

Using and validation of a novel spectrophotometric method for determination of ceftriaxone sodium in pharmaceutical dosage forms via its complexation with Cu (II)

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□ ABSTRACT □

A simple, accurate and sensitive UV-Visible spectrophotometric method have been used and validated for the quantitative determination of ceftriaxone sodium in either pure form or in its pharmaceutical vials. The method is based on the formation of a complex with copper(II). Appropriate conditions were examined for the reaction to obtain maximum absorptivity and sensitivity. Under the optimum reaction conditions, linear relationship with good correlation coefficient (0.999) was found between the concentrations and the absorbance of the formed complex. The color of the produced complex is measured at 635nm. The molar absorptivity is $1.16 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and Sandell's sensitivity is $0.057 \mu\text{g/cm}^2$. The method is applicable over concentration range of 8 – 70 $\mu\text{g/ml}$. The results of analysis have been validated statistically, and recovery studies confirmed the accuracy of the proposed method which was carried out by following the ICH guidelines. Furthermore, the developed method hold its accuracy and precision well when applied to the determination of ceftriaxone sodium in its pharmaceutical vials.

Key words: ceftriaxone sodium, complex, optimum reaction, molar absorptivity, Sandell's sensitivity, validation.

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استخدام طريقة طيفية جديدة ذات مصداقية لتحديد سيفترياكسون الصوديوم بتشكيل معقد مع شوارد النحاس الثنائية

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□ ملخص □

تم في هذا البحث استخدام طريقة بسيطة، حساسة، ودقيقة لمعايرة سيفترياكسون الصوديوم بشكل النقي وفي الأشكال التجارية الحاوية عليه من خلال تشكيل معقد مع شوارد النحاس الثنائي باستخدام مقياس الطيف الضوئي في المجال المرئي. تم فحص الظروف المناسبة للتفاعل للحصول على الحد الأقصى من الامتصاصية وحساسية الطريقة وبعد تحديد الشروط المناسبة للتفاعل وجد هنالك علاقة طردية بين تركيز الدواء والامتصاصية مع معامل ارتباط 0,999. أعطى المعقد الناتج امتصاصية عظمى عند طول موجة 635 نانومتر مع معامل امتصاص مولي $10^4 \times 1.16$ ل مول⁻¹ سم⁻¹ وحساسية ساندال 0.057 مكغ / مل. تم التحقق من مصدوقية الطريقة التحليلية المتبعة فكانت خطية ضمن المجال 8 - 70 مكغ / مل مع حد كشف كيميائي 0.84 مكغ / مل وحد كمي 2.55 مكغ / مل. تتمتع الطريق المتبعة بدقة ومضبوطة ونوعية عالية مع نسبة استرداد قريبة من 100% وذلك وفق مبادئ ICH كما حافظت الطريقة التحليلية على دقتها أثناء فحص الأشكال الحقنينة المدروسة.

الكلمات المفتاحية: سيفترياكسون الصوديوم، معقد، معامل الامتصاص المولي، حساسية ساندال، المصدوقية.

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Introduction:

Ceftriaxone (CF) (6R,7R,Z)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza bicyclo [4.2.0]oct-2-ene-2-carboxylic acid is a third-generation cephalosporin antibiotic (fig 1)[1].

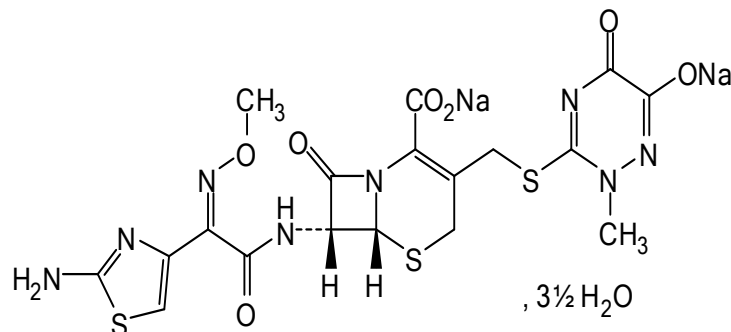


Fig (1): Structure of ceftriaxone sodium

Ceftriaxone sodium powder, which is ready to be injected, is available within vials containing about 250mg, 500mg, 1g or 2g of ceftriaxone [2], it is given as a salt sodium by slow intravenous or intermittent intravenous infusion or deep injection into muscle[3], by complete dissolution of vial contents and by suitable quantities from the compatible solvent. It is worth mentioning that the results reached by Lee and his colleagues have given the chance to ceftriaxone sodium antibiotic to be taken as an oral dosage, so we can predict that other oral dosages may come to light in the coming few years[3-6].

Ceftriaxone has a wide spectrum on both negative and positive gram bacteria, it is active upon neisseria gonorrhoeae which are unresponsive to penicillin and to haemophilus as well as streptococci, staphylococci, pneumococci and so on[3-10]. Generally, we can say that cephalosporin's third generation members like ceftriaxone are active in treating lots of intractable infections which other antibiotics can't do anything about it.

Cephalosporins are distributed in a good way in the tissues and body fluids. It is distributed in the pleural fluid around the heart and in the synovial fluid. Although the previous generations of cephalosporins (1st and 2nd) have failed in penetrating the central nerve system and in reaching therapeutic concentrations there, but the 3rd generation compounds -except cefoperazone and oral cephalosporins- were able to reach central nerve system in good concentrations and they were considered to be enough to treat Meningitis caused by negative gram aerobic bacteria. Ceftriaxone reaches the bones as well as the nerve system and distributes in a therapeutic concentrations. It crosses the placenta and distributes in breast milk. It reaches its highest concentration in the serum after 2-3 hours by injecting it intramuscularly. ceftriaxone half age is between 7-8 hours and is excreted mainly by biliary tract[10].

There are many analytical ways in order to determine the quality of ceftriaxone sodium compound in its pharmaceutical forms containing it as the only effective substance or within therapeutic mixture and even in vital circles. Most of these ways are under one of these provisions: the spectrum[11-37] and chromatographic ways[38-57].

The spectrum ways classified into: visible field and UV field of the spectrum. We can classify photolysis methods for ceftriaxone sodium antibiotic within the visible field depending on the interaction occurring between the antibiotic and the detector into the

following: metal complexation, oxidation and return reactions, interactions with colored detectors after inciting the sabotage of antibiotic.

In the present work, spectroscopic analytical study for the analysis of ceftriaxone sodium in pure and its pharmaceutical formations through complexation with Cu(II) using potassium-sodium tartrate –in water, has been applied.

Materials and methods:

Pure ceftriaxone sodium, three commercially available vials were purchased from the local market in Syria (A, B, C), Copper (II) sulfate, potassium-sodium tartrate, hydrochloric acid, sodium hydroxide, distilled water. In addition to instruments like: UV-Vis spectrophotometer and pH measurement.

Preparation of stock standard solution of ceftriaxone sodium:

Ceftriaxone sodium stock solution (500 μg / ml) was prepared by dissolving 50 mg of the drug substance in a 100 ml distilled water with good shaking.

General procedure for ceftriaxone sodium determination:

Accurately measured aliquots of ceftriaxone sodium stock solution in the range of 8-70 $\mu\text{g}/\text{ml}$ of drug were transferred into separate 25ml volumetric flasks and then 1 ml copper (II) sulfate(100mg/100ml) solution was added to each flask followed by 0.5mlpotassium–sodiumtartrate(100mg/100ml). The contents of each flask were shaken thoroughly and each mixture was diluted to 25mL with distilled water. A blue-green solution is formed and the absorbance of the resulting solution was measured at λ max against blank prepared in the same manner without addition of the examined drug.

Study of spectral characteristics of (ceftriaxone – copper) complex in distilled water:

After enabling the initial adjustment and blank correction, the absorption of ceftriaxone solution (45 $\mu\text{g}/\text{ml}$) was scanned in the range from 400 to 700 nm to obtain the wavelength of maximum absorption (λ max).

Optimization of reaction conditions:

Effect of pH:

The absorbance of (ceftriaxone – copper) complex was measured at different pH values (2-7).

Procedure: To 0.6 ml of ceftriaxone sodium standard solution (in 25ml volumetric flask), 1 ml of copper (II) and 0.5 ml potassium–sodium tartrate were added followed by different volumes of hydrochloric acid(1N) or sodium hydroxide (1N) solutions to attain different pH solutions. The solutions were diluted to 25 ml with distilled water and the absorbance was measured at λ max.

Effect of reaction time:

The absorbance of (ceftriaxone – copper) complex was measured after 5, 10, 15, 20 25,30 minutes.

Procedure: 1 ml of copper (II) solution, 0.5 ml potassium– sodium tartrate were added to 0.6ml ceftriaxone sodium standard solution and the mixture was diluted to 25 ml with distilled water and the absorbance was measured at λ max after 5, 10, 15, 20, 25 and 30 minutes.

Effect of concentration of copper (II) and sodium-potassium tartrate solutions:

Different concentrations of copper (II) and potassium-sodium tartrate were examined.

Procedure: To 0.6 ml ceftriaxone sodium standard solution, 0.5mlpotassium-sodium tartrate and different volumes of copper (II) solution (0.5, 1, 1.5, 2,2.5and 3ml) were added into different volumetric flasks. The solutions were diluted to 25ml with

distilled water and the absorbance was measured at λ max. The method was repeated by changing potassium-sodium tartrate concentrations.

The sequences of additions:

The sequences of addition of ceftriaxone sodium solution, copper (II), and potassium-sodium tartrate to each others were studied in order to attain the best sequence in complex formation.

Composition of ceftriaxone : Cu(II)complex:

Formation of ceftriaxone: Cu(II) complex were employed to determine by mole-ratio and Job's method of continuous variation as follows:

a-Molar ratio method:

The stoichiometry of ceftriaxone: Cu(II) complex by molar ratio method according to following equation: $A_{max} = f([\text{Cu(II)}]/[\text{ceftriaxone}])$, where the concentration of ceftriaxone is constant (1×10^{-3} M) and the concentrations of Cu(II) are changing from (0 to 5×10^{-3} M).

b-Continuous variation:

The nature of ceftriaxone: Cu(II) complex was determined using Job's method of continuous variation. Master equimolar (1×10^{-3} M) aqueous solutions of ceftriaxone sodium and copper (II) sulfate were prepared. Series of 1ml portions of the master solutions of ceftriaxone sodium and copper (II) sulfate were made up comprising different complementary proportions (0.2:0.8, 0.4:0.6, 0.6:0.4, 0.8:0.2). These 1 ml portions were transferred to 25ml volumetric flasks, 0.5ml potassium-sodium tartrate was added to each flask. The contents of each flask were shaken thoroughly and each mixture was diluted to 25ml with distilled water. The absorbance of the resulting solution was measured at λ max against blank prepared in the same manner without addition of the examined drug. A graph of absorbance then plotted versus mole fraction.

Analytical method validation:

Analytical method validation was performed according the ICH guidelines (ICH, 2005) with respect to accuracy, precision, linearity, limit of detection and limit of quantitation.

a- Linearity:

The linearity of the proposed method was determined by measuring the absorbance of five concentrations covering the range (8, 12, 24, 45 and 70 $\mu\text{g/ml}$), each concentration was measured in triplicate. Then the plot of absorbance against concentration was examined visually and statistically by calculating correlation coefficient.

b- Accuracy:

Three concentrations covering the range were measured and each of them was repeated three times to calculate accuracy and precision.

Accuracy of the method was ascertained by performing recovery studies using standard addition method. To a pre-analyzed sample, standard drug aliquots were added at three different levels viz. 8 $\mu\text{g/ml}$, 24 $\mu\text{g/ml}$, 45 $\mu\text{g/ml}$, to baseline amount of vial powder. These mixtures were transferred into separate 25 ml volumetric flasks and then 1 ml copper (II) sulfate solution was added to each flask followed by 0.5 ml potassium-sodium tartrate. The contents of each flask were shaken thoroughly and each mixture was diluted to 25 ml with distilled water. The absorbance of the resulting solution was measured at λ max against blank prepared in the same manner without addition of the examined drug.

c- Precision:

Intraday and interday precision were assessed by triplicate analysis of three different concentrations (8, 24 and 70 $\mu\text{g/ml}$). Precision of the analytical method is

expressed as SD or RSD of series of measurement by replicate estimation of drugs by the proposed method.

d- Method sensitivity (LOD and LOQ):

The values of LOD and LOQ were calculated by using SD (standard deviation of response) and S (the slope of the calibration curve) and by using equations:

$$\text{LOD} = 3.3 * \text{SD}/\text{S} \text{ and } \text{LOQ} = 10 * \text{SD}/\text{S}.$$

e- Specificity:

The specificity of the method was investigated by observing any interference encountered from common excipients or drugs that may be added to ceftriaxone sodium formulations like sulbactam. To fulfill this purpose, a mixture was prepared by mixing known amounts of sulbactam and ceftriaxone sodium.

Procedure: A quantity of the prepared mixture containing 50 mg ceftriaxone sodium was dissolved in distilled water (50 ml) with good shaking, filtered and the volume was completed to 100 ml with distilled water. Accurately measured aliquots of ceftriaxone sodium filtrate were transferred into separate 25ml volumetric flasks and then 1 ml copper (II) sulfate solution was added to each flask followed by 0.5ml potassium-sodium tartrate. The contents of each flask were shaken thoroughly and each mixture was diluted to 25 ml with distilled water. The absorbance of the resulting solutions were measured at λ max against blank prepared in the same manner without addition of the examined drug.

Assay of ceftriaxone sodium vials:

The contents of ten vials of each brand were emptied and triturated and a known weight of the powder equivalent to 50 mg of the drug was dissolved in 50ml distilled water with good shaking for 10 minutes and any remaining residue was removed by filtration. The filtrate solution was then transferred into 100 ml calibrated flask and diluted to 100 ml with distilled water. Different volumes (0.4, 0.6 and 1.2 ml) of the filtrate solution were transferred into separate 25 ml volumetric flasks and then 1 ml copper (II) sulfate solution was added to each flask followed by 0.5ml potassium-sodium tartrate.

The contents of each flask were shaken thoroughly and each mixture was diluted to 25 ml with distilled water. The absorbance of the resulting solution was measured at λ max against blank prepared in the same manner without addition of the examined drug. A triplicate measurements were done for each concentration and the percentage of recovery was calculated from the calibration graph.

Results and Discussion:

The reaction between ceftriaxone sodium and copper(II) and potassium-sodium tartrate was performed, and the absorption spectrum of the product was scanned in the range of 400-700 nm against a reagent blank. It was found that the product exhibiting λ max at 635 nm, and the absorbance increased directly with ceftriaxone sodium concentration.

Optimization of reaction conditions:

Effect of pH:

The effect of pH on the absorbance of ceftriaxone : Cu(II)complex was studied using HCL(1N) and NaOH (1N) in the range 2-7 of pH and also without any addition (pH = 4.5). Values of pH were examined only in the range of 2-7 because of dissociation of the complex and precipitation of copper (II) at pH >7nm. Also because the color of the complex will disappear at pH <2. The maximum absorbance was achieved at pH 2.7 as shown in fig2.

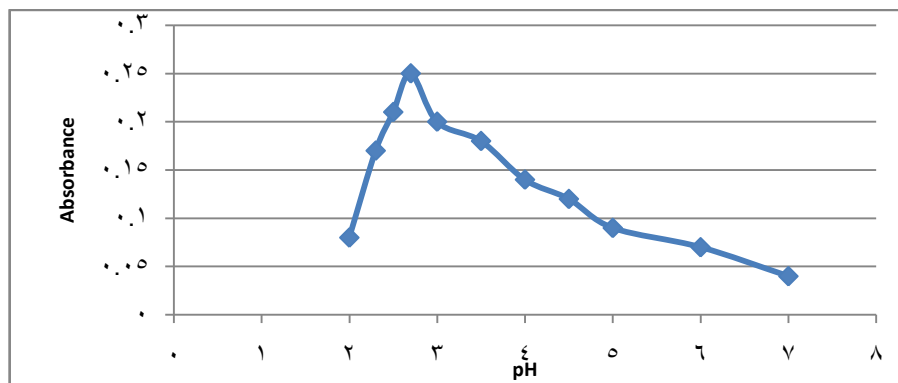


Fig (2):Effect of pH on ceftriaxone : Cu(II) complex

Effect of reaction time:

It was found that the reaction went to completion in 15minutes after dilution at reached maximum and constant absorbance as shown in fig 3. The color formed under the above mentioned optimum conditions was constant for at least 30minutes.

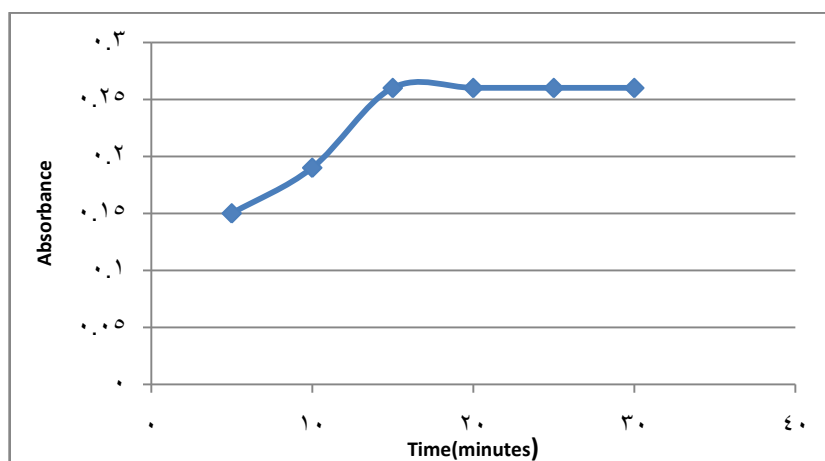


Fig (3):Effect of time reaction.

Effect of concentrations of copper (II) and potassium-sodium tartrate solutions:

Concerning the effect of Cu(II) concentration on the absorbance of the complex formed, the optimum results i.e., maximum and constant absorbance were obtained using 1 ml of copper sulfate (100mg/100ml) (Fig 4). Also the effect of potassium-sodium tartrate concentration was studied by keeping the concentration of Cu (II) constant and varying potassium-sodium tartrate concentration. Optimum results were obtained by using 0.5 ml of potassium-sodium tartrate (100mg/100ml). Higher concentration of reagents did not affect the color intensity (Fig 5).

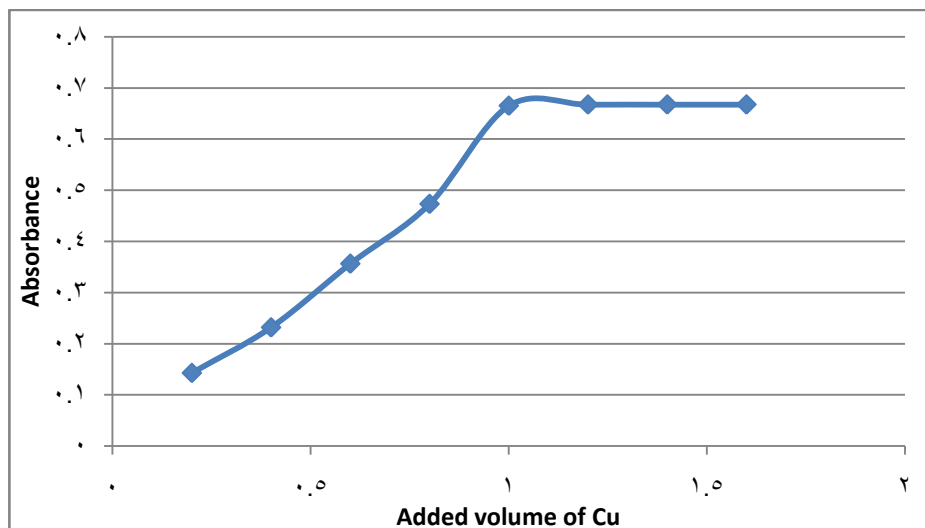


Fig (4):Effect of Cu (II) concentration

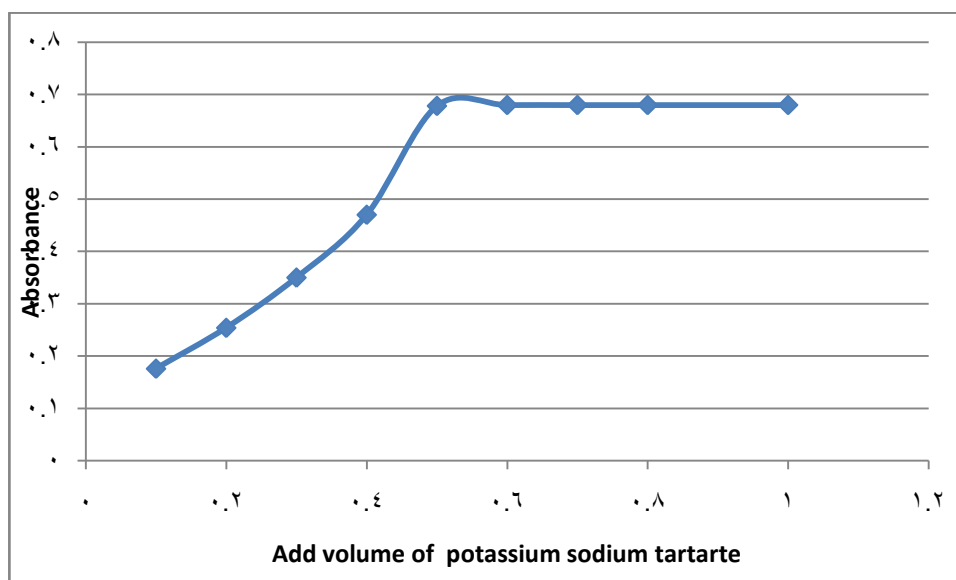


Fig (5):Effect of concentration of potassium-sodium tartrate

The sequences of additions:

The optimum results i.e., maximum and constant absorbance were obtained when adding 0.6ml ceftriaxone sodium solution firstly in the volumetric flasks, then adding 1ml copper (II) solution, then adding 0.5 ml potassium-sodium tartrate, and finally adjusting pH of the solution to 2.7 and dilution to 25 ml using distilled water.

Composition of ceftriaxone : Cu(II)complex:**a-Molar ratio method:**

The stoichiometry of ceftriaxone: Cu(II) complex by molar ratio method according to following equation: $A_{max} = f([Cu(II)]/[ceftriaxone])$ confirms that the ratio of complex ceftriaxone: Cu(II) is equal to 1:1, where the concentration of ceftriaxone is constant (1×10^{-3} M) and the concentrations of Cu(II) is changing from (0 to 5×10^{-3} M) (fig6).

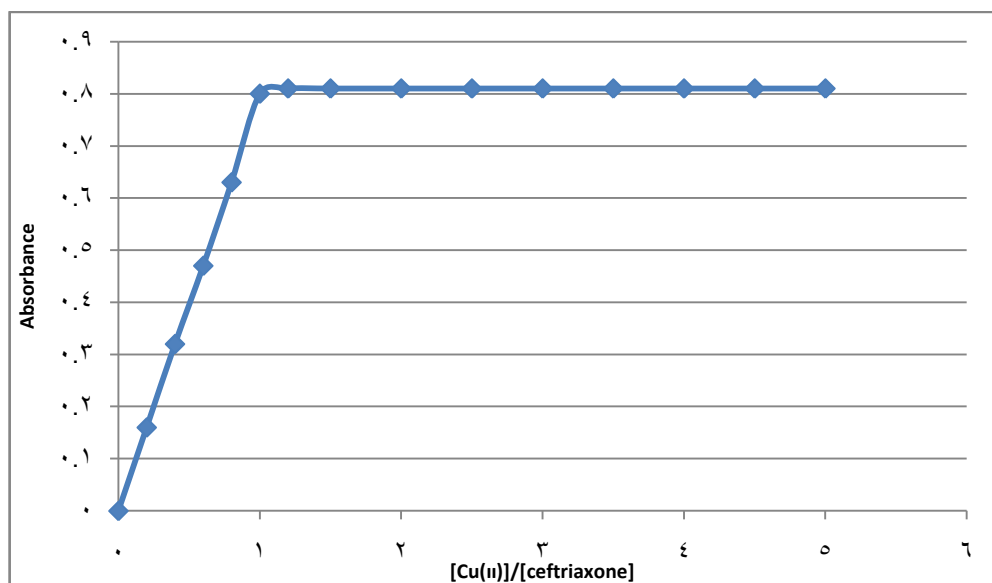


Fig (6): Molar ratio method to calculate coupling ratio for ceftriaxone: Cu(II) complex

b-continuous variation:

The nature of the complex (ceftriaxone: Cu(II)) was determined using Job's method of continuous variation. The result of applying this method showed that the [ceftriaxone: copper(II)] ratio was 1:1 as shown in fig 7.

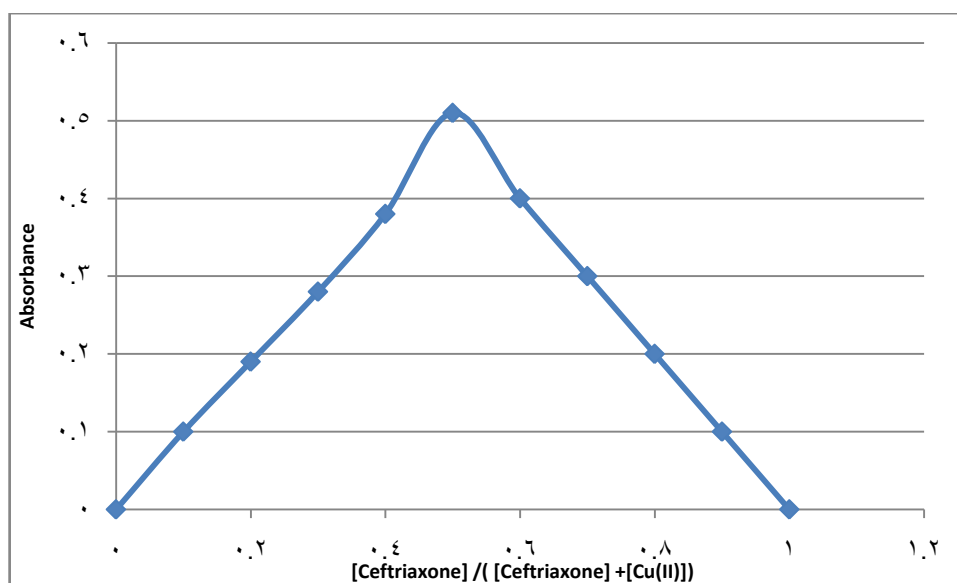


Fig (7): Job's method of continuous variation of ceftriaxone:Cu (II) complex

Analytical method validation:

a-Linearity:

Under the above experimental conditions, the calibration curve was constructed by plotting concentration of ceftriaxone versus absorbance (Fig8). A linear calibration graph was found between absorbance and concentration in the range of 8-70 μg/ml. The correlation coefficient, intercept and slope for the calibration data for ceftriaxone were calculated using the least squares method (Table 1).

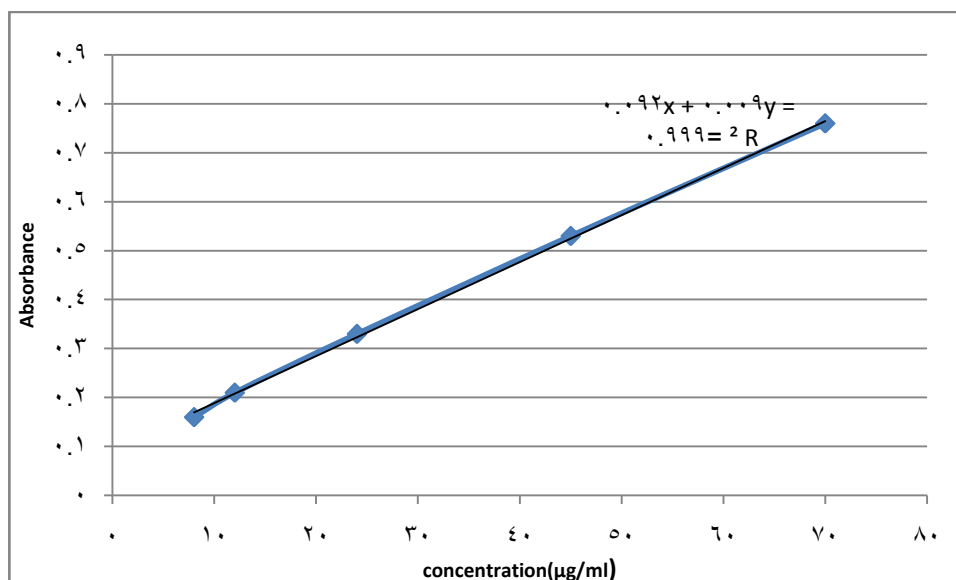


Fig (8): Calibration curve for ceftriaxone sodium

Table (1): characteristics and statistical data of the regression equation for the complex formation with ceftriaxone sodium

Parameters	Ceftriaxone Spectral Data
λ max nm	635
Beer's law limits, µg/ml	8 – 70
Molar absorptivity, $L \text{ mol}^{-1} \text{ cm}^{-1}$	1.16×10^4
Sandell's sensitivity, $\mu\text{g}/\text{cm}^2$	0.057
Limit of detection µg/ml (LOD)	0.84
Limit of quantitation, µg/ml (LOQ)	2.55
Regression equation	$Y = 0.0092X + 0.092$
Intercept (a)	0.092
Slope (b)	0.009
Correlation coefficient (r)	0.999

$Y = a + bX$, where Y is the absorbance, a intercept, b slope and X concentration in µg/ml

b-Sensitivity (LOD, LOQ):

The limit of detection (LOD) and the limit of quantitation (LOQ) for the proposed method were calculated using the following equations:

$$\text{LOD} = 3.3 \text{ SD}/S \quad - \quad \text{LOQ} = 10 \text{ SD}/S$$

SD is calculated as the standard deviation of the residuals around the regression line, S is the slope of calibration curve. LOQs and LODs for ceftriaxone are listed in Table 1.

Sandell's sensitivity is the concentration of the drug in µg/ ml which will exhibit an absorbance of 0.001 in 1 cm cell, and is expressed as $\mu\text{g cm}^{-1}$.

c-Precision:

The precision and accuracy of the proposed method were tested by means of replicate measurements of the tested drug within Beer's law limits. The precision of the analytical procedure is usually expressed as the standard deviation of a series of measurements.

Intraday and interday precision were assessed using three concentration and three replicates of each concentration. The calculated relative standard deviation values were found to be very small (<1%) indicating good repeatability and reliability of the proposed methods. The results and their statistical analysis were summarized in Table2.

Table (2): evaluation of precision of the analytical procedure of ceftriaxone sodium

Statistical Parameters		8 µg/ml	24 µg/ml	45 µg/ml
Intraday	1	7.985	23.991	44.983
	2	8.012	24.045	44.981
	3	7.993	24.021	45.012
	Mean recovery	7.996	24.019	44.992
	Mean% recovery	99.95	100.07	99.98
	S.D.	0.013	0.027	0.017
	R.S.D. (%)	0.62	0.11	0.037
Interday	1	7.889	24.015	44.987
	2	7.992	23.994	45.018
	3	7.990	23.986	45.021
	Mean recovery	7.957	23.998	45.008
	Mean% recovery	99.46	99.99	100.01
	S.D.	0.058	0.014	0.018
	R.S.D. (%)	0.72	0.05	0.039

d-Accuracy

Accuracy of the proposed method was further confirmed by performing recovery studies using standard addition method. A fixed amount of drug from dosage form was taken and pure standard drug at three different concentrations within Beer's range was added. The total concentration was found by the proposed method. The determination with each concentration was repeated three times and average percent recovery of the added standard was calculated and results are tabulated in Table 3. The results obtained showed excellent mean recovery percent values, close to 100%, and low standard deviation values ($S.D < 1.0$) which indicate high accuracy of the proposed analytical methods.

Table(3): results of recovery study for ceftriaxone sodium

Base level (µg/ml)	Amount spiked (µg/ml)	Amount recovered* (µg/ml)	% Recovery±SD
8	8	7.995	99.93±0.05
8	24	23.993	99.97±0.03
8	45	45.011	100.02±0.02

* Mean value of three determinations

e-Specificity:

The commonly drug (sulbactam) that maybe added to ceftriaxone sodium vials was found not to interfere in the analysis of ceftriaxone sodium, as shown in table 4.

Table (4): results of specificity study for ceftriaxone sodium

Concentration(µg/ml)	Amount recovered* (µg/ml)	% Recovery ±SD
8	7.992	99.9±0.09
24	23.991	99.96±0.12
45	44.992	99.98±0.13

* Mean value of three determinations

Application to pharmaceutical vials:

The assay for the marketed vials (brand A, brand B, brand C) of ceftriaxone was established using the present optimized spectrophotometric conditions and it was found to be accurate and reliable. The results are shown in table 5. The assay values of ceftriaxone vials ranged from 98.31 % to 99.35%, with relative standard deviation of not more than

1%. The assay values for the formulations were very close as mentioned in the label claim, indicating that the interference of excipients is insignificant in the estimation of ceftriaxone by the proposed analytical method. Recovery percentage obtained by the proposed method was similar to other methods (101% was obtained by Qasim et al, 2011)[20].

Table 5: results of application of spectrophotometric method to the determination of ceftriaxone sodium from pharmaceutical vials

Brand	Label claim($\mu\text{g/ml}$)	Amount recovered*($\mu\text{g/ml}$)	% Recovery \pm SD
A	8	7.933	99.16 \pm 0.09
	12	11.913	99.27 \pm 0.07
	24	23.844	99.35 \pm 0.03
B	8	7.921	99.01 \pm 0.07
	12	11.909	99.24 \pm 0.05
	24	23.802	99.17 \pm 0.05
C	8	7.907	98.83 \pm 0.12
	12	11.798	98.31 \pm 0.09
	24	23.799	99.16 \pm 0.08

* Mean value of three determinations

Conclusion:

An accurate spectrophotometric method was used and validated for the determination of ceftriaxone sodium through complex formation with copper (II). The method was suitable to determine concentrations in the range of 8-70 $\mu\text{g/ml}$ precisely and accurately. The limits of detection and quantitation for the drug was (0.84;2.55). Furthermore, the mean relative standard deviation (RSD) and the mean relative analytical error can be considered to be very satisfactory. The sample recovery from the formulation was in good agreement with its respective label claim. Finally, this method had proven its sensitivity and specificity in determination ceftriaxone sodium, so the proposed method can be used as alternative to the reported ones for the routine analysis and the quality control of ceftriaxone sodium in pure form and in pharmaceutical dosage forms.

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