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A NEW KINETIC SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CEFIXIME IN PHARMACEUTICAL PREPARATIONS USING SAFFRON EXTRACT AS NATURAL REAGENT

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Abstract: A new simple and sensitive kinetic spectrophotometric method has been proposed for the determination of cefixime in pure form and pharmaceutical formulations. The proposed method is based on the formation of yellow coloured product (λ_{max} = 390nm) that resulted after addition of the mixture, which is composed of saffron extract, sodium hydroxide and potassium permanganate, to cefixime aqueous solution. The experimental conditions have been optimized. Beer's law is obeyed over the concentrations of 10-0.6µg/ml, with a linear regression correlation coefficient of 0.994. The proposed method has been successfully applied in the determination of cefixime in pharmaceutical dosage forms.

Keywords: Cefixime, Saffron, Spectrophotometric analysis, Pharmaceutical analysis.

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1. INTRODUCTION

Cefixime is an orally third generation cephalosporin antibiotics [1]. It is used for the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary tract infections [2]. Literature survey reveals that cefixime in its formulations has been quantified with a number of methods such as spectrophotometric [3, 4], HPLC [5-10], HPTLC [10, 11], LC-MS [12], HPCPE [13], Voltametric [14], calorimetric method [15], capillary zone electrophoresis [16], and selective membrane electrode [16, 17].

In the late 1900's and early 2000's, a new concept has been introduced to the analytical chemistry technique that is green analytical chemistry by which natural or unrefined reagents are used as alternatives for highly refined or purified reagents [18]. Since then, the use of this method has widely spread and has been used for quantitative analysis of many materials [19-23]. Green chemistry concepts have led to simpler analytical processes that are lower in operational cost and more favourable in reducing energy consumption [24-26].

In this work we aim to use a mixture of potassium permanganate and saffron extract as a medium for determination of cefixime spectrophotometrically. Saffron one species of genus *Crocus, C. sativus L.* is the most fascinating and intriguing species [27]. It has been reported that compounds of crocin (1, 2, 3 and 4) are the main and most effective components in saffron extracts [28]. No reports in literature have been found for the use of saffron as natural reagents in chemical analysis and hence this work might be the first attempt to do so. Various factors related to the proposed method have been tested to select the optimum conditions. The stoichoimetry and kinetics of the reaction have also been investigated.

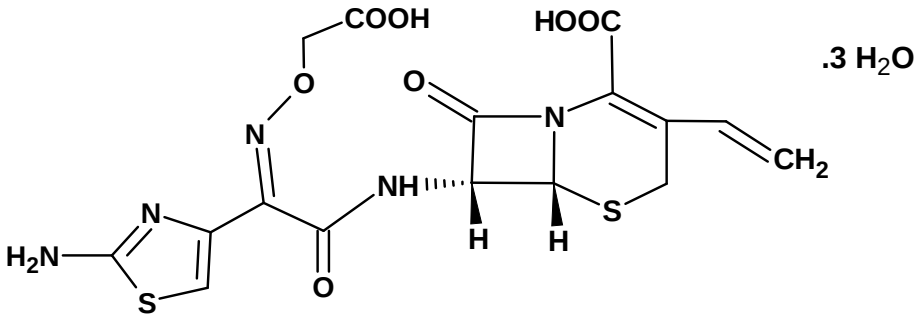


Fig 1. Chemical structure of cefixime

MATERIALS AND METHODS

Apparatus

The absorbance measurements were performed on Spectronic-20 Visible spectrometer, Fisher Scientific U. K. A Lutron digital pH-meter was used for pH adjustment, Jenway U. K.

Chemicals and reagents

Potassium permanganate and sodium hydroxide were of analytical grade and obtained from Sigma Aldrich. Pure Cefixime was obtained from Modern Pharma, Sana'a, Yemen. Saffron powder: local market in Ibb City- Yemen, which labelled to be a mixture of Spanish and Iranian saffron**.** The reagents solution was prepared by mixing proper volumes of Saffron, sodium hydroxide and potassium permanganate. Water was used to prepare extracts of Saffron, 0.0713 g dried red stigmas of saffron were immersed in double distilled water (100 mL) for 15 min and filtered. The final mixture of the reagents solution (Saffron extract, NaOH, $KMnO_4$) was of green colour and used after standing time that is estimated experimentally.

