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Article

Development of a Sensitive Spectrophotometrric Quantitative Determination of Cefotaxime in Pharmaceutical Formulations using Diazotization and Coupling Reactions with Thiosimicarbazone

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Abstract: A simple, accurate, and Ultrasensitive Spectrophotometrric determination of cefotaxime (CEF) in pure form and in pharmaceutical formulations was analyzed and developed. The proposed method is based on the conjugation reaction between denaturing cefotaxime and thiosimcarbazone in a basic medium, resulting in the formation of a stable, water-soluble, bright red azo dye exhibting maximum absorbance wavelength 514 nm. The method demonstrated compliance with Beer's law over the concentration range of (5-65 µg/ml)µg/ml, achieving a limit of detection (LOD) of 0.214 µg/ml, with an (LOQ) of 0.677 µg/ml. a Sandell sensitivity of 0.084 µg/cm², and a molar absorbance of 5.681x103 $L \cdot mol^{-1} \cdot cm^{-1}$. The stoichiometric ratio of the drug substance to the reagent in the resulting stain was determined using both the Job method and the molar ratio, and was found to be 1 : 1 Cefotaxime levels in pharmaceutical formulations and pure samples were successfully determined using the suggested method.demonstrating high analytical efficiency and reliability.

Keywords: Cefotaxime, spectrophotometry, Diazotizayion reaction; thiosimcarbazone, pharmacodynamics analysis, pharmaceuticl Formulations.

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Introduction

It is a third-generation cephalosporin antibiotic[1,2] It works by killing bacteria in the body. Cefotaxime is used to treat a variety of serious infections, including life-threatening ones, that are brought on by bacteria that are resistant to antibiotics. Cefotaxime is also used to prevent infection in persons undergoing surgery. [3]. Cefotaxime injections are used to treat certain infections caused by bacteria[4]. meningitis (infection of the membranes surrounding the brain and spinal cord), lower respiratory tract pneumonia, gonorrhea (an STD), and other infections of the brain and spinal cord, abdomen (stomach area), female genitalia, skin, blood, bones, joints, urinary tract infections, and respiratory tract infections [5]. Recently, an oral medication was brought into medical practice. [6]. Cefotaxime has positive and negative properties and has clinical and analytical importance. Among its beneficial properties is its tolerance to the penicillinase enzyme [7]. It may be used to treat diseases resistant to penicillin and its derivatives. It has numerous adverse symptoms, including headache, nausea, vomiting, cramping in the stomach, and diarrhea. [8].

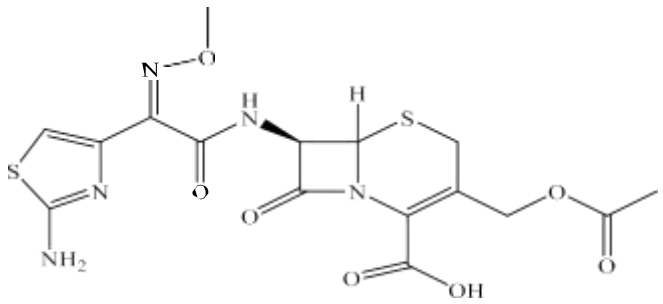


Figure 1.

Several analytical methods have been reported for the determination of cefotaxime , including spectrophotometry methods [9,10,11]. HPLC technology [12,13,14]. Potentiometer methods [15,16,17]. Electroanalytical techniques [18,19]. Flow injection technique [20]. Spectroscopic methods based on the formation of colored products using organic reagents are important analytical methods in the estimation of pharmaceutical compounds, due to their simplicity of procedure, speed of execution, good sensitivity, and low cost compared to some other analytical techniques [21]. Thiosemicarbazone derivatives have also received widespread attention in the field of analytical chemistry due to their ability to react with many compounds to form stable colored products that can be measured spectroscopically [22]. Based on the above, this research aims to develop a simple and sensitive spectroscopic analytical method for estimating cefotaxime in pharmaceutical preparations using the drug's Diazotization-Coupling reaction using sodium nitrite in an acidic medium, followed by a conjugation reaction with a prepared organic reagent of the thiosimicarbazone type in a basic medium to form a stable Azo Chromogen. The absorption intensity of this product is measured using ultraviolet-visible spectroscopy at a suitable wavelength, while studying the analytical properties of the suggested approach in terms of accuracy, sensitivity, and linearity and compatibility, in addition to the possibility of successfully applying it in the estimation of Cefotaxime in some pharmaceutical preparations.

Experimental

Apparatus

UV-Visible Spectrophotometer: (Shimadzu UV-1601, Kyoto, Japan) was used to record the absorption spectra of standard solutions and samples at the maximum wavelength (λmax = 514 nm) utilizing a quartz cell with a one-centimeter optical path length.

Electronic Balance: Type (Sartorius BL 210, Germany), with an accuracy of (±0.0001 g), and it was used to weigh chemicals and samples.

Materials and Reagents

All standard solutions and samples were prepared and diluted using distilled water, and all chemicals and reagents were of Analytical Reagent Grade (AR) or high purity As for the thiosemicarbazone reagent, it was dissolved in dimethyl sulfoxide (DMSO) due to its poor solubility in water**.**

Cefotaxime Standard Powder was obtained from the Samarra General Business for Medical Supplies and Pharmaceutical Industries (SDI), Iraq, and used without any further purification.

Preparation of Standard Solutions:

1- Standard Cefotaxime Sodium Solution (1.1×10^{-3} M) 500 μg/m³

This solution was prepared by dissolving 0.500gm of cefotaxime powder in a100 mL volumetric flask and bringing the volume up to the mark with distilled water. It should be stored in an opaque bottle.

2- Thiosimacarbazone Solution 1×10^{-2} M

This solution was prepared by dissolving 0.2277 g of thiosimacarbazone powder in a given amount of DMSO and then filling a 50 ml volumetric flask to the mark with DMSO.

3- Sodium nitrite solution $NaNO_2$ 1×10^{-2} M

The solution was prepared by taking a weight of 0.0345 g and dissolving it in a specific quantity of distilled water, then bringing the volume up to the mark in a 50 ml volumetric flask with distilled water.

4- 1M hydrochloric acid solution

Prepare by adding 8.28 ml of hydrochloric acid solution (12.077 M) to an amount of distilled water in a 100mLvolumetric bottle and supplement the made up to volume with distilled water.

5-Sodium hydroxide solution (1M)

Sodium hydroxide solution was prepared by dissolving 5.6g of it in 100ml of distilled water.

6- Solution (starch - glucose - magnesium citrate - sodium chloride - lactose) 1000 mcg/ml

These solutions were prepared by dissolving 0.1 g of them in 100 ml of absolute water from which diluted solutions were then prepared.

7- 500 µg/mL Pharmaceutical Solutions

Cefotaxim injection Cefotaxime injection was obtained from the general company for the manufacture of medicines and medical supplies (Spain) containing 1 gram of cefotaxime, which was dissolved in distilled water and diluted to 1L in a volumetric flask to prepare a stock solution of 1000ug mL^{-1} . This solution was prepared from a 500 µg/ml solution of the pharmaceutical preparation.

Results and discussion

Absorption spectra.

The principal step of the developed method involved the reacting the Cefotaxim drug using the thiosimcarbazone reagent resulting in the formation of a colored product, whose absorbance was maesured against the white (blank) solution; Figure (2). shows the maximum absorbance was measured at a wavelength of 514 ηm.

Optimization of reaction variables.

The several factors pertaining to the formation of the chromogenic product were studied by varying one factor at a time and keeping the other factors constant, and the optimal and stable conditions were selected.

1- - Effect of Reagent Addition Order: The effect of four different ingredient addition orders on the formation of the colored compound was studied by altering the sequence of the reactants were analyzed in quadruplicate, as shown in table (1). Based on the results, the third order (number 4) was chosen as it gave the highest absorption intensity and was adopted in the subsequent experiment.

No	Squence	Abs
1	C + T + HA + SN + SH	0.437
2	T + SN + C + HA + SH	0.526
3	C + HA + SN + T + SH	0.590
4	C + SN + HA + T + SH	.0.614

So: (C) cefotaxime, (T) thiosemacarbazone, (SN) the ionizing agent sodium nitrite, (HA) hydrochloric acid, and (SH) the base sodium hydroxide.

2- **Effect of acid type and volume:** A range of acids (HCl, H_2SO_4, HNO_3, CH_3COOH) were tested at a concentration of 1 M. The results showed that hydrochloric acid (HCl) was superior in providing the optimal medium for producing the highest absorbance. Optimal

volume: By gradually increasing the volume of the acid, 1.2 mL was found to be the ideal amount, as shown in Figure 2.

