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Spectrophotometric determination of cefixime using hydralazine as coupling reagent

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***Abstract*---**A sensitive, accurate, and easy indirect spectrophotometric method was developed for the determination of cefixime in its pure form and in the pharmaceutical preparation (capsules). The method depended on the oxidation of the mixture of the medicinal compound cefixime by the oxidizing agent N-Bromo succinimide, followed by the addition of the reagent hydralazine. A brown complex was obtained and the absorption was measured at 360 nm for the medicinal compound cefixime, its molar absorbance was 2038.6 liters. Mol^{-1}. cm^{-1}. With a recovery rate of 99.33, the method was developed to be simple, inexpensive and fast because it does not include any solvent extraction. The method was successfully applied to the pharmaceutical preparation.

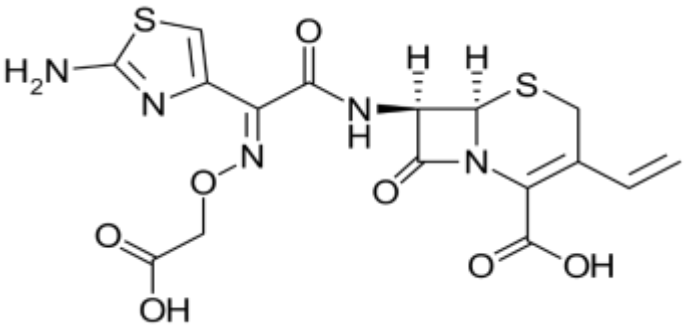
***Keywords*---**spectrophotometric determination, hydralazine, coupling reagent.

Introduction

Cefixime

Cefixime trihydrate is a semi-synthetic antibiotic which belongs to the third generation of the cephalosporin group (1), its chemical name is: 5-thia-1-azibicyclo [4,2,0] Oct-2-ene carboxylic acid, 7-[(2-amino-4-thiazolyl) {(Carboxy methoxy) imino] acetyle} amino] 3ethynyl-8-oxo-trihydrate (2) (3) It is an antibacterial by inhibiting the formation of mucopeptide in the bacterial cell wall (4) and is used in the treatment of infections of the upper respiratory tract, urinary tract, skin and middle ear (5), it is a white, yellowish powder that does not dissolve in water, but dissolves in hydrochloric acid, slightly soluble in ethanol

and partially soluble in ethyl acetate and has the following chemical composition (6)



Several analytical methods have been used to estimate cefixime, including spectrophotometric methods (8-7), chromatography methods (15-9) as well as electrochemical methods (18-16). Spectrophotometry is the best tool for identifying drugs in research laboratories, hospitals and pharmaceutical industries due to its low inherent cost.

Practical Part

The Devices Used

Absorption was measured and spectrum was graphed in a dual-beam T92 + UV Spectro photometer PG IOSNTRUMNTS equipped with a pair of 1 cm wide quartz cells. Samples were weighed with a BEL ENGINEERING scale and heated with a STAR WHITE heater.

The Chemicals

All chemicals and reagents were used with high purity

1- Cefixime Solution
   Cefixime was prepared at a concentration of 1000 µg/ml with a weight of 0.1 g, dissolved in 5 ml of hydrochloric acid (1 molar) and bring the volume up to the mark in a volumetric bottle of 100 ml.
2- Hydralazine Solution
   Prepare hydralazine at a concentration of 100 µg/ml with a weight of 0.01 g of hydralazine and dissolve it in 2 ml of concentrated hydrochloric acid and 3 ml of distilled water and bring the volume up to the mark in a 100 ml vial.
3- N-Bromo succinimide Oxidizing Agent Solution
   The oxidizing agent NBS was prepared at a concentration of 3.8×10^{-2} M, with a weight of 0.6764 g, and dissolved it in 10 ml of hot distilled water and bring the volume up to the mark in a volumetric bottle of 100 ml.
4- Solutions Of surfactant active substances
   SDS and CTAB were prepared at a concentration of 5×10^{-3} M and triton (x-100) at a concentration of 1%.

Working Method and Standard Curves

(2.5 ml) of the oxidizing agent NBS was added at a concentration of 3.8×10^{-2} M, and increasing amounts of the medicinal compound cefixime (0.25-2.5 ml) were added at a concentration of (25-250) µg/ml, and then (1.5 ml) of the reagent hydralazine was added at a concentration 100 µg/ml and the solutions were left for (5 minutes) after completing the volume to the mark with distilled water in volumetric bottles of (10 ml) and the absorbance of the solutions was measured against its sham solution at the wavelength 360 nm, it was found that the method followed Beer's law up to the mentioned extents, followed by a deviation from the law.

Pharmaceutical solutions

Pharmaceutical capsule (Winex 200 mg cefixime)

The contents of eight capsules were weighed and the equivalent of 1000 µg/ml of the pharmaceutical product cefixime was weighed and dissolved in 5 ml of hydrochloric acid with heating the solution for 10 minutes, then cooled and filtered with washing the filter paper (4-5) times with distilled water and bring the volume up to the mark in Volumetric bottle of 100 ml.

Results and discussion

The optimum conditions necessary for the formation of the cefixime complex were set by changing one of the conditions and stabilizing the other and studying the effect of these conditions on the absorbance as we will notice in the following steps:

Studying the best quantity of hydralazine reagent

The effect of the best amount of hydralazine reagent on the absorbance of the formed complex was studied. It was found that adding (1.5 ml) of the reagent is the best amount as shown in the table below:

Volume of ml reagent 100 ppm	0.5	1	1.5	2	2.5	3
Absorbance	0.359	0.361	0.416	0.400	0.389	0.385

Table (1) study of the best quantity of hydralazine

Studying the effect of the best oxidizing agent used

Some types of oxidizing agents have been studied and their effect on the absorption of the formed complex has been studied. It has been found that the use of the oxidizing agent N-bromo succinimide is the best oxidizing agent as shown in the table below:

Oxidant 0.1 M	KIO_4	KIO_3	$K_2Cr_2O_7$	NBS
Absorbance	0.033	0.034	0.018	0.418

Table (2) Study of the type of oxidizing agent

Studying the effect of the best amount of oxidizing agent

The effect of different amounts of the oxidizing agent N-bromo succinimide (3.8×10^{-2} M) on the absorbance of the colored compound formed, it was found that the use of (2.5 ml) of the oxidizing agent gave the best absorption as shown in the table below:

Volume of 0.1M NBS 3.8×10^{-2} M	0.5	1	1.5	2	2.5	3
Absorbance	0.146	0.367	0.371	0.418	0.463	0.458

Table (3) Amount of oxidizing agent

Study of the oxidation time

The effect of the time required for the oxidation of the hydralazine reagent was studied using NBS, followed by the addition of the drug cefixime and leaving the solutions for different periods of time. It was noted that after 5 minutes, the highest absorption was obtained as listed in the table below:

Time/min	1	5	10	15	20	25	30
Absorbance	0.476	0.478	0.464	0.449	0.436	0.417	0.403

Table (4) Oxidation time measurement

Studying the effect of acids and bases

The effect of different types of acids with the base NaOH was studied to find out their effect on the absorption of the formed product. It was found that the inefficiency of acids and bases in increasing the intensity of absorption led to their exclusion in the following steps as shown in the table below:

Acid or Base 1M	without	H_2SO_4	HCl	NaOH
Absorbance	0.473	0.432	0.445	0.050

Table (5) Effect of acids and bases

Studying the surfactant active substances

The effect of different types of surfactant active substances was studied, as 1 ml was added to each SDS and CTAB at a concentration of 5×10^{-3} M and triton (x-100) at a concentration of 1% and measured for different periods of time. The results indicate that they are insufficient in increasing the intensity of absorption, so they were excluded from the following steps as shown in the table below:

Time	without	SDS	CTAB	triton (x-100)
1	0.473	0.395	0.330	unstable output
5	0.475	0.452	0.364	
10	0.465	0.441	0.342	
15	0.450	0.435	0.339	
20	0.439	0.421	0.336	
25	0.420	0.408	0.321	

30	0.408	0.401	0.317	

Table (6) Study of the surfactant active substances

Study the effect of temperature

The effect of time and three different temperatures on the absorbance and stability of the resulting solution were studied. It was found that the best temperature was the laboratory temperature, which gave the highest absorption of the colored product after 5 minutes of dilution and was stable for at least half an hour as shown in the following table:

Time/min	Absorbance		
	R.T	40°c	50°c
2	0.459	0.443	0.419
5	0.473	0.442	0.352
10	0.463	0.405	0.270
15	0.448	0.369	0.240
20	0.435	0.333	0.221
25	0.421	0.305	0.200
30	0.412	0.208	0.183

Table 7 Study the effect of temperature

Studying the effect of the addition sequence

The effect of choosing the best addition sequence that affects the absorbance intensity was studied, and it was found that the best sequence is (III) as shown in the table below.

Order Number	Order Of Addition	Absorbance
I	R+O+D	0.478
II	R+D+O	0.497
III	D+O+R	0.528

D= cefixime O=NBS R= hydralazine

Table 8 Studying the effect of the addition sequence

Final Absorption Spectrum

It was shown through the absorption spectrum of the reaction after stabilization the optimal conditions for the reaction of the medicinal compound Cefixime with a concentration of 1000 µg with the oxidizing agent N-bromo-succinimide with the reagent Hydralazine, where 2 ml of the drug was used, and it was found that the highest absorption was at a wavelength of 360 nm.

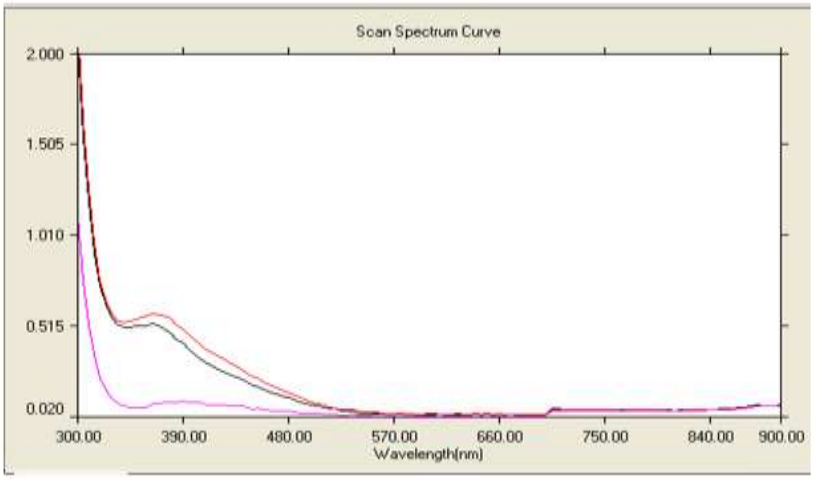
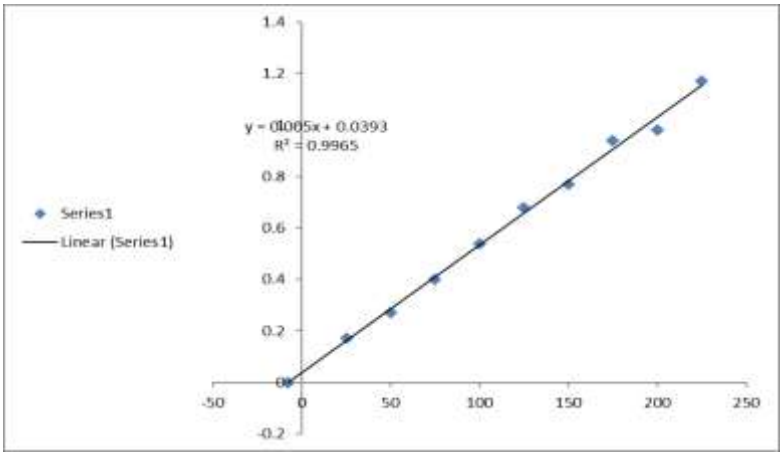


Figure ( ) shows the standard curves of the medicinal compound, which indicates the possibility of estimating (25-250) µg/ml for the medicinal compound Cefixime and that there is a deviation from Beer's law after the upper estimated limits, where the estimation factor of the standard curves reached (0.9963) for the medicinal compound, which indicates that it has Excellent linear specifications, while the molar absorptivity reached (2038.5) liters. $mol^{-1}.cm^{-1}$, which indicates the high sensitivity of the method.



Standard curve for the estimation of cefixime. The limits of detection and quantification were calculated by taking replicas of Planck and measuring their absorption against distilled water and by applying the equation LOQ = $\frac{10\,\sigma B}{S}$ and the equation LOD = $\frac{3\sigma B}{S}$ where **σ** represents the standard deviation of the sham solution absorption and **S** represents the slope of the standard curve

Method accuracy and compatibility

The accuracy and compatibility of the method was calculated by calculating the relative standard deviation and the recovery rate for a number of replicas at three different concentrations as in the table, where it was found that the method has good accuracy and compatibility, as the recovery rate was 99.33 and the relative standard deviation rate RSD% =2.662

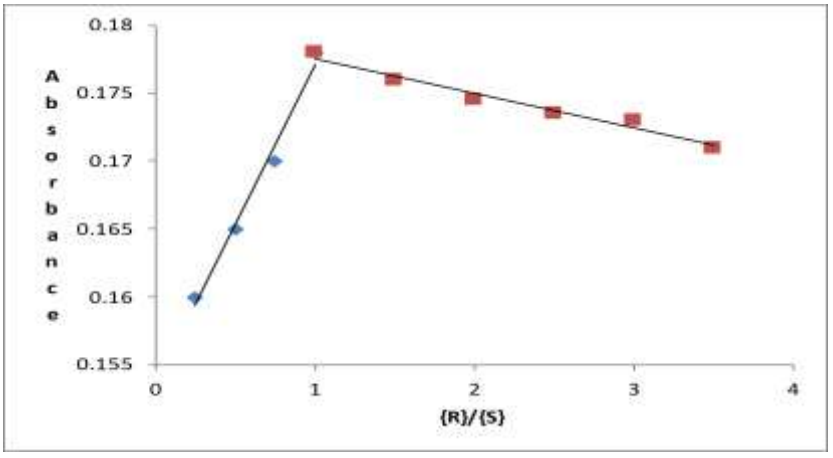
Compound	Amount added Mg.ml^{-1}		Recovery%	Average Recovery %	RE%	RSD%
	Taken	Found				
Cefixime	50	52.7	98.224	99.33	-1.776	1.586
	100	103.4	99.89		-0.1232	0.495
	150	148	99.88		0.1174	0.581

Table ( ) shows the linear specifications, slope, range, molar absorbance, detection limits, and quantitative estimation in the estimation of cefixime.

The measured values of the proposed method	
Beer's law limits (Mg.ml^{-1})	(25-250)
Molar absorptivity (L.mol^{-1}.cm^{-1})	2038.5
LOD (ug.m^{-1})	1.7256
LOQ (µg.m^{-1})	5.75
Correlation coefficient (R^2)	0.998
Intercept	0.017
Slop	0.0045
Sandells sensitivity (µg.cm^{-2})	0.222

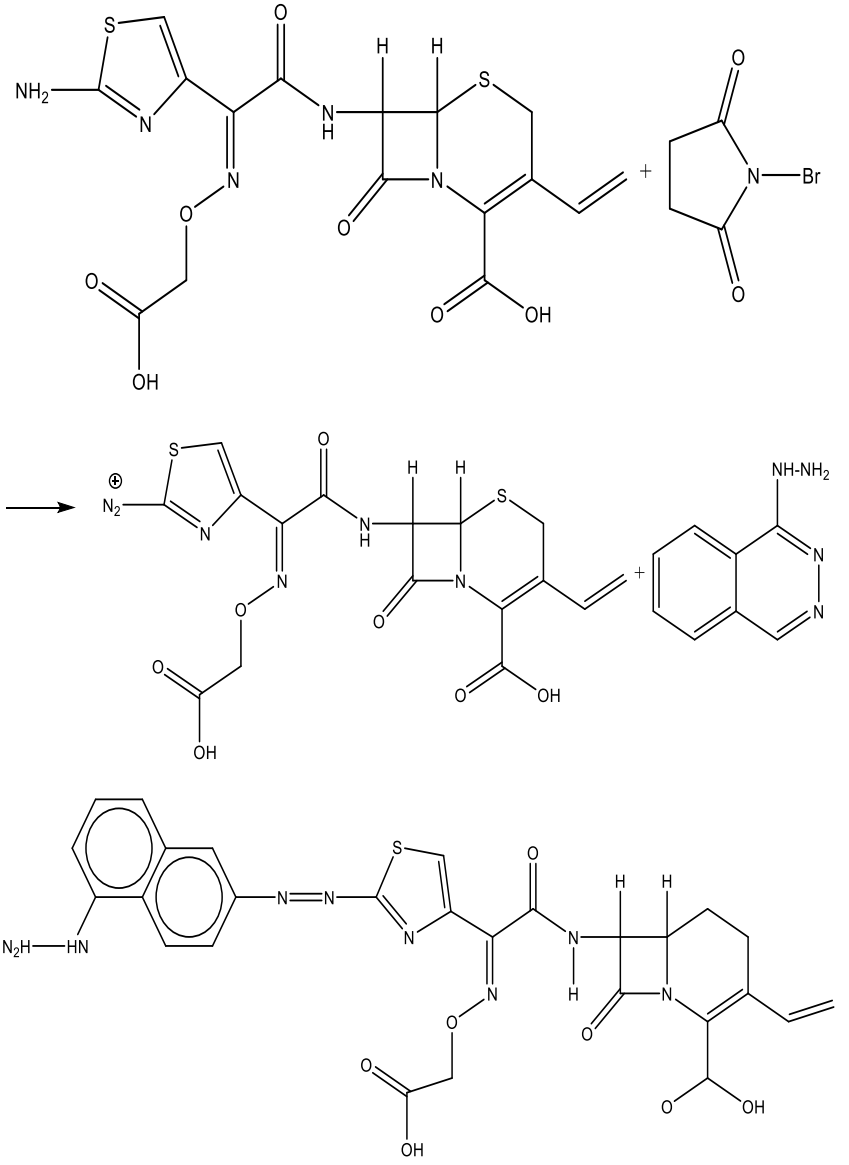
Ratio of complex composition and stability constant

The composition ratio of the complex formed between cefixime and the hydralazine reagent was studied by applying the molar ratio method, using two equal solutions with molar concentration (2.2×10^{-3} M) . Through the results obtained, it was found that the composition ratio of cefixime to the hydralazine reagent is 1:1, as shown in the figure:



Suggested interaction

The medicinal compound cefixime is oxidized by using the oxidizing agent N-bromosoxanamide as an oxidizing agent in aqueous solution, followed by coupling it with the reagent hydralazine to form a colored complex, the binding ratio was 1:1 by molar ratio method and it has the highest absorption at 360 nm. Through previous spectroscopic studies, the proposed chemical equation is similar to the following form:



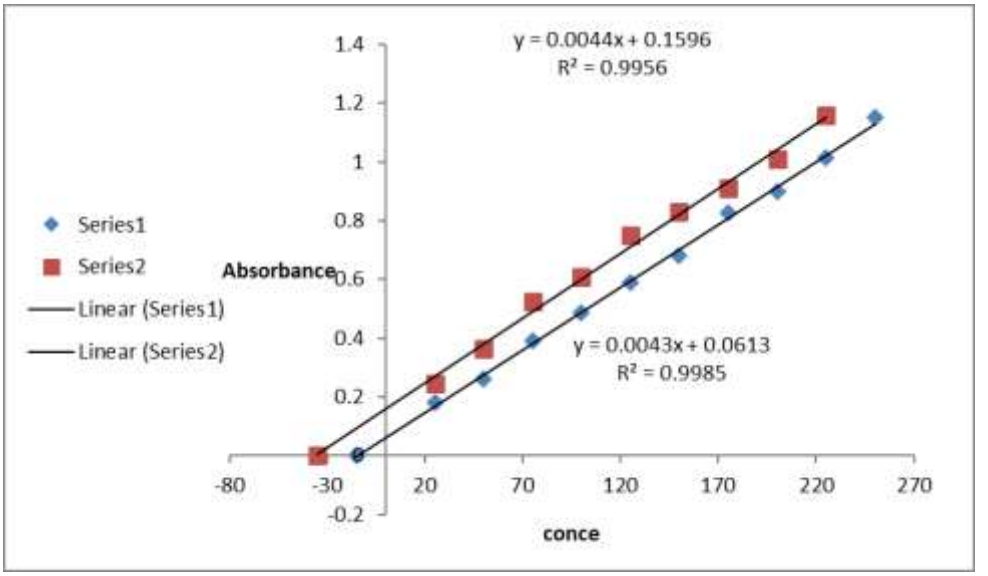
Applying of the developed method to the pharmaceutical product of the medicinal compound cefixime

Table ( ) Determination of the medicinal compound cefixime on the pharmaceutical product

Pharmaceutical preparation	certified value (mg)	Amount present ($\mu g.ml^{-1}$)		Drug content found	Recovery%	Average Recovery%
		Taken	Found			
Capsule	200	50	46.2	199.22	99.61	99.75
		100	99.23	199.58	99.79	
		150	144.34	199.7	99.85	

Method Evaluation

The proposed method was applied to the medicinal capsule for the determination of cefixime for the purpose of proving the efficiency of the developed method and its free from overlapping additives. Figure ( ) shows that the method is well and selectively compatible with the approved method of work in a satisfactory manner.



Conclusion

A sensitive, easy and accurate indirect spectrophotometric method was proposed for the determination of cefixime, which was based on the interaction of the medicinal compound with the oxidizing agent NBS in the presence of the hydralazine reagent, it was found that the method is of high accuracy, as the recovery rate (99.33%) and good agreement RSD≥0.887, and the proposed method was successfully applied for the determination of the medicinal compound cefixime in its pharmaceutical products in the form of a capsule.

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