Preparation of stock solutions

A stock solution of saffron has been prepared by dissolving 0.0713 g in 100 ml distilled water. Double distilled water was used throughout all experiments. Solutions of Potassium permanganate (0.00263 M) and sodium hydroxide (0.00615 M) were always prepared freshly before the experiments. A stock solution of cefixime (100µg/ml) was prepared by dissolving the required amount of cefixime in double distilled water and shaking the solution until the complete dissolution. This solution was always kept refrigerated and used within 72 hours.

General Procedure

About 1 mL of (0.6-10 μgmL^{-1}) for cefixime (previously heated at 80^oC for five minutes and quenched to room temperature) were transferred to a series of 10 ml volumetric flask. Then 0.7 ml of the reagent solution was added to each flask with gently shaking and completed to volume with double distilled water. The absorbance of the resulting solution was measured at 390 nm after 12 min at 25±1 °C against reagent blank. The optimum conditions has been fixed by measuring the absorbance while varying one condition and considering that value that gives the highest and/or stable absorbance.

Sample Solutions

The pharmaceutical formulations of cefixime in the form of capsule and tablet were procured from local market. Three capsules was weighed, finely powdered and mixed thoroughly. The average weight for each capsule or tablet was determined. An accurately weighed amount of the powder obtained from capsules or tablet equivalent to 0.01g of pure drug was dissolved in 100 mL double distilled water. The contents of the flask were swirled, sonicated for 5 min,

and then filled to volume with double distilled water. The contents were mixed well and filtered. This prepared solution was diluted quantitatively with distilled water to obtain a suitable sample solution of 10µgmL^{-1} for the analysis.

Results and discussion

Absorption spectra

Figure 1 shows the spectrum of the reaction product as well as that of saffron reagent. The reaction product shows λ_{max} of 390nm while the saffron at 420nm, which confirms that the reaction product is distinguished from the reagent and the wavelength of 390 nm can be considered for this further investigation.

Beer - Lambert's law

Figure 2 shows the calibration curve, which resulted from the application of this method in concentrations range 0.6 - 10µg/ml. The curve shows that Beer-Lambert's law is obeyed in cefixime concentrations range of 0.6-9 µg/ml.

