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Spectrophotometric Determination of Cefixime by Using Diazotization Reaction with Resorcinol Reagent

Deena Mazin Abd Al-Jawad

dina.abduljawad@uomosul.edu.iq

College of Education for Women, Dept. of Chemistry, Univ. of Mosul-Iraq

Abstract

A simple and sensitive spectroscopic technique was developed to determine the purity of cefixime and its pharmaceutical preparations using the diazotization reaction. The technique relies on the coupling of diazotized cefixime with the reagent resorcinol under basic conditions, resulting in a stable, light-orange dye that is measured spectroscopically at a wavelength of 436nm. The method was in compliance with Beer's law in the concentration range (0.4-7 µg/ml) and the molar absorptivity value was (1.44637×10^4 $L.mol^{-1}.cm^{-1}$), and Sandel's sensitivity (0.0350 µg/cm^2), while the recovery rate was (100.18%) and the relative standard deviation rate was <0.8%, with (0.196 µg/ml) detection limit. The nature of the formed dye was also studied and it was found that the compositional ratio of Diazotized cefixime and Resorcinol was (1:1). The suggested method is excellent and has good applicability to pharmaceutical preparations, as evidenced by the successful application of the technique in the determination of cefixime in pharmaceutical preparations (tablets, capsules), where it was found to be in good agreement with the pure content of pharmaceutical preparations. The standard method used is the standard addition method.

Introduction:

Cefixime (trihydrate) is a chemical compound whose scientific name is: (6R,7R)-7-[[2-(2-Aminothiazol-4-yl)-2-[(carboxymethoxy) imino] acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid.

Molecular formula C16H15N5O7S2.3H2O

Molecular weight 507.5 g/mol, and its structural formula is shown in Figure 1:

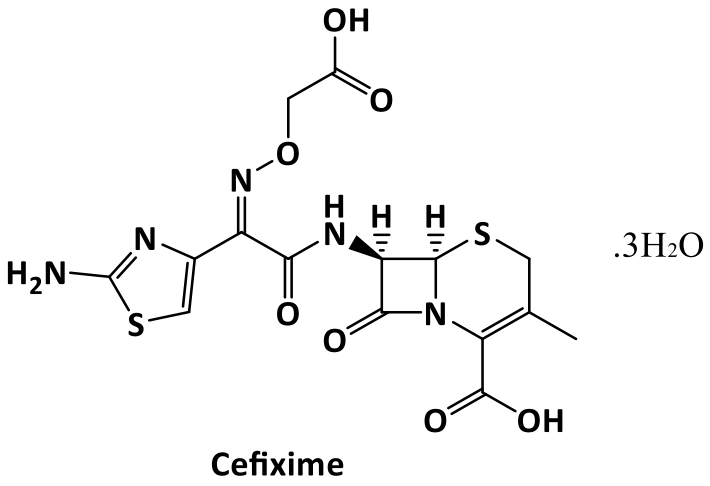

Figure 1: The structural formula of cefixime.

Practical part:

Materials and chemical solutions used:

Cefixime solution (100µg/ml):

To prepare the solution, (0.0100 g) of pure cefixime was dissolved (supplied by the state enterprise for drug industries and medical appliance SDI-Samarra-Iraq) in methanol (5 ml), then distilled water was added to a (0.1L) volumetric flask until the volume was reached.

Resorcinol reagent solution (0.1%):

The solution was prepared by dissolving (0.1000 g) of resorcinol in ethanol, and the final volume was completed with ethanol.

Sodium nitrite solution (1%):

The solution was prepared by dissolving sodium nitrite (1 g) in distilled water, and then distilled water was added to a 0.1 L volumetric flask until the desired volume was reached.

Sulfamic acid solution (1%):

It was prepared by dissolving sulfamic acid (1g) in distilled water, then distilled water was added to a 0.1L volumetric flask until the volume was reached.

Sodium carbonate solution (1 M):

(10.6 g) Sodium carbonate was dissolved in distilled water, and then distilled water was added to a 0.1 L volumetric flask until the volume was reached.

Hydrochloric acid solution (1 M):

(8.3 ml) A concentrated solution of hydrochloric acid is placed in a 0.1L volumetric flask, and the volume is completed with distilled water to the mark.

Results and Discussion:

General principle of the technique:

In the presence of hydrochloric acid, cefixime reacts with $NaNO_2$ to form a diazonium salt, and excess nitrite is subsequently eliminated by adding sulfamic acid.

In the second step, the diazonium salt is coupled with resorcinol reagent in a basic medium, forming an orange azo dye.

Preliminary study:

The purpose of the study is to develop a method for the determination of cefixime by diazotizing it and then coupling it with the resorcinol reagent to form an orange azo dye that can be measured spectroscopically, by mixing (1ml) of cefixime (100µg/ml) with (0.5ml) of hydrochloric acid (1 molar), then waiting for four minutes after adding (1 ml) of sodium nitrite (1%), then adding (0.5ml) of sulfamic acid (1%). After making the medium basic by adding (1ml) of sodium carbonate (1 molar), then adding (0.5 ml) of resorcinol reagent (0.1%), it was observed that an azo dye of a light orange color was formed, showing its maximum absorption intensity at 436nm wavelength.

Study of the optimum conditions:

To produce a dye with a high and consistent absorbance, the effect of various variables affecting the intensity of absorption of the resulting dye and its color was studied. In the subsequent experiments, 1 ml of (100 µg/ml) of cefixime solution was used in 25ml volumetric flasks, and the absorbance was measured at 436nm wavelength.

1- Studying the effect of the acid type:

It is clear from the preliminary study that the formation of diazonium salt occurs in the presence of acid. The effect of several acid types - strong and weak - was studied by adding fixed amounts (0.5 mL) at a concentration of 1 M of each one separately. Table 1 outcomes show that hydrochloric acid is the desired acid for giving the highest absorption intensity, and therefore, it was used in subsequent studies.

Table (1): Choosing the appropriate type of acid

Type of acid (1 M)	**H_2SO_4**	**CH_3COOH**	**HCl**
Absorbance	0.049	0.058	0.093

2- Studying the effect of the base type:

Since the dye is only formed in the basic conditions, the effect of various base types (strong and weak) was investigated by adding (1ml) of bases with concentrations (1M) of each of them separately. The results in Table 2 show that sodium carbonate yields the highest absorption intensity, and therefore, it was used in subsequent studies.

Table (2). Choosing the appropriate type of base.

Type of base (0.5 M)	KOH	NaOH	Na_2CO_3
Absorbance	0.013	0.014	0.103

3- Study the effect of the hydrochloric acid amount:

To examine the effect, hydrochloric acid with a concentration of 1 M was added in varying quantities (0.25-2 mL) to the reaction mixture, and then the absorption of each solution was assessed in relation to the blank solution. The outcomes in Table 3 showed that the volume of 1 ml gave the highest absorption intensity; therefore, it was used in subsequent studies.

Table (3): Amount of acid affecting absorption.

Volume of HCl (1M)(ml)	0.25	0.5	0.75	1	1.5
Absorbance	0.053	0.073	0.101	0.105	0.099

4- Studying the effect of the amount of sodium carbonate:

Different volumes (0.1-1) ml of sodium carbonate at a concentration of (1 molar) was added to the reaction mixture to study the effect, then the blank solution was used to compare the absorption of these solutions and the results in Table (4) showed that the volume of 0.5 ml gives the highest absorption intensity therefore it was used in subsequent studies.

Table (4): The amount of base affecting absorption.

Volume of Na_2CO_3 (1M)(ml)	0.1	0.2	0.3	0.5	1
Absorbance	0.061	0.089	0.091	0.109	0.057

5- Studying the effect of the amount of nitrite and time:

Nitrite serves as the diazotizing agent in the pharmaceutical compound; therefore, this effect was studied by adding different volumes of sodium nitrite (1%) for varying periods of time to diazotize the drug. Then, after coupling with the detector, the absorption intensity of the colored solutions was measured against their blank solutions at 436nm wavelength. Using 1.0 mL of nitrite solution for 3 min produced the best absorption of the formed dye, as indicated by the data in Table 5, and was therefore adopted in subsequent studies.

Table (5): The effect of the amount of sodium nitrite and time on the absorption of the dye.

Volume of $NaNO_2$(1%)(ml)	Absorbance/min. standing time			
	0	2	3	4
0.1	0.015	0.018	0.025	0.012
0.25	0.071	0.101	0.104	0.039
0.5	0.083	0.108	0.112	0.060
1	0.087	0.109	0.115	0.071
1.5	0.021	0.029	0.107	0.025

6- Study of the effect of the amount of reagent:

After making the medium basic, diluting to the appropriate level, and measuring the absorbance of the solutions, varying amounts (0.25-2 ml) of the coupling reagent (resorcinol) were added to a fixed amount of the diazotizing medication in order to determine the ideal concentration of the reagent (0.1%). Subsequent investigations employed a 1 mL volume of the reagent solution, as it yielded the highest absorption intensity, as indicated by the practical data displayed in Table 6.