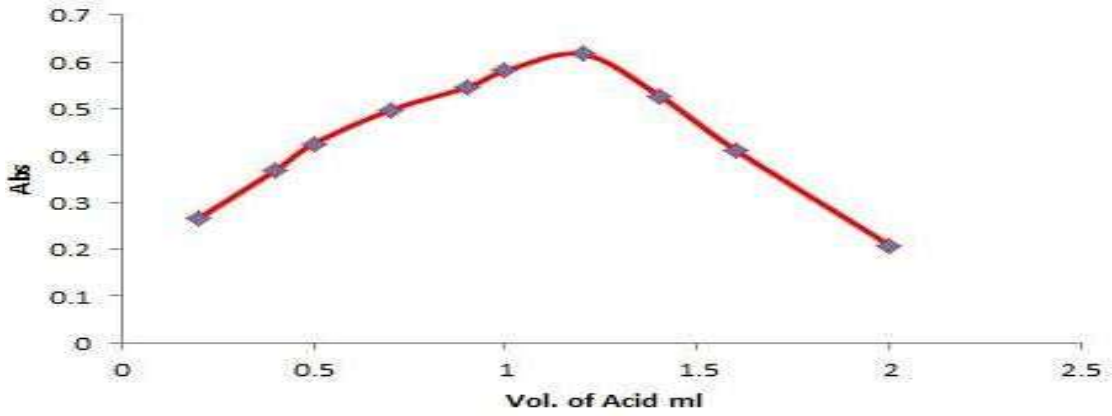


Figure 2. Effect of acid volume on color development.

3- **Effect of Base Type and Volume:** A range of bases (NaOH, KOH, Na_2CO_3, NH_4OH) were tested at a concentration of 1 M to determine the most suitable medium for completing the conjugation reaction. The results showed that sodium hydroxide (NaOH) was the best. Optimal Volume: By studying the effect of different volumes of NaOH, it was found that 1.8 ml is the optimal volume for best absorbance, as shown in Figure(3)..

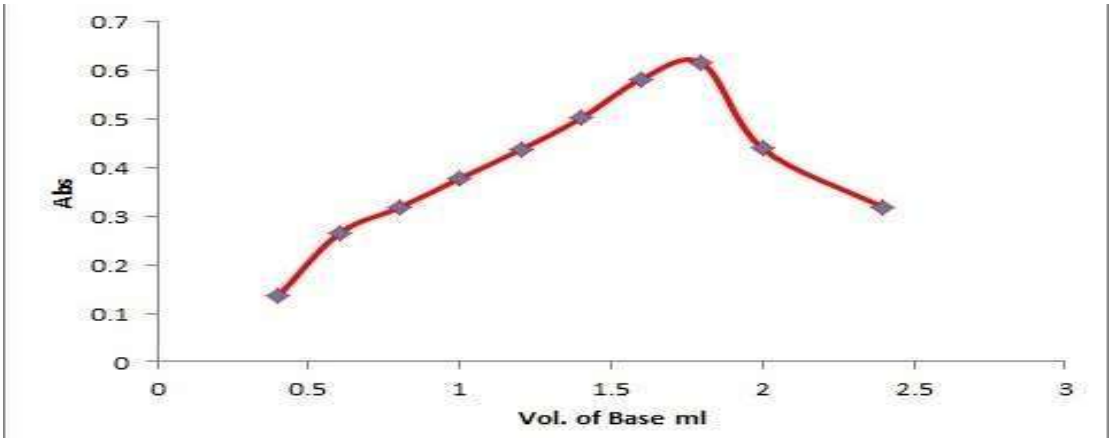


Figure 3. Effect of bace Volume on color Development.

4- **The effect of sodium nitrite volume was investigated using different volumes of sodium nitrite** from 0.1 - 2.5 mL, of sodium nitrite ($NaNO_2$) at a concentration of 0.01 M on the maximum absorbance of the colored product. the highest absorbance was obtained at a volume of 1 ml of sodium nitrite ($NaNO_2$). Above this value, the absorbance reading decreased, as shown in Figure (4).

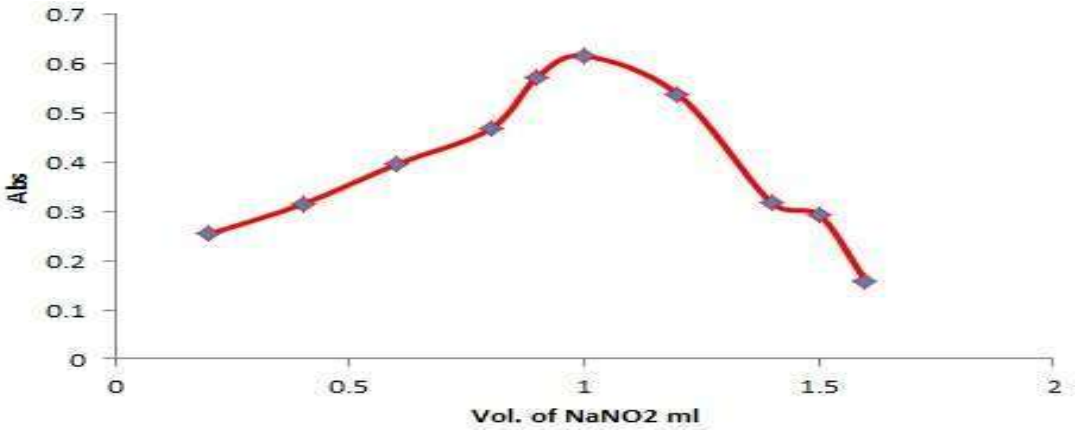


Figure 4. Effect of Sodium Nitrite Volume on color development.

5- Effect of Thiosimacarbazone Reagent Volume: The effect of different volumes of thiosimacarbazone reagent on the absorbance of the fomed colored product was studied over the range of 0.1 - 2mL at a concentration of 0.01 M. as shown in figure (5). The maximum absorbance was observed at a volume of 1.4mL OF Reagent . Beyond this value, the absorbance was found to decrease therefore,, 1.4 mL of thiosimacarbazone reagent at a concentration of $1x10^{-2}$M was used in subsequent work.

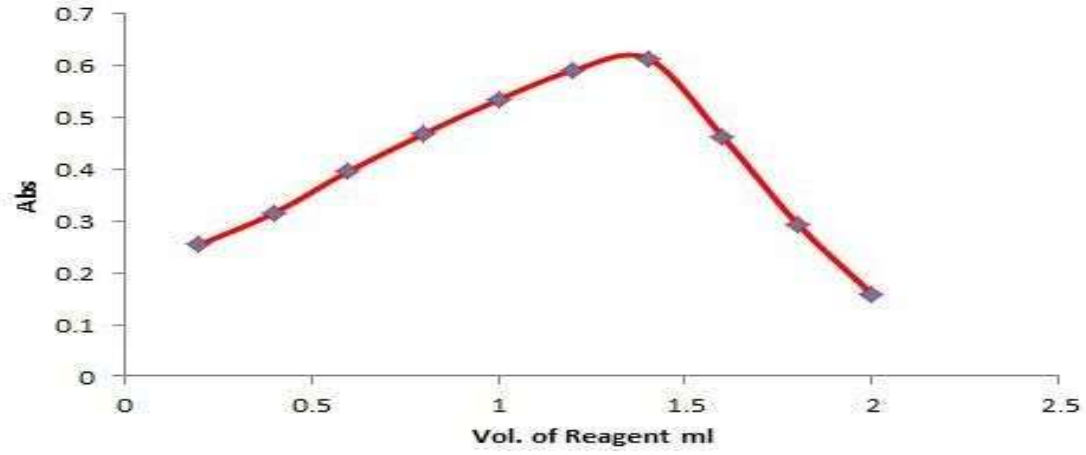


Figure 5. Effect of the Thiosimacarbazone Volume on color development.

6- Stability Time: was carried out conducted by spectrophotometric measurement of the colored product at different times. the solution's colored peaked after 5 minutes and remained almost stable for more than 30 minutes, as shown in Table (2). The absorption spectrum of the colored compound was recorded under optimal conditions and showed the highest absorbance at a wavelength of 514 nm compared to the white (blank) solution.

Table 2. The stability.

Time/min	direct	5	15	25	30	35	40	45	50
Absorbance	0.612	0.617	0.614	0.614	0.613	0.611	0.608	0.601	0.595

Final Absorption Spectrum

After achieving optimal conditions, using 1.5mL of cefotaxime A working solution of 500 μg ml^{-1}, 1ml of the nitrogenous agent $NaNO_2$ at a concentration of 1×10^{-2} M was added, followed by the adding 1.2mL of 1M HCl. After 5 min with continuous shaking , 1.4mL OF The reagent was added 1×10^{-2}M thiosimcarbazone reagent solution was added, and then 1.8 ml of 1 M NaOH solution was added. The solution was left for 5 minutes to complete and stabilize the reaction. The volume was then filled and made up then mage up to volume in a 10 mL volumetric flask using distilled water. The final absorbanc spectrum The absorbance of the resulting red- colored product was measured against the corresponding reagent blank. that of its analogous solution. It was found that the product exhibited the highest absorption at a wavelength of 514 nm, while its analogous solution did not. Any absorption in this area is shown in Figure (6).

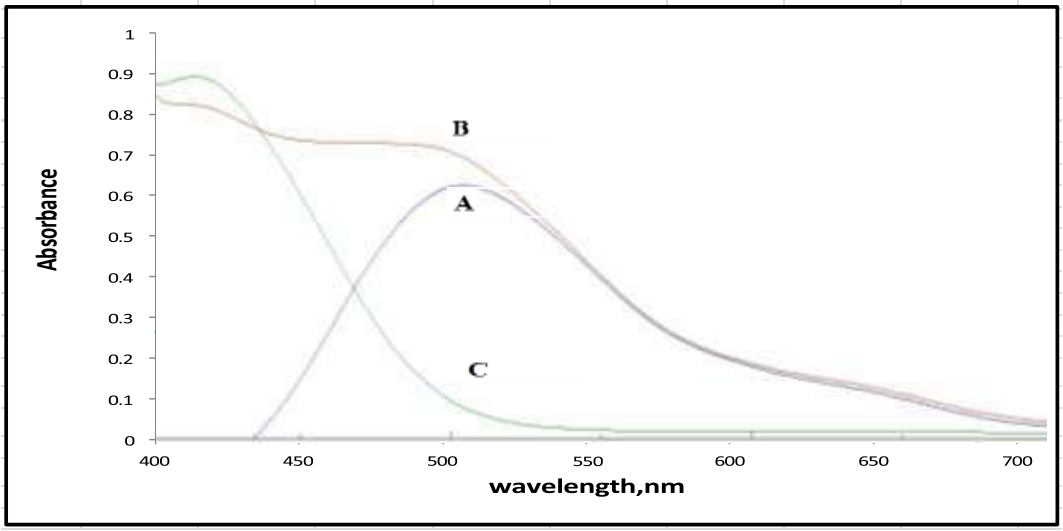


Figure 6. Final absorbance spectrum of cefotaxime: (A) cefotaxime solution versus the reagent blank, (B) cefotaxime solution versus distilled water. (C) reagent blank versus distilled water.

Calibration curve Construction

A series of solutions were prepared with increasing final concentrations of cefotaxime (5-65 μg mL^{-1}), equivalent to volumes of 0.1-1.3 ml of 500 μg/ml. subsequently, 1mL of $1x10^{-2}$M $NaNO_2$ solution was added, then 1.2 mL of 1 M HCl. After waiting 5 min with occasional shaking, 1.4 ml of 1×10^{-2} M Thiosemicarbazone reagent was added, followed by 1.8 ml of 1 M sodium hydroxide. The solution was left to stand for 5 minutes to complete and stabilize the reaction. The volume was then topped up and diluted to the mark with distilled water in 10mL volumetric flask. the absorbance of the developed colored solutions was measured at 514nm against the reagent blank . the calibration graph shown in figure (7) exhibited good linearity and complied with Beer's law over the concentration range of 5-65μg mL^{-1} of cefotaxime and the correlation coefficient value for the estimate is 0.9967, and the molar absorbance reached (5681.65 $L.mol^{-1}.cm^{-1}$) and the Sandell significance was (0.084 μg mL^{-1}).

