



Spectrophotometric Determination of Cefixime Diazotization and Coupling with Terephthalaldehyde

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Abstract

Cefixime (CFX) was treated with sodium nitrite and hydrochloric acid for diazotization reaction followed by coupling with Terephthaladeyhde in alkaline medium to form, a red colored azo dye compound at 523 nm where the concentration of (CFX) was determined spectrophotometrically. The optimum reaction conditions and analytical parameters were evaluated. Beer's law was obeyed in the concentration range of (2.5-30) $\mu\text{g.mL}^{-1}$ with a molar absorptivity of 11774 $\text{L.mol}^{-1}.\text{cm}^{-1}$. The limit of detection was found to be 0.06146 $\mu\text{g.mL}^{-1}$ and the Shandell's sensitivity value was 0.0431 $\mu\text{g.cm}^{-2}$. The proposed method could be successfully applied to the determination of (CFX) in pharmaceutic formulations. .

Keywords: Spectrophotometric determination, Cefixime, Diazotization reaction, Terephthaladeyhde, Coupling reaction.

التقدير الطيفي للسيفكسيم بتفاعلات الازوتة والاقتران مع الكاشف ترفتالديهايد

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الخلاصة

طورت طريقة سهلة وبسيطة لتقدير السيفكسيم (CFX) بتفاعلات الازوتة والاقتران، واعتمدت الطريقة على ازوتة السيفكسيم بعد إضافة نترت الصوديوم وحامض الهيدروكلوريك اليها حيث يتكون ملح دايزونيوم وبعدها يتم تفاعل الملح المتكون مع الكاشف ترفتالديهايد بوجود المحلول القاعدي حيث يتكون ناتج بلون احمر ويعطي اعلى امتصاص عند الطول الموجي 523nm مقابل المحلول الصوري، ويتبع قانون بير في مدى من تراكيز بين (2.5-30 $\mu\text{g/ml}$) ، وكانت قيمة الامتصاصية المولارية 11774 L/mol.cm وكانت قيمة دلالة ساندل $0.0431 \mu\text{g/cm}^2$ ، وقيمة حد الكشف 0.061465 $\mu\text{g/ml}$. وكانت الطريقة على درجة عالية من الدقة والتوافق، وقيمة الاسترجاعية بلغت 100.1376%. وقد طبقت الطريقة بنجاح على المستحضر الصيدلاني.

الكلمات المفتاحية: تقدير طيفي، السيفكسيم ، ترفتالديهايد ، الازوتة والاقتران.

1- Introduction

Cefixime is third generation cephalosporin antibiotic. It is under the category of Beta Lactum Antibiotic/ Cell wall inhibitors. It acts by inhibiting an enzyme

transpeptidase, involved in the building of bacterial cell walls. It is used in lower respiratory tract infection. It is helpful in acute urinary tract infection, biliary tract infection, sinusitis, acute otitis media, peptic ulcer and many more⁽¹⁾. Chemically cefixime (Fig. 1) is (6R, 7R)-7-[2-(2-amino-4-thiazolyl) glyoxylamido]- 8- oxo-3-vinyl-5-1-azabicyclo [4.2.0] oct-2- ene-2-carboxylic acid, 7-9z)-[o carboxymethyl)-oxime] trihydrate⁽²⁾.

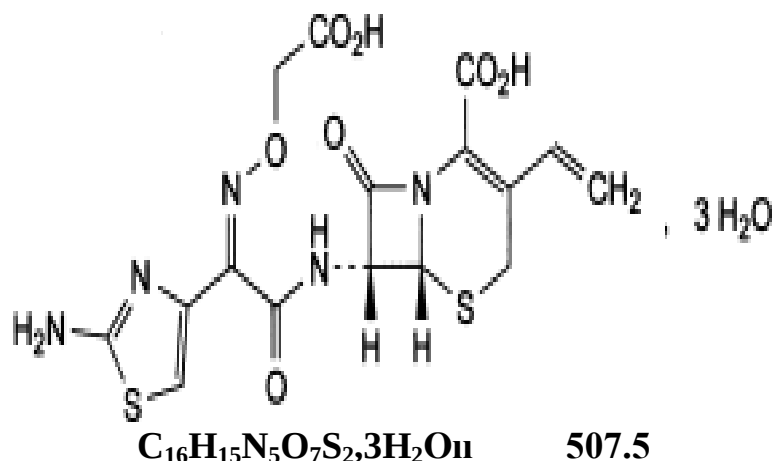


Fig [1]: Chemical Structure of Cefixime Hydrate

Cefixime hydrate was determined by several methods , including nitrogenation coupling⁽³⁾ , oxidative coupling , as well as Schiff's base method⁽⁴⁾, point cloud method⁽⁵⁻⁷⁾, High performance Chromatography method⁽⁸⁾ , and derivatives method⁽⁹⁾.

2- Materials and Methods

2-1 Instrument used

A Double beam – Shimadzu UV- Visible UV -1800 spectrophotometer was used, with quartz cells with a width of 1 cm. The weighing was carried out using a four order sensitive balance.

2-2 Reagents

All chemical used are of the highest purity available.

2-3 Preparation of solutions

2-3-1 Solution Cefixime hydrate Standard 1000 µg./mL

The solution was prepared by dissolving 0.1000g of Cefixime hydrate in 5ml of ethanol with continuous stirring. After dissolution, the volume was completed with



distilled water using a 100 ml volumetric vial.

2-3-2 Cefixime hydrate 250 µg/ml

The 250µg/ml solution was prepared by with drawing 25 ml of the prepared Cefixime hydrate standard solution at a concentration of 1000 ppm in to a 100 ml volumetric vial and supplementing the volume with distilled water to the mark.

2-3-3 A solution of Terephthaladeyde of 0.01 M

Prepare by dissolving 0.1340 gm of the in 5 ml of ethanol and then completing the volume to the mark in a 100 ml volumetric vial with distilled water.

2-3-4 A solution Sodium nitrite of 0.01 M

Prepared by dissolving 0.069 gm of the pure substance in a small amount of distilled water and then completing the volume to the mark in the volumetric bottle of 100 ml.

2-3-5 A Sodium hydroxide of ~ 1M

Prepared by dissolving 4.00 gm. of the pure substance in a small amount of water and then completing the volume to 100 ml of distilled water.

2-3-6 A hydrochloric acid HCl of ~ 1M

The acid solution was prepared by diluting 8.6 ml of concentrated (11.64) to 100 ml of distilled water.

3- Results and discussion.

3 -1 Study of the Optimum Reaction Conditions

For the subsequent experiments 1ml Cefixime hydrate (250 µg/ml) is taken in final volume 10 ml and absorbance measurements are performed at 523 nm.

3-1-1 Volume effect of Sodium nitrite

The effect of the amount of sodium nitrite was studied by taking different 0.01M by mixing 1ml of HCl 1M , 1ml of volumes of (0.2- 1.6) ml of Cefixime 250 µg/ml , 1ml reagent 0.01M and 1ml NaOH 1M. It was noted in Table 1.



Table 1: Effect of the amount of NaNO_2

ml of NaNO_2 $1 \times 10^{-2} \text{M}$	Absorbance
0.2	0.212
0.4	0.274
0.6	0.393
0.8	0.491
1	0.533
1.2	0.473
1.6	0.390

From the results that the best volume that gives the highest absorption of the colored product is 1ml of sodium nitrite , so it was adopted in subsequent experiments.

3-1-2 Effect of acid type

Different acids (HCl , H_2SO_4 , CH_3COOH) were used with a concentration of 1M for each of them ,as well as the same added volume of 1ml to find out which acid gives the best absorption when forming the product , as shown in table 2.

1M of 1ml acid	Absorbance
HCl	0.533
H_2SO_4	0.520
CH_3COOH	0.440

It was noted from the results that the best acid that gives the highest absorption of the colored product is HCl so it was adopted in subsequent experiments.

3-1-3 Effect of the amount of HCl

The effect of the amount of HCl was studied by taking different volumes of (0.2-1.4) ml of HCl solution with a concentration of 1M by mixing , 1 ml sodium nitrite (0.01)M , 1ml Cefixime (250) $\mu\text{g/ml}$ and 1ml of reagent (0.01)M , 1ml of NaOH 1M. It was noted in Table 3 from the results that.

Table 3: Effect of acid tybe

ml of 1 M Acid	Absorbance
0.2	0.429
0.3	0.438



0.4	0.520
0.5	0.567
0.6	0.559
0.8	0.540
1	0.533
1.2	0.512
1.4	0.478

It was noted from the result that the best volume that gives the highest absorption of the colored product is 0.5 ml of HCl so it was adopted in the subsequent experiments.

3-1-4 Effect of Teteraladehyde (0.01M) amount

The effect of the a mount of reagent was studied by taking different volumes of (0.3- 2) ml of Terephtaladehyde solution with a concentration of 0.01 M by mixing 1ml of sodium nitrite 0.01M , 0.5 ml HCl 1M , 1ml Cefixime 250 µg/ml and 1ml of NaOH 1M. It was noted in table 4.

Table 4: Effect of reagent amount

Amount (ml) of Reagent $1 \times 10^{-2} \text{M}$	Abs	
	BW	SB
0.3	0.027	0.314
0.5	0.048	0.410
1	0.065	0.566
1.5	0.080	0.573
1.8	0.057	0.521
2	0.043	0.498

3-1-4 Effect of base type

Different bases (NaOH, KOH, NH_4OH) were used with a concentration of 1M for each of them , as well as the same added volume of 1ml to find out which base gives the best absorption when forming the product, as shown in table 5.

Table 5: Effect of base type

1ml of 1M base	Abs
NaOH	0.573
KOH	0.522
NH_4OH	0.499

It was noted from the results that the best base that gives the highest absorption of the colored product is NaOH so it was adopted in subsequent experiments.

3-1-5 Effect of the amount of NaOH

The effect of the amount of NaOH was studied by taking different volumes of (0.1-2) ml of NaOH solution with a concentration of 1M by mixing , 1ml of NaNO₂ 0.01M, 1ml CFX µg/ml 250, 0.5 ml HCl 1M and 1.5 ml of reagent 0.01M. It was noted in Table 6 from the results that.

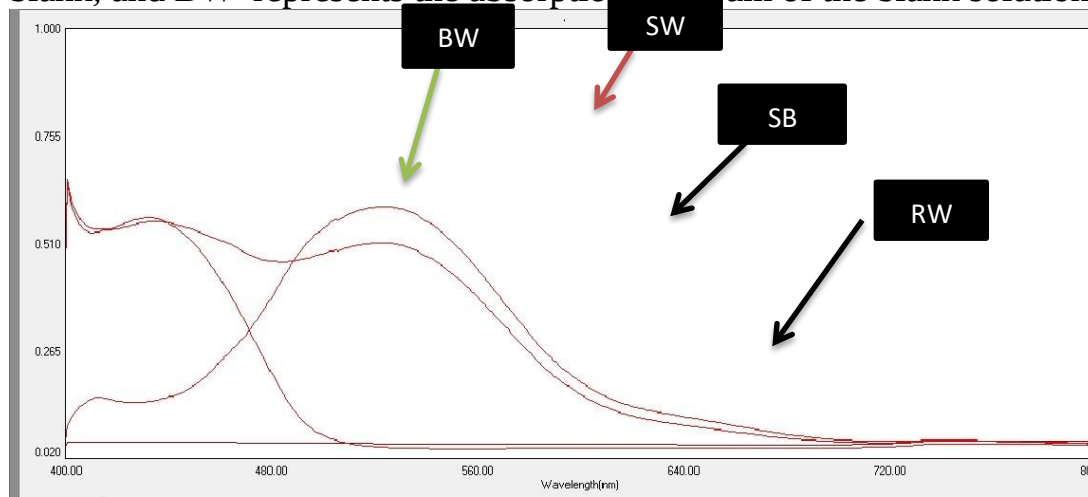
Table 6: Effect of the amount of base NaOH