Table (6): Effect of the reagent amount.

Volume of reagent (0.1%) (ml)	Absorbance
1/4	0.063
1/2	0.104
1	0.121
1.5	0.112

7- Study of the effect of surfactants:

By adding different volumes (0.5–2 mL) of surfactants (positive, negative, and neutral) to the reaction mixture, the impact of these surfactants on the absorption intensity of the azo dye generated was investigated. Since the use of surfactants reduces the absorption intensity of the colored product, as indicated in Table 7, they were not further explored in this research.

Table (7): Effect of surfactants

Surfactant	Absorbance /ml of surfactant utilized			
	1/2	1	1.5	2
CTAB 0.1%	0.036	0.090	0.047	0.085
SDS 0.1%	0.022	0.065	0.052	0.074
Triton X-100 0.1%	0.001	0.014	0.020	0.015
Without Surfactant	**0.123**			

8- Examining how time affects the final product's stability:

By monitoring the absorption intensity of the azo dye generated at various times, the impact of time on the stability of the final product was investigated. Based on Table 8, the azo dye is generated instantly after the base is added, stabilizes 5 minutes after the additions and dilution are finished, and stays stable for 30 minutes at room temperature.

Table 8. The effect of time on dye stability.

Cefixime 4µg. mL^{-1} Room Temperature	Absorbance/time (minutes)
After addition	0.120
5	0.122
10	0.121
15	0.120
20	0.119
25	0.118
30	0.117
35	0.115
40	0.112
45	0.110
50	0.109
55	0.106
60	0.105

Final absorption spectrum:

The final absorption spectrum of the azo dye formed by the coupling reaction of diazotized cefixime with resorcinol reagent in basic medium was studied. The absorption spectrum plot displayed the highest absorption intensity of the dye at a wavelength of 436 nm, as shown in the figure below.

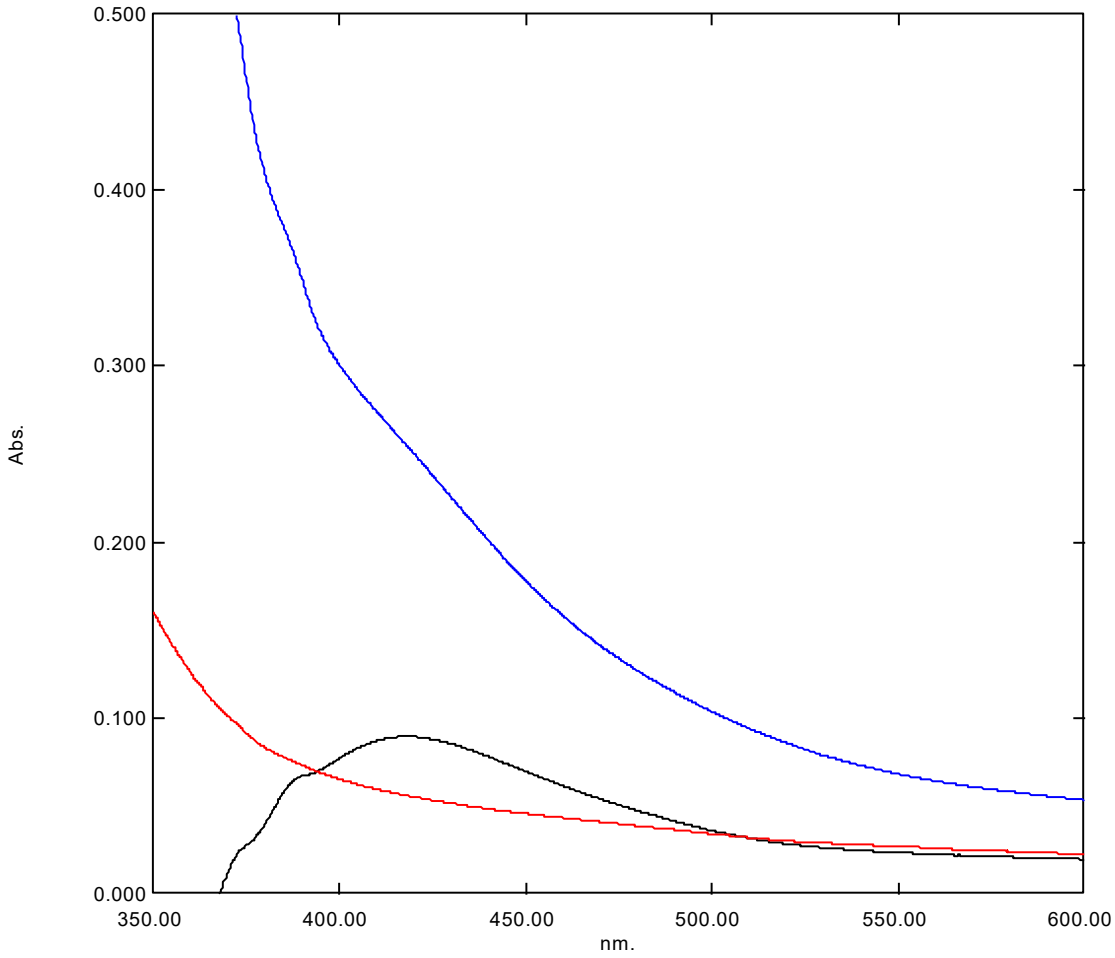

Figure (2): Absorption spectrum of the dye resulting from the coupling of a solution (4 µg/ml) of cefixime with the resorcinol reagent. a. Absorption spectrum of the dye versus the blank solution. b. Absorption spectrum of the dye versus distilled water. c. Absorption spectrum of the blank solution versus distilled water.

Method of work and preparation of the standard curve:

1 ml of hydrochloric acid (1M) was added to 1 ml of cefixime (100 µg/ml), then 1 ml of sodium nitrite (1%) was added, and after 3 minutes, 0.5 ml of sulfamic acid (1%) was added. After that, add (0.5 ml) of sodium carbonate (1 molar) to make the basic solution. A light orange azo dye was formed after adding 1 ml of resorcinol reagent (0.1%), exhibiting its maximum absorption intensity at 436nm.

The standard curve for determining cefixime is shown in Figure 3. It follows Beer's law in the concentration range of 0.4-7 µg/mL, but deviates negatively from Beer's law after exceeding the upper estimated limit. The value of the determination coefficient shows that the standard curve's linear specifications are excellent.

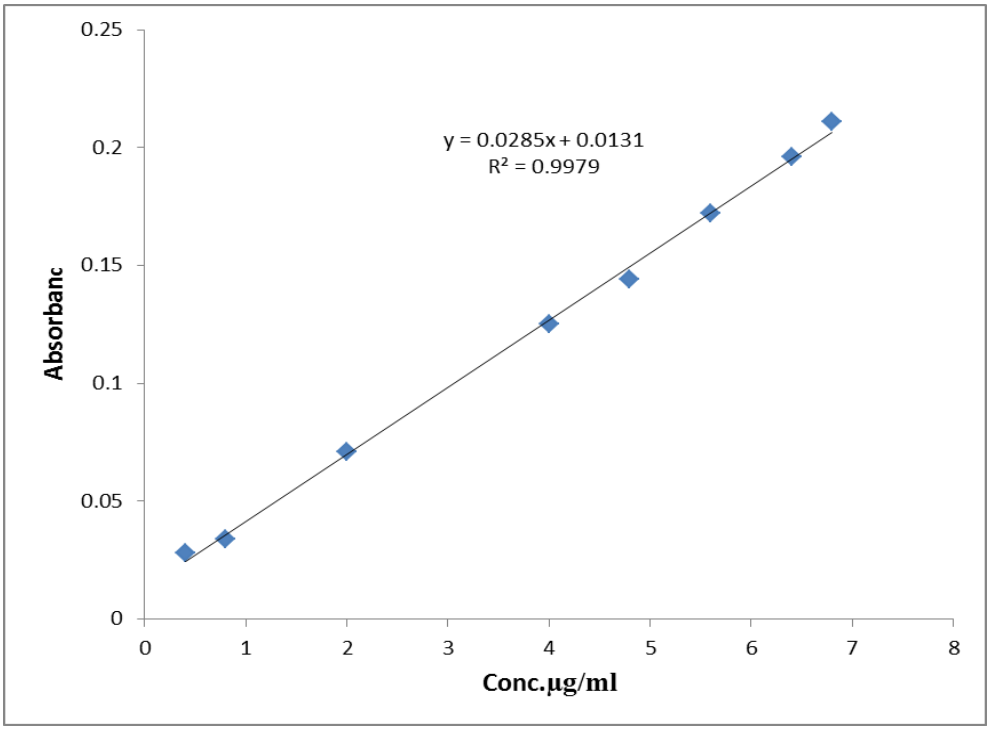

Figure (3): Standard curve for determining cefixime.

Limit of detection (LOD) and limit of quantification (LOQ) were also determined by measuring the absorbance of ten samples of the blank solutions against distilled water at a wavelength of 436 nm, in order to find the arithmetic mean (Ẋ) and the standard deviation (σ) as shown in the following table:

Table (9): Calculations of the limits of detection and quantification for the proposed method.

Absorbance (Xi)	(Xi- Ẋ)	(Xi- Ẋ)²
0.016	$10^{-3}\times3.3$	$10^{-5}\times1$
0.015	$10^{-3}\times2.3$	$10^{-6}\times5.29$
0.012	$10^{-4}\times-7$	4.9×10^{-7}
0.011	$10^{-3}-1.7\times$	$10^{-6}\times2.89$
0.010	$10^{-3}-2.7\times$	$10^{-6}\times$ 7.29
0.013	$10^{-4}\times3$	9×10^{-8}
0.011	$10^{-3}\times-1.7$	2.89×10^{-6}
0.014	$10^{-3}\times1.3$	$10^{-6}\times1.69$
0.012	$10^{-4}\times7-$	$10^{-7}4.9\times$
0.013	$10^{-4}3\times$	$10^{-8}\times9$

$\Sigma Xi=0.127$ $\Sigma(Xi-\dot{X})^2=3.12\times10^{-5}$

Through the equations resulting from Table 9, using the following mathematical relationships, the limit of detection (LOD) was calculated as follows:

$$\sigma = \sqrt{\frac{\sum(Xi-\dot{X})^2}{n-1}}$$

$$\sigma = \sqrt{\frac{3.12\times10^{-5}}{10-1}} = 1.86\times10^{-3}$$

$$LOD = \frac{3\sigma_B}{slope}$$

$$= \frac{10^{-3}\times1.86\times3}{0.0285} = 0.196 \ \mu g/ml$$

The LOQ was calculated in the same way using the following relationship:

$$LOQ = \frac{10\sigma_B}{slope}$$

$$= \frac{10^{-3}\times1.86\times 10}{850.02} = 0.653 \ \mu g/ml$$

The molar absorptivity and Sandell's sensitivity were also determined, and the results are shown in Table 10.

Table (10): Linearity range, detection limits, molar absorptivity, Sandell's sensitivity, slope, intercept, and correlation coefficient

Linearity range (µg/ml)	0.4-7
LOD*(µg/ml)	0.196
LOQ*(µg/ml)	0.653
Molar absorptivity ($I.mol^{-1}.cm^{-1}$)	1.44637×10^4
Sandell's sensitivity ($\mu g/cm^2$)	0.0350
Slope	0.0285
Intercept	0.0131
Correlation coefficient	0.9979

Accuracy and consistency of the method:

Five replicates of three different concentrations (2, 4, and 5.6 µg/ml) of cefixime solution were measured and treated using the approved method to examine the accuracy and consistency of the method under ideal conditions. The results, presented in Table 11, demonstrate the method's good accuracy.

Table 11. Accuracy and consistency of the method.

Conc. of Cefixime µg/ml	Recovery*(%)	Relative error* (%)	RSD* (%)
2	99.82	-0.175	1.110
4	99.91	-0.087	0.248
5.6	100.81	0.814	0.933

Study of the formed product nature:

1- The continuous variation method (Job method):

The final product nature was studied through applying the Job method to determine the molar compositional ratio between the pharmaceutical compound (cefixime) and the reagent (Resorcinol). The pharmaceutical compound and reagent solutions were prepared at a concentration of 1.97 × 10^ (- 4) M. Multiple solutions were then created by mixing different volumes of cefixime and the reagent until the final volume of the two components was constant and equal to 6 mL. Following the ideal conditions and diluting to the mark in a 25 mL volumetric flask, the absorption intensity of the solutions was measured against their blank solutions at a wavelength of 436nm. The coupling ratio between cefixime and the reagent resorcinol is (1:1), as shown in Figure 4.