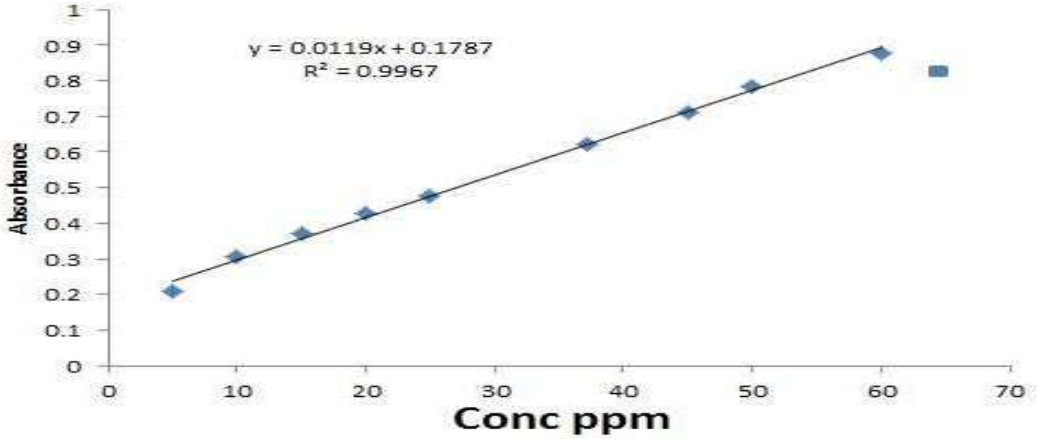


Figure 7. Calibration curve for the determination of CEF under optimum condition.

Accuracy and Precision

The accuarcy and precision of the proposed method were evaluated cefotaxime were calculated by measuring Three different concentrations of the drug under the optimal conditions shown in the method of operation. The results obtained and shown in Table (3) Indicate the method has good accuracy, and conformity.

RSD%	Average Recovery.%	Recovery.%	RE %	Conce μg/ml

1.23	99.03	98.53	1.47	10
0.78		99.13	0.87	15
0.41		99.43	0.56	20

* (Each value is the average of 5 readings)

The limit of detection (LOD) and limit of quantity (LOQ) for this method were also calculated from the previous relationship, where the LOD = 0.214 μg/ml and the LOQ = 0.677 μg/ml were found.

Composition of the product

The Job method and the molar ratio were used to determine the compositional ratio of the colored complex. In the Job method, two solutions of equal molar concentration (2 x 10^{-2}M) were prepared for both the drug (CEF) and the thiosimcarbazone reagent. different volumes of the drug, ranging from (0.5-4.5mL), were added into 20 ml volumetric bottles, and decreasing volumes of the reagent, starting from (4.5-0.5) ml, were added to keep the total volume of the mixture constant. the absobavce was measured at 514 nm. the obtained results are graphically presented in figure (8)(9), gave a maximum at the mole fraction X max=0.5 , X max=1 in the Job method and the mole ratio method respectively, indicating a 1:1 binding ratio between cefotaxime and thiosimcarbazone. The possible composition of the final dye is suggested in diagram (1).

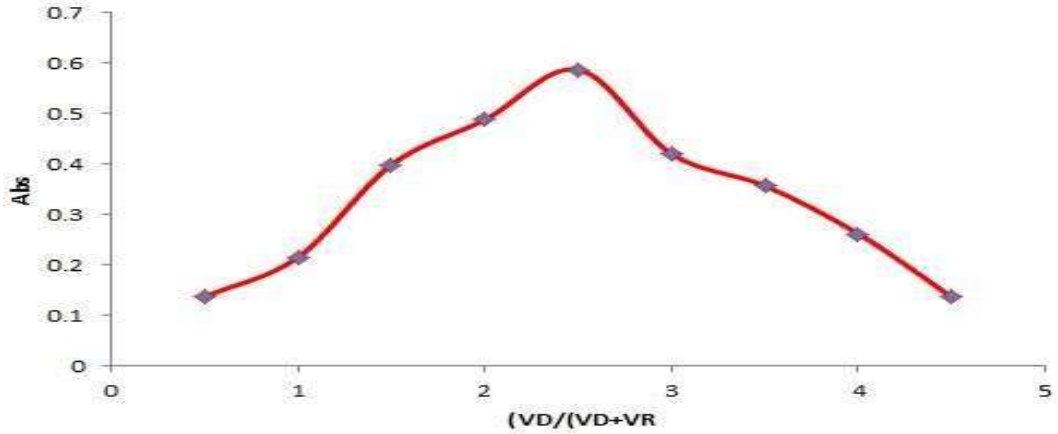


Figure 8. Job's corve of 2 x 10^{-2} M CEF and thiosimcarbazone.

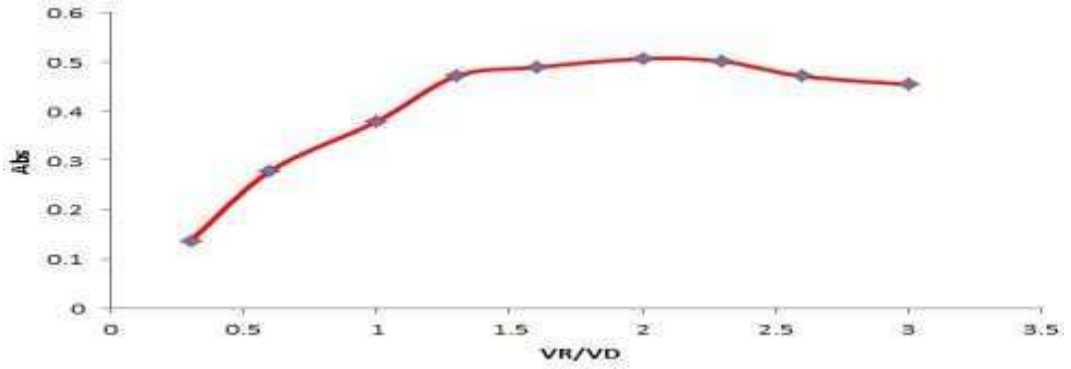
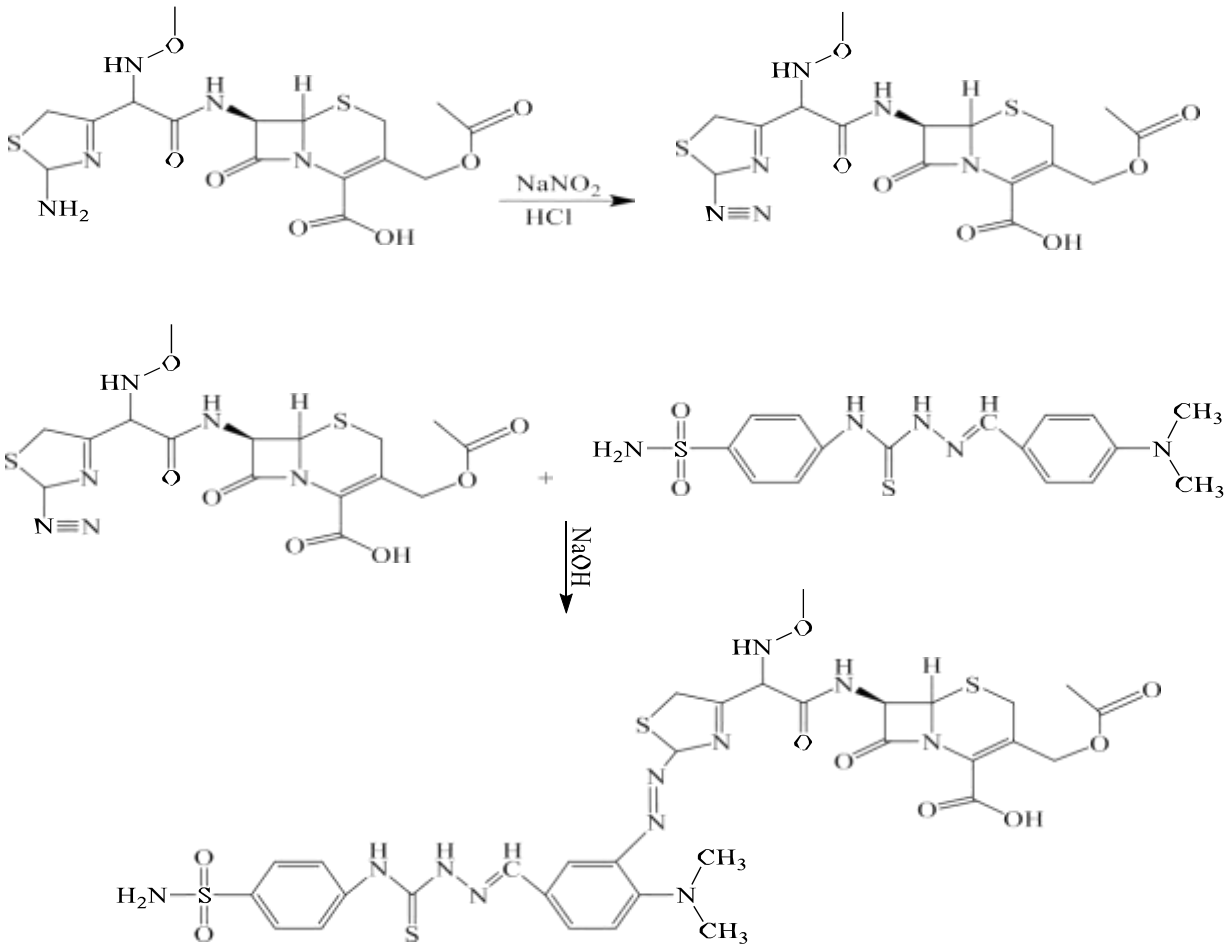


Figure 9. Mole ratio of 2 x 10^{-2} M for each CEF and thiosimcarbazone.

Both methods confirmed that the drug-to-reagent binding ratio is 1:1, and the proposed reaction can be according to the following equation:



scheme 1. proposed reaction mechanism between CEF and thiosimcarbazone.

Interference study

The degree of interference caused by about additives that frequently accompany Pharmaceutical formulations were analyzed by measuring the absorbance of their solutione containing 10 μg/ml of the drug (CEF) and different volumes of the additive at a concentration of 1000 μg/ml^{-1} to obtain two different concentrations in a final volume of 25 mL. The results listed in Table (4) show that the appropriate additives do not interfere with the determination of the drug (CEF).

Table 4. Effect of Potential interfering substances for the determination of cefotaxime using the proposed method.

RE%	Conce added μg/ml^{-}	RE%	Conce added μg/ml^{-}	Foreign Compound 1000 μg/ml^{-1}
2.16	100	0 .37	50	Starch.
4.98-	100	4.42	50	Lactose.
3.29	100	2.45-	50	Sodium sulfate.
2.44	100	3.54-	50	Magnesium stearate.
0.99-	100	1.76	50	Glucose.

Application in pharmaceutical forms

The proposed method for estimating cefotaxime in its pharmaceutical preparations in the form of injections was applied, where the weight of the injection was taken, which was (1g), and it was dissolved in 1000ml of water. Then, 3 concentrations were taken from

the vial to estimate cefotaxime in the pharmaceutical preparation, and the results are shown in Table (5).

Table 5. Recovery data obtained by applying the proposed method to pharmaceutical formulations.

Drug	Amount Taken μg mL^{-1}	Amount Found μg mL^{-1}	RE%	Rec%	Average Recovery %	Company
Cefotaxime	10	9.98	-0.2	99.8	100.65	Spain
Normon	15	15.1	0.66	100.66		
injection	20	20.3	1.5	101.5		

Comparison of the methods

In this study, the proposed method for estimating cefotaxime was compared with other spectroscopic methods, and the results are shown in Table (6).

Table 6. Comparison of the proposed method.

Analytical performance characteristics	Reported method [23]	Proposed method
λ_{max} [nm]	626	514
Reagent	Crestal violet day	Thiosemicarbazone
medium	Acid	*basic*
solvent	water	*Water*
Molar absorbance (L.mol^{-1}.cm^{-1})	12660	5681
correlation coefficient (r)	0.9982	0.9967
Sandal Index (μg.cm^{-2})	0.036	0.084
Limit of Detection (μg/ml)	0.0591	0.214
Limit of quantification(μg/ml)	0.1992	0.677
Beer's law (μg/ml)	1.2 -36	5-65
Slop	0.0279	0.0119
intercept	0.2734	0.1787
colour	Blue	Red
RSD%	0.486	1.23%

Conclusion

A straightforward, sensitive, and precise spectroscopic analytical technique was created for the assessment of of cefotaxime via nitrogenization and conjugation reactions. The method relies on the nitrogenization of the drug by Sodium nitrite in acidic medium, producing a red -colored product upon conjugation with the reagent thiosimcarbazone in a basic medium (NaOH). The highest absorption of the colored product is observed at a wavelength of 514 nm . Beer's law is followed within a concentration range of 5–65 μg mL^{-1}. The molar absorbance 5420.093 L·mol^{-1}·cm·1, the Sandal significance was 0.084 μg cm^{-2}, the recovery rate was 101.47%, the standard deviation was less than 0.80%, The Limit of detection (LOD) was found to be 0.214 μg mL^{-1}, and the estimation coefficient was 0.9967. The method has proven to be accurate and compatible, and it is characterized by its simplicity. The reaction is completed and stabilizes after 5 minutes and remains stable for more than 30 minutes, which is sufficient time to conduct many measurements. the proposed method been successfully applied to pharmaceutical preparations.

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