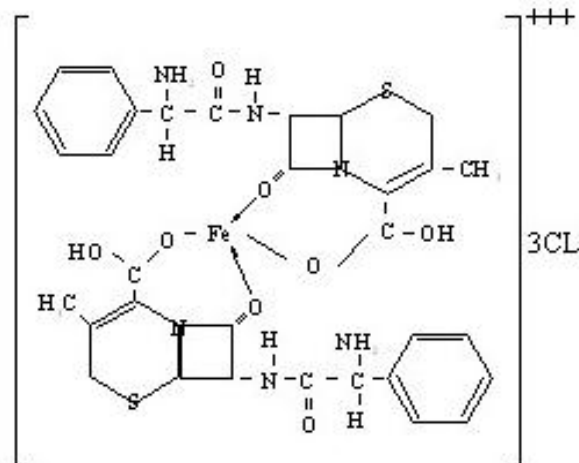
A0 Blank	0.005
A2 (Ex.no.2)	0.085
A1 (Ex.no.1)	2.84
PH	2
Fe III Mg/ml	60
CEX Mg/ml	800

Mole Ratio of complex:

determination the mole ratio for complexation it means percent of moles of drug to the moles of metal , different volumes of CEX between (0.5-3.5)ml with constant volume of metal with all optimum conditions of complexation , it was found (1:2) (M:L) . Figure 10 show the relationship between absorbance . and V_L / V_M .The reaction of FeIII with drug occur in this equation :-



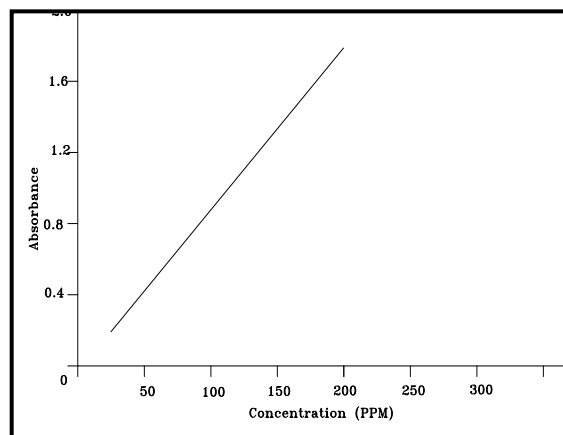
The suggested chemical structure for the reaction of FeIII with CEX. :



Fig(10) mole ratio of complex CEX-FeIII by using UV Spectrum

Calibration curve of the spectrum :

For determination of direct calibration curve for (CEX-FeIII),a different concentration for complex were used as in figure (11) and the relationship between Absorbance and concentration PPM was draw, it was found that the maximum concentration which obey Beer-Lumbert law $200\mu\text{g} / \text{ml}$.



Fig(11) : Calibration curve for CEX-Fe III by using UV spectrum

Determination of the drug concentration in pharmaceutical preparation :

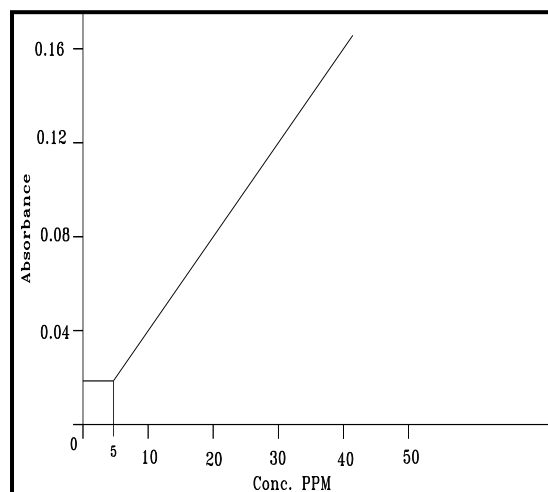
6 capsules were mixed with each other then one capsule was weighted , and the Absorbance was measured of its (CEX-FeIII) using optimum condition then from the calibration curve determine the concentration , we carried the procedure for CEX . (SDI ,Ajanta, Germany) .Table (3)show the result of work .From these result , we suggested that the CEX . concentration in Germany capsules is less than the real concentration which is 500 mg while Ajanta and SD Iis O.K.

Table(3):The concentration of Cefalexin capsule in 500mg cefalexin of different trade marks

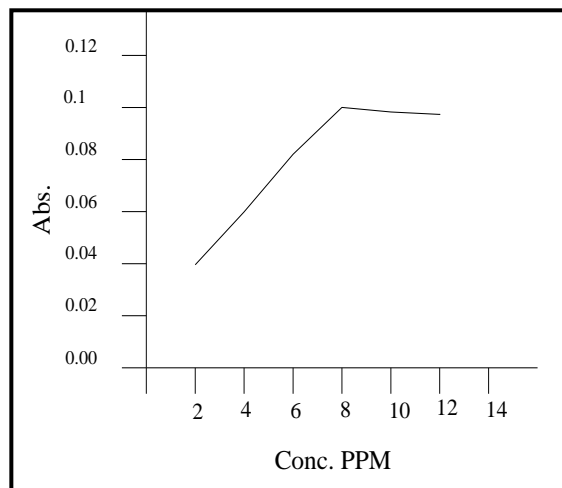
Germany	Ajanta	SDI	Capsules
1.29	1.3	1.4	Abs.
487	495	502	Conc.PPM
500	500	500	Standard

Determination of drug CEX – FeIII by using Flame Atomic Absorption Spectrophotometer:

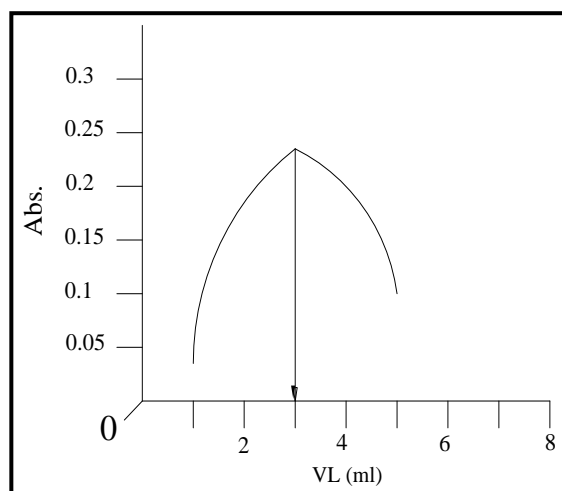
To be sure about the result obtained by U.V , we used another technical method, Flame Atomic Absorption Spectrophotometer (FAAS) , by indirect measurement the absorbance of Fe III in the complex to detect the cefalexin conc. as in figure (12) . The complex CEX-FeIII was prepared by using optimum condition of pH , temperature , proper solvent etc . (the same conditions mentioned previously in U.V spectro - photometer) except changing the conc.of ferric ion and phase ratio, it was found the best conc. of FeIII to give maximum absorbance 8 µg /ml , and 5ml of aqueous layer and 3ml of organic layer is enough to get higher absorbance for complex as in figure (13) and (14) .Also we measured the concentration of cefalexin in these pharmaceutical preparations using calibration curve of indirect (FAAS), we got the same result which obtained by U.V method



Fig(12) : Calibration curve for (CEX) by indirect FAAS by using UV spectrum



Fig(13): Effect of FeIII on complex by using FAAS



Fig(14): Effect of phase ratio on absorption of complex by using FAAS

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