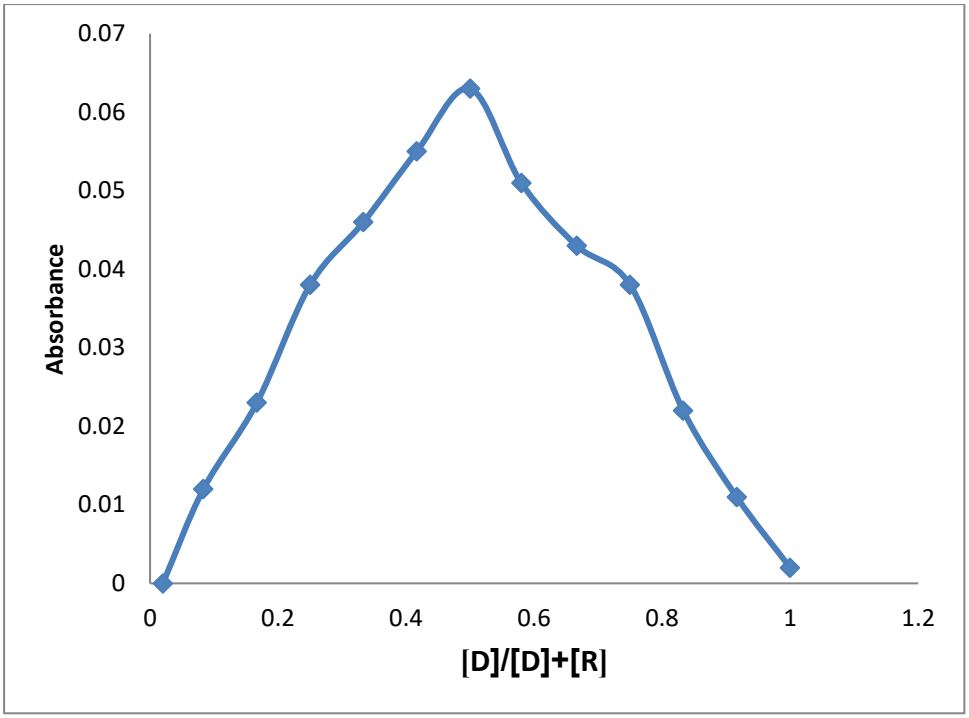

Figure 4: The continuous changes of the interaction of cefixime with the resorcinol reagent in the basic medium.

Study of the effect of interferences:

Six different amounts (100, 500, 1000, 2000, 2500, and 3000 µg/ml) of pharmaceutical preparations were used to determine the effect of various interferences. Based on the results shown in Table 12, we found that no interference from the compounds under study occurred, indicating that they have no effect on the determination of cefixime using the suggested method, even when they are present in high quantitative multiples.

Table 12: The interferences with the determination of cefixime

Foreign Compound	Recovery % Of 100µg Of Cefixime Per µg Foreign Compound Added				
	100	500	1000	2000	2500
Sucrose	99.87	99.88	99.54	100.24	100.87
Starch	98.17	100.67	99.75	99.32	99.69
Lactose	99.4	100.66	98.07	98.21	100.31
Gelatin	99.1	100.2	99.61	98.71	97.34

Application of the method to pharmaceutical preparations:

The suggested method was applied to pharmaceutical preparations of cefixime and the sample was from pills, through taking (3) different concentrations of the solutions of the pharmaceutical preparations mentioned above and all steps were applied according to the approved method under optimum conditions

and the relative error's value, the recovery rate and the relative standard deviation were determined and Table (13) displays that the suggested technique is successful in determining cefixime in pharmaceutical preparations in the form of pills and that the technique has good accuracy and compatibility.

Table (13): Calculations of cefixime in pharmaceutical preparations

Pharmaceutical preparation	Amount taken µg/ml	Amount measured µg/ml	Recovery (%)	Relative error* (%)	RSD* (%)	Drug content found (mg)
Cefixime Tabuk-Saudi Arabia	2	2.035	101.75	1.75	1.22	407
	4	3.992	99.82	-0.175	0.99	399.2
	5.6	5.607	100.12	0.125	1.19	400.4
Sancaklar/duzce/ Turkey	2	1.992	99.64	-0.350	0.98	398.5
	4	3.989	99.73	-0.263	1.16	398.9
	5.6	5.614	100.25	0.250	1.27	401

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