1M of NaOH	Abs
0.1	0.353
0.3	0.429
0.5	0.583
0.8	0.579
1	0.573
1.5	0.520

It was noted from the results that the best volume that gives the highest absorption of the the colored product is 0.5 ml of NaOH so it was adopted in the subsequent experiments.

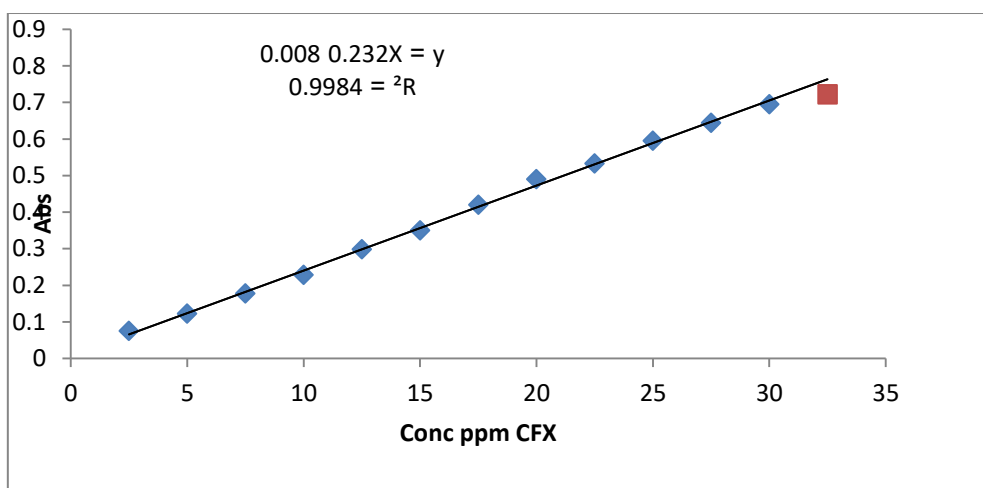
3-2 Final absorption spectrum

After stabilizing the optimal conditions, which is the use of 1ml of sodium nitrite , 0.5 ml of HCl , 1ml of Cefixime, 1.5 ml of reagent and 0.5 ml of NaOH in a 10 ml volumetric vial. After 10 minutes, the absorption spectrum of the solution was measured , as it gave highest absorption at the wavelength of 523 nm , as shown in figure 3. As SW representa the absorption spectrum of Cefixime solution verus distilled water, SB represents the absorption spectrum of Cefixime solution versus the blank, and BW represents the absorption spectrum of the blank solution versu



3-3 Approved of work and calibration curve

Taking a series of volumetric bottles of 10 ml containing volumes of (0.1-1.3) ml Cefixime solution at a concentration of 250 $\mu\text{g/ml}$. Figures 4 represent the calibration curve and the absorption spectrum, as it was found that the concentrations of Beers law (2.5- 30) $\mu\text{g/mL}$ Cefixime solution, with a correlation coefficient of 0.9984 and the value of the molar absorptivity was calculated, and its value was $11774 \text{ L.mol}^{-1} \cdot \text{cm}^{-1}$.



3-4 Accuracy and Precision of the method

Optimum conditions were used in the working method to test the methods accuracy and precision. Six reading were taken for three different concentrations (12.5, 22.5, 25) $\mu\text{g/mL}$ of Cefixime solution within the limits of Beers law in the calibration curve. The recall rate and relative standard deviation were calculated. The method has high accuracy and precision, as the results show in Table (7).

Amount of CFX $\mu\text{g/ml}$	Found CFX $\mu\text{g/ml}$	RE. %	Recovery. %	Average of Recovery	RSD. %
12.5	12.4928	- 0.05747	99.9425	100.6310	0.42494
22.5	22.622	0.5427	100.5427		0.14254
25	25.3520	1.4080	101.4080		0.26582

3-5 Detection limit and quantitative limit

Detection Limit was calculated by measuring the absorption for the blank at optimal conditions at 443nm. The results were shown in Table (8)

Table 8: detection limit and quantitative limit

Slop	$\bar{X}$	S	L.O.D $\mu\text{g/ml}$	L.O.Q $\mu\text{g/ml}$
0.0232	0.0325	0.0006053	0.0614631	0.1862520

3-6 Composition of the azo dye

The stoichiometry of the azo dye is studied under the established conditions by applying the continuous methods Jop s and molaratio methods. The experimental data in both methods (Fig. 3 and 4) show that the azo dye has been formed by a 1:1 combining ratio.

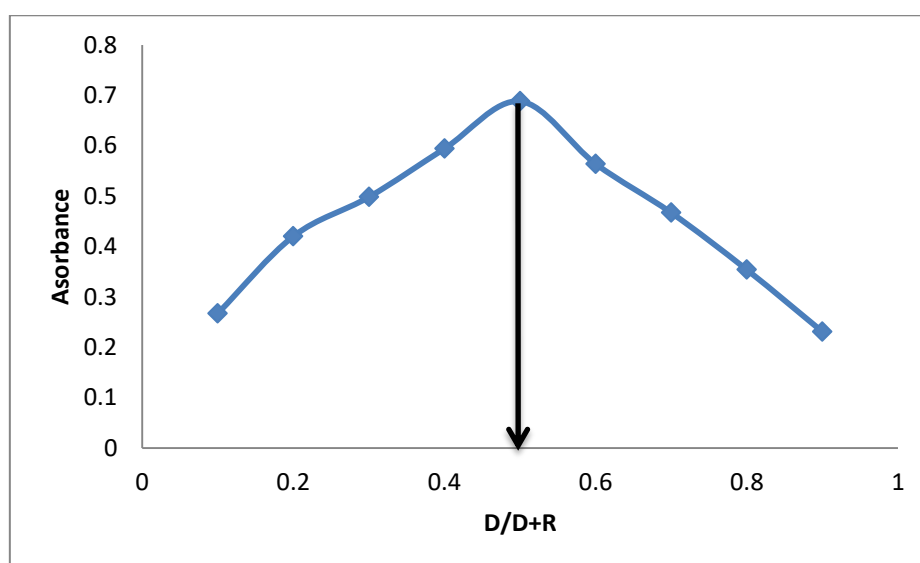


Figure 3: Jops method

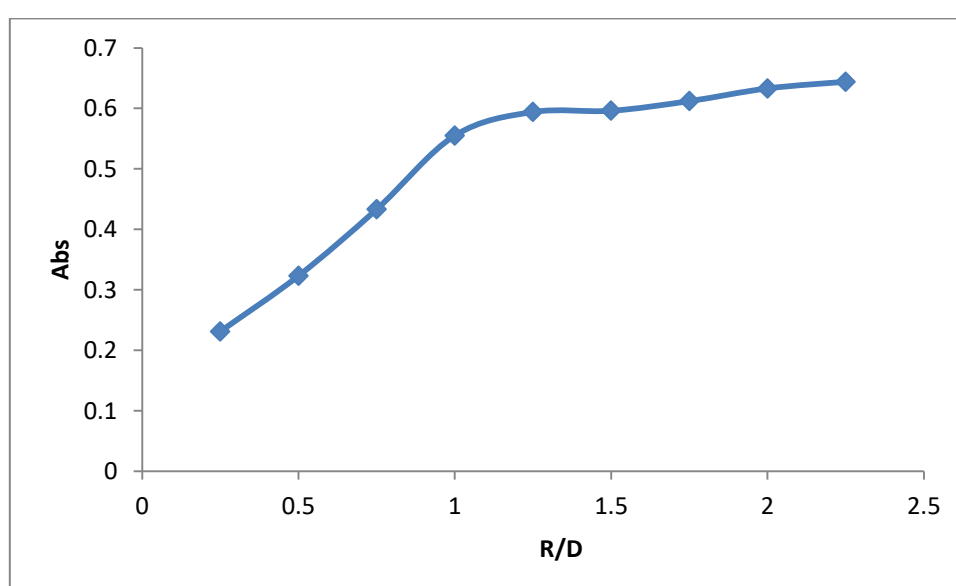
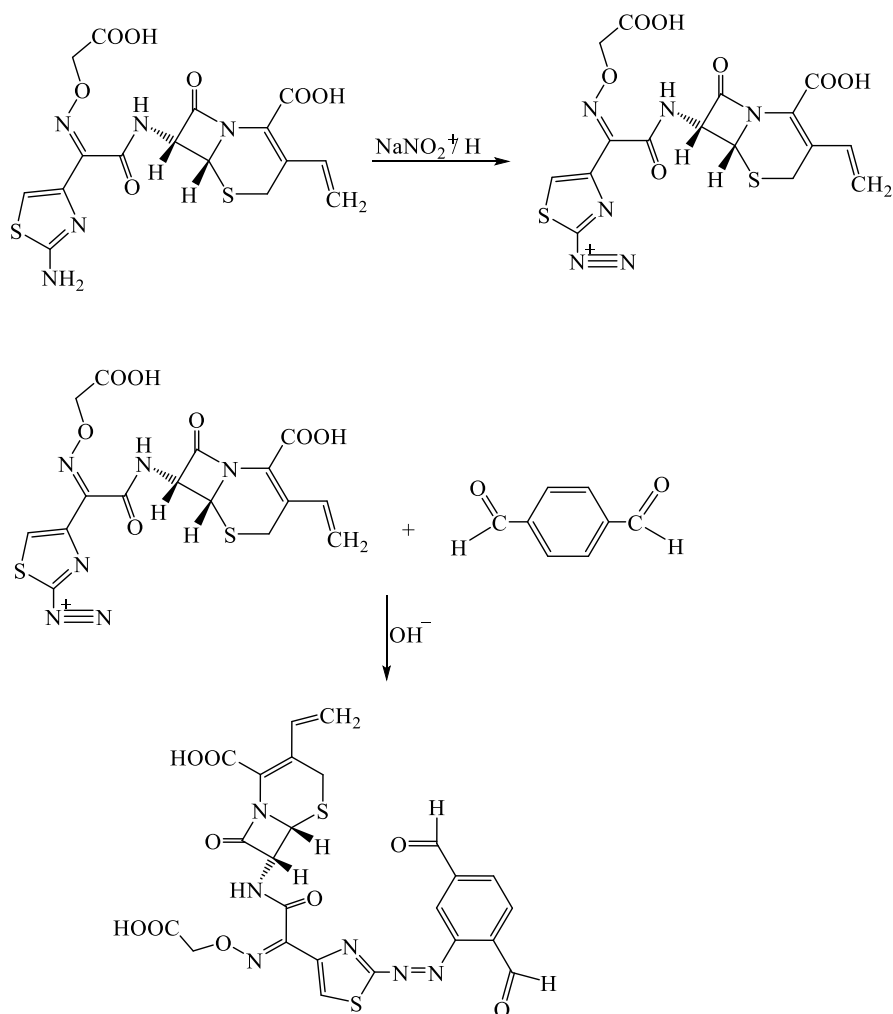


Figure 4: molaratio method

Therefore, the proposed reaction equation is as follows⁽¹⁰⁾:



Applications

The pharmaceutical preparation Winex 200 Cefixime Capsules - Saudi Arabia is prepared in the following way, which is the weight of eight capsules, where each capsule is equivalent to 2000 $\mu\text{g/ml}$, and the total weight of the capsules is equal to 1.9886gm, so about 0.03107gm is taken, which is equivalent to 250 $\mu\text{g/m}$. It is dissolved in a small amount of ethanol and then diluted with water to 100ml.

3-7-1 Direct Method

Three different concentrations of the solution of each preparation (12.5, 20, 25) $\mu\text{g/ml}$ were taken and the solutions were treated with the same steps followed when preparing the calibration curve and then the absorption was measured for them at the wavelength 523 nm and an average of six readings was calculated for each concentration in addition to calculate the retrospective and the results are shown in the table 9.

Table 9 : of direct additives to the preparation

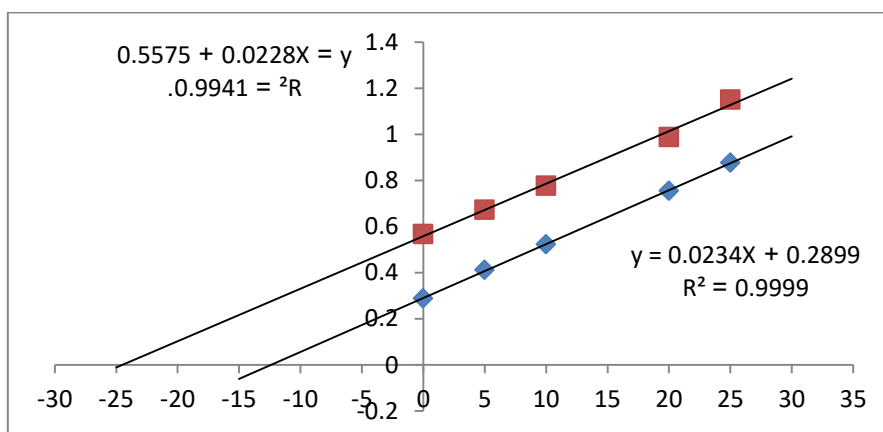
Cone of CFX $\mu\text{g/ml}$	Absorbance of pure CFX	Absorbance of CFX	RE %	Recovery, %	Average recovery %
12.5	0.298	0.30625	2.8448	100.8448	99.0159
20	0.473	0.465	-1.5086	98.4013	
25	0.596	0.57525	-2.1982	97.8017	

3-7-2 Standard addition method

In order to show that the above proposed method is free of interference, The standard addition method was applied in the determination of Cefixime in its pharmaceutical preparations. The method included adding fixed quantities (0.5, 1) ml of the solutions of the previously prepared pharmaceutical preparations at a concentration of 250 $\mu\text{g/ml}$, in two series of volumetric bottles. Capacity of 10 ml, then adding increasing volumes (0.2, 0.5, 0.8, 1) ml of the standard solution of 250 $\mu\text{g/ml}$ pure Cefixime solution. At a wavelength of 523nm, and the results are shown in the Table 10, and Figures 5.

Table 10: standard addition method

Capsules of Drg	CFX Present $\mu\text{g/ml}$	CFX measured $\mu\text{g/ml}$	Recovery, %
Capsules' (CFX) 200mg. Saudi Arabia	12.5	12.3888	99.1111
	25	24.4517	97.8070



conclusion

An easy, accurate and sensitive method has been developed for the spectrophotometric determination of the drug cefixime 250 in $\mu\text{g/ml}$ its pure form and in pharmaceutical formulations by the azo method and coupling, where the azo dye is formed as a result of coupling between the reagent and the Diaz onium salt formed from the reaction of sodium nitrite 0.01M with cefixime 250 $\mu\text{g/ml}$. Where it gave the highest at the wavelength 523nm and follows Beer's law A range of concentrations between (2.5-32) $\mu\text{g /mL}$ and the value of molar absorbance was 11774 L/mol.cm, Sandal significance 0.04310, detection limit 0.02966, recovery rate 100.6310, and the relative standard deviation between 0.1435-0.4249. It turns out that the method has good compatibility and accuracy.

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