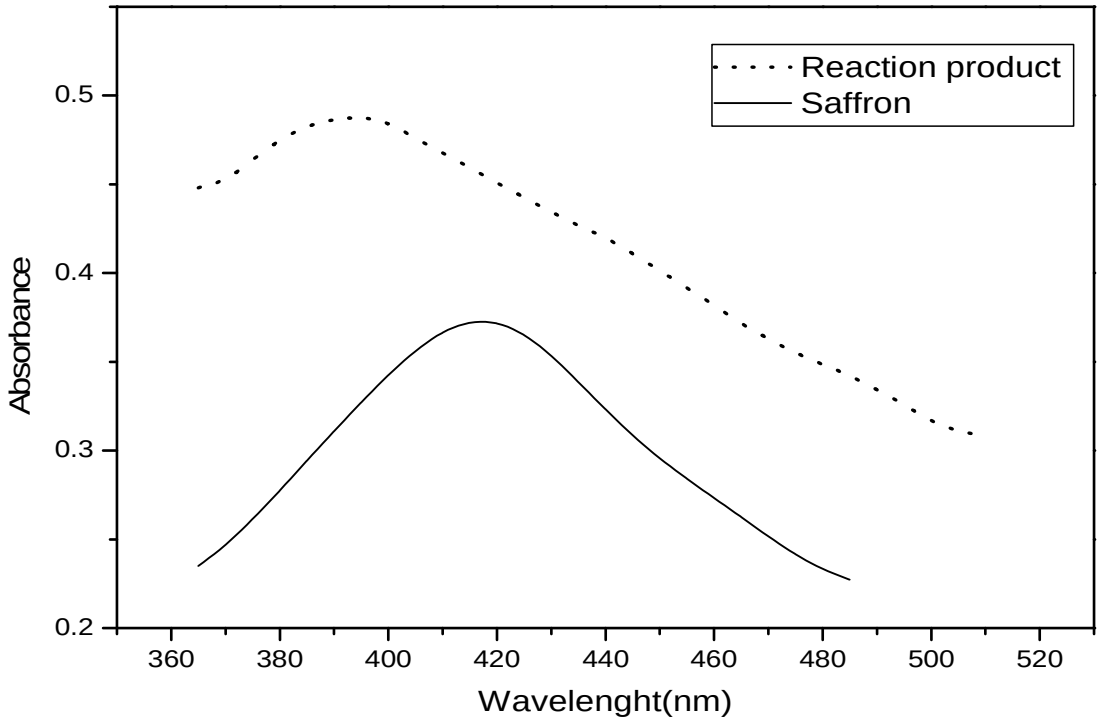


Fig 1. Spectrum of Cefixime at 390 nm Absorption spectra of (**.**) reaction product ;(**-**) Saffron

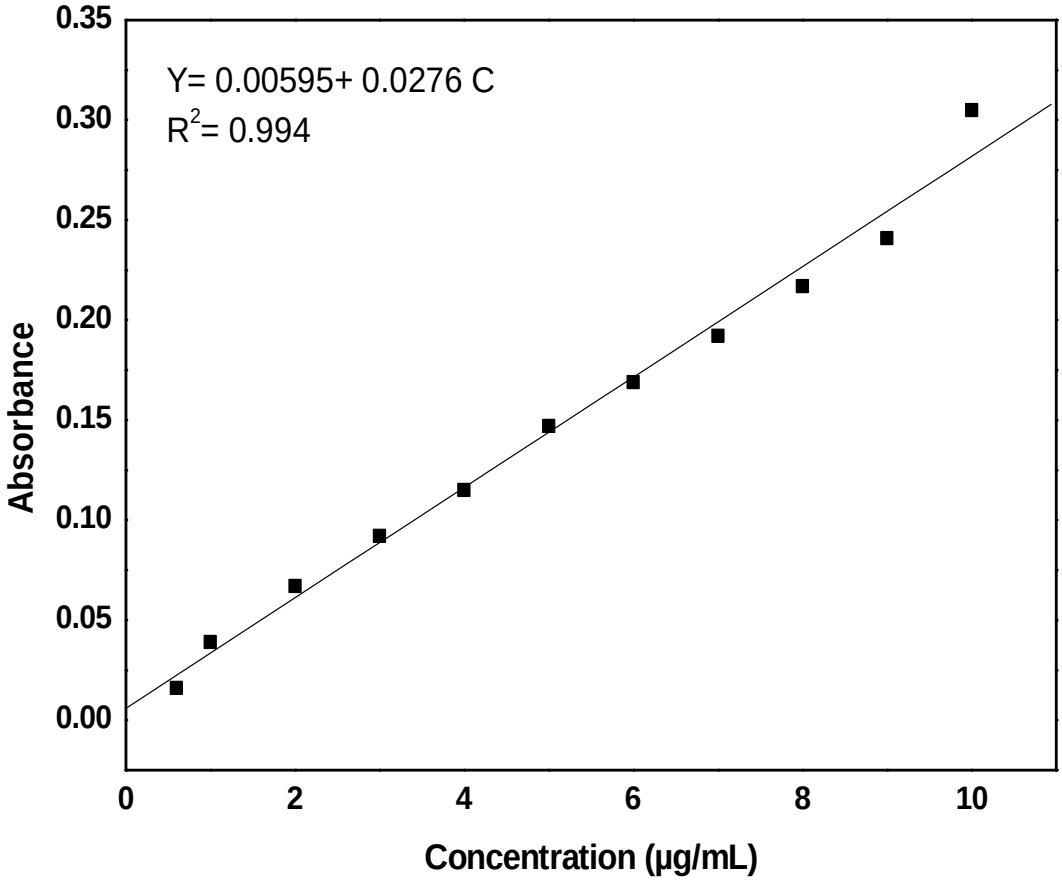


Fig 2. Calibration curve of Cefixime at 390 nm

The optimum composition of the reaction

The optimum composition of the reagent has been investigated by varying the concentration of one of the components (saffron, sodium hydroxide and potassium permanganate) and performing the procedure that has been described earlier. Figures (3-5) show how the absorbance varied with these parameters and those that give the highest absorbance have been selected as the optimum composition of the reagent. The results indicated the a composition of (0.6ml saffron, 0.9 sodium hydroxide and 0.8ml potassium permanganate) can be adopted as the optimum composition. The effect of reagent volume on the reaction was also studied by varying its volume from 0.1 to 1.5 mL. The highest absorbance was attained when the volume of reagent was 0.7 mL Figure 6.

The first standing time, the time required to allow attaining stable reagent, has been measured through measuring the absorbance at 420nm with time. The absorbance attained its maximum value after 3 minutes (Figure 7). The second standing time, the time for the absorbance to attaining its maximum and stable value after addition of the reagent of cefixime solution was measured. Figure 7. shows that the second standing time is about 12 minutes. The colour obtained was stable for at least 8 hours.

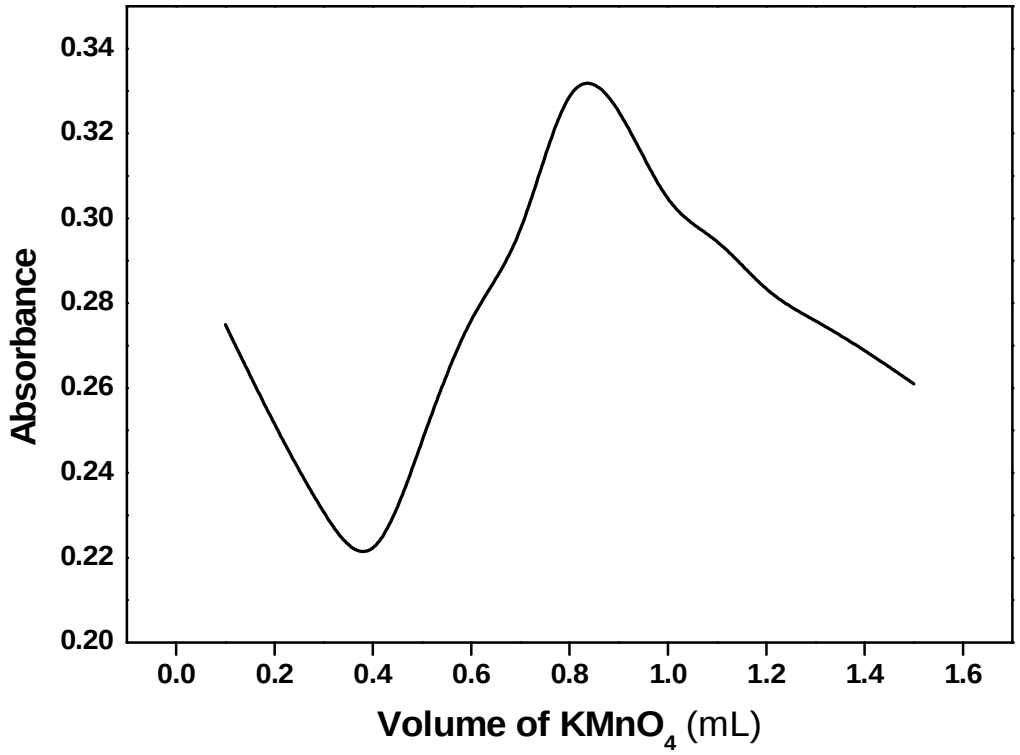


Fig 3. Effect of volume of $KMnO_4$ on absorbance of cefixime

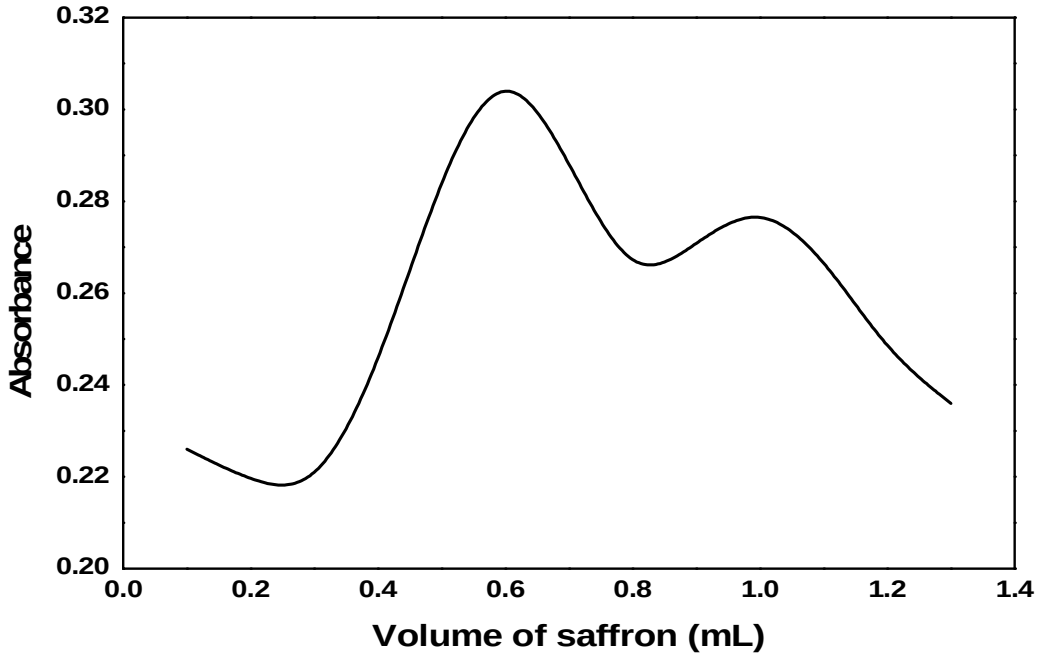


Fig 4. Effect of volume of saffron on absorbance of cefixime

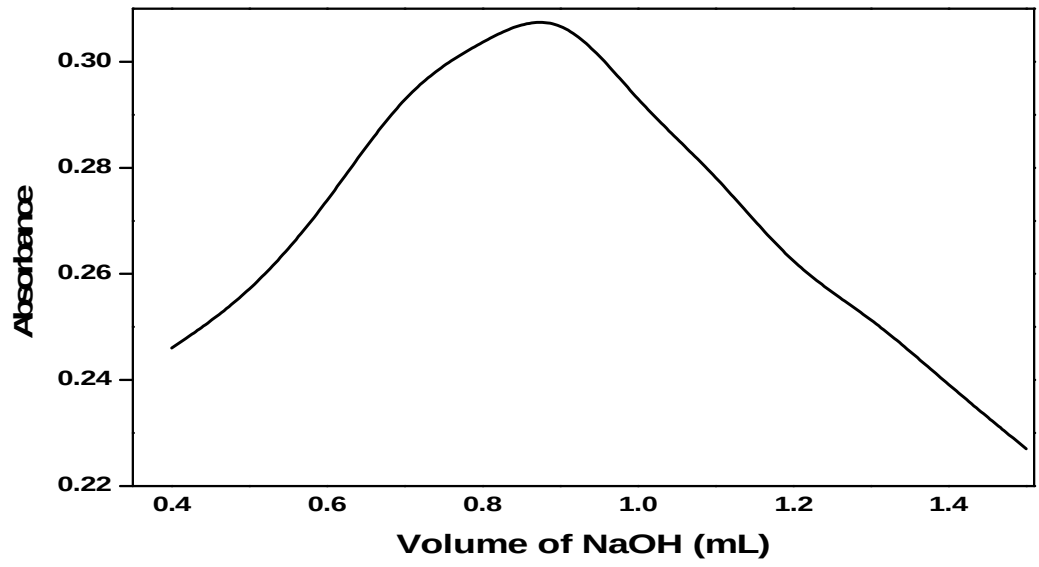


Fig 5. Effect of volume of NaOH on absorbance of cefixime

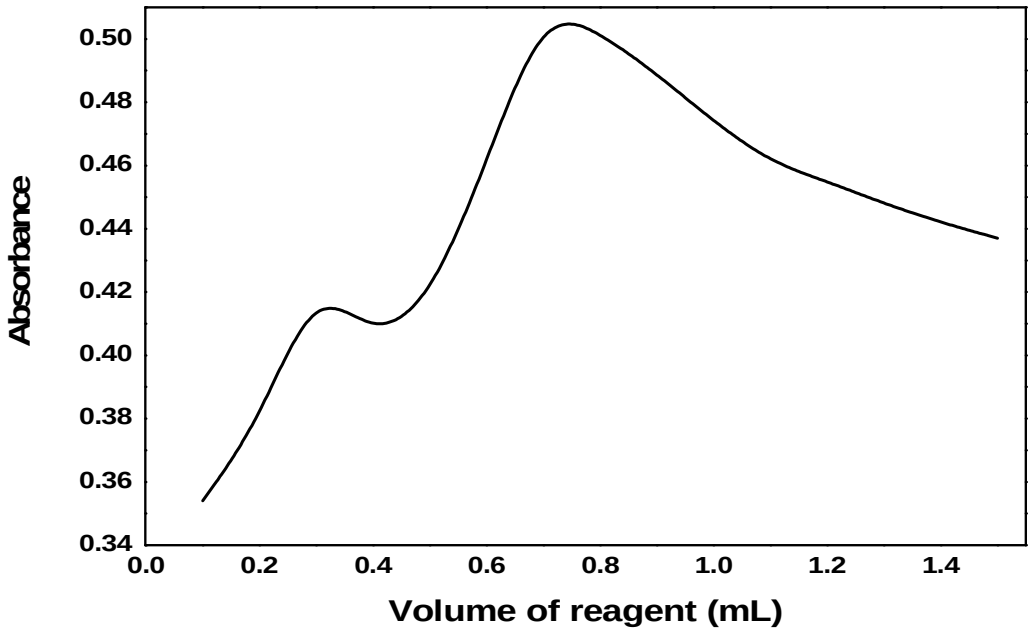


Fig 6. Effect of volume of reagent on absorbance of cefixime

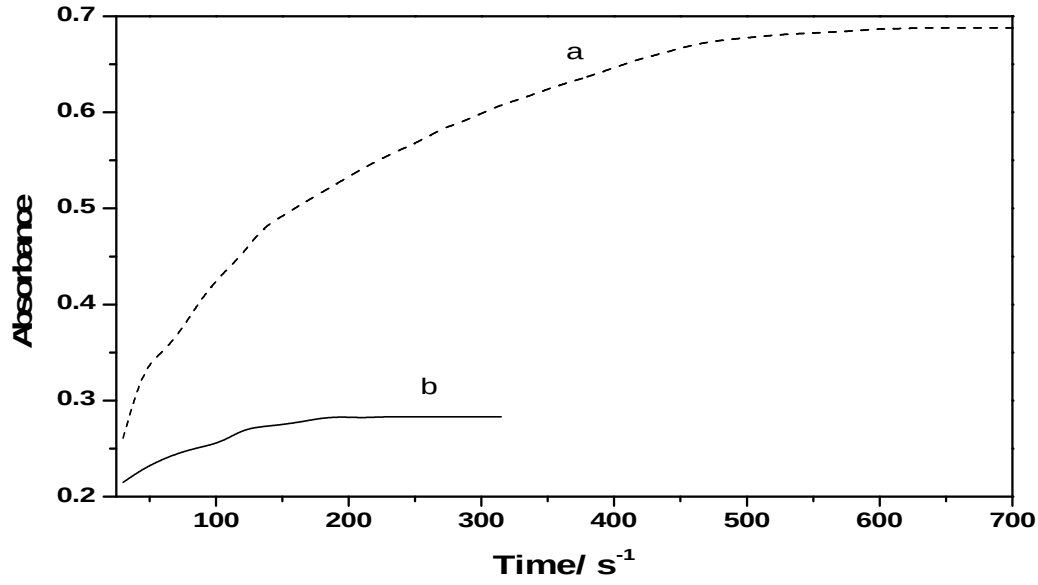


Fig 7. Plots of Absorbance vs. time of (a) after addition of cefixime (10 µg mL^{-1}) to the reagent; (b) before addition of the drug to the reagent at 390nm

Stoichiometry

The stoichiometry of the reaction has been estimated using continuous variation method [29]. Under the optimum conditions shown in Table 1. The stoichiometry of the reaction with respect $KMnO_4$ to potassium permanganate and cefixime was found to be 1:2 (Figure 8).

Fig 8. Continuous variations graph for the reaction between1.93×10^{-3} M cefixime and 2.63×10^{-3}

Mechanism of the reaction

The proposed mechanism of the reaction is depicted in Scheme 1. As reported in literature, the main effective chromogens are crocin groups that have similar structures. Permanganate ion converts Saffron compounds into product A and mangante ion that have green colour in solution. After addition of this reagent solution (Saffron, $KMnO_4$, and NaOH) to cefixime solution the produced divalent manganese reacts with the compound A to form a complex (compound C; yellow color appeared).

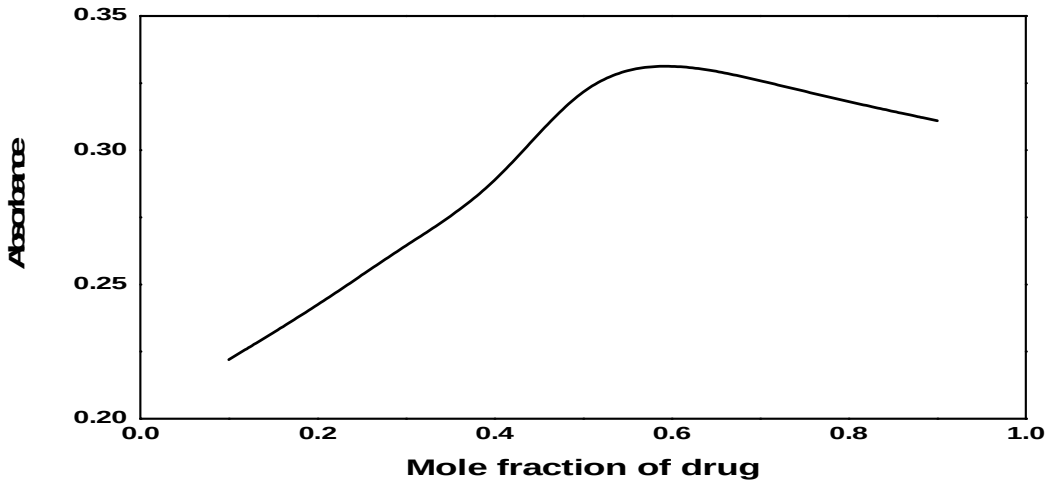
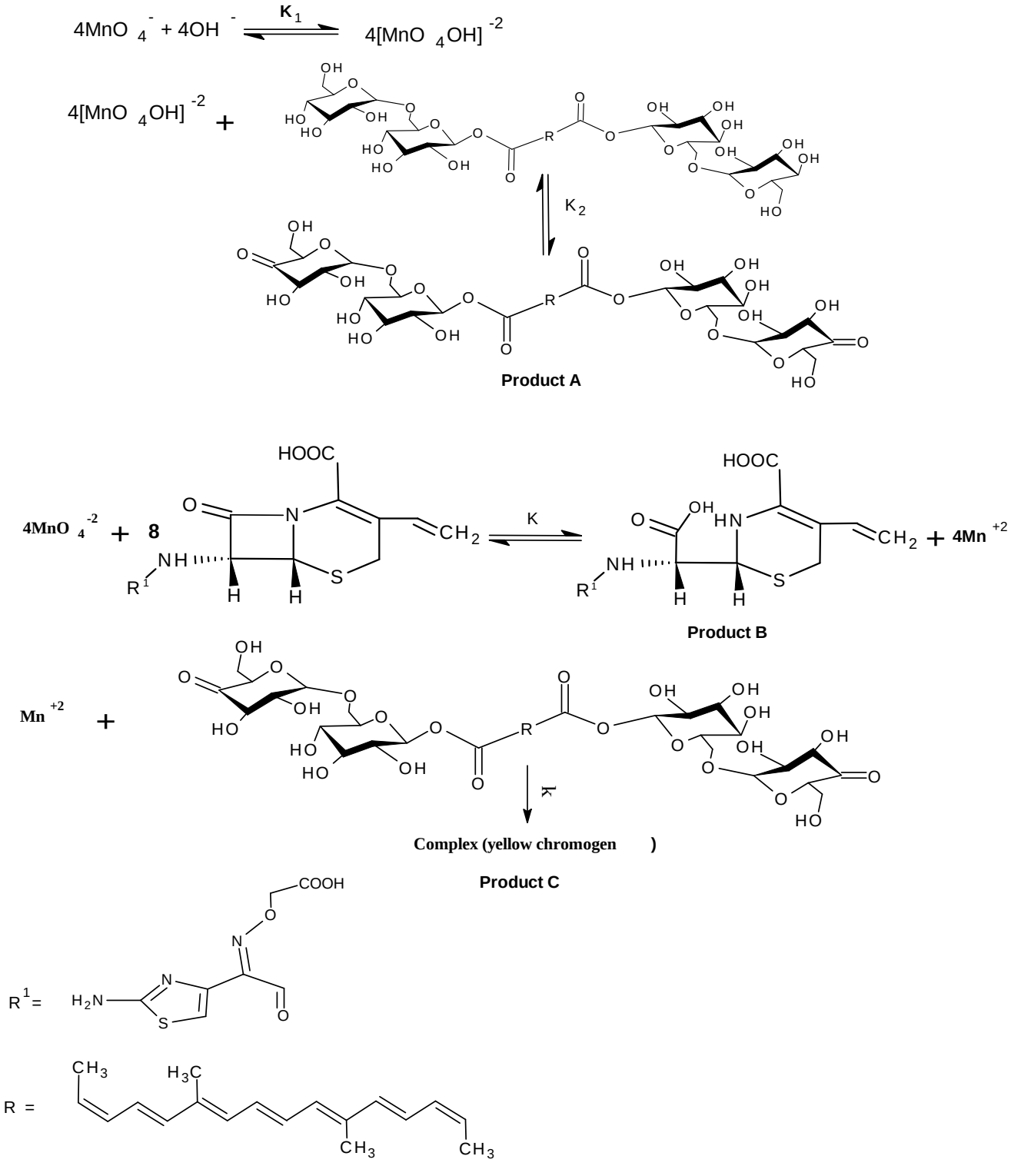


Fig 8. Continuous variations graph for the reaction between1.93×10^{-3} M cefixime and 2.63×10^{-3} $KMnO_4$


4MnO 4 - + 4OH - K1 4[MnO 4OH] -2
4[MnO 4OH] -2 +
K2
Product A
4MnO 4 -2 + 8
K
4Mn +2
Product B
Mn +2 +
Complex (yellow chromogen)
Product C
R 1 =
R =

Scheme 1.

Analysis of pharmaceutical preparations

Table 3 shows the results of analysis of some pharmaceutical preparations collected from Yemeni markets. The reproducibility of the method was found to be acceptable as can be concluded from the reasonable range of relative standard deviation (RSD). In general the results show some consistence between the labelled and found values for some preparations while divergence is observed for others. The non-consistence between the labelled and found assays implies that some companies do not stick strictly to the labelled ones. Similar deviations were reported by Rind et al. for some pharmaceutical preparations of ceftriaxone available in local market of Pakistan [30].

Recovery studies

The accuracy of the proposed methods was checked by recovery studies with the addition of standard drug solution to pre-analyzed sample solution at two different concentration levels (7 and 3) within the range of linearity for the drugs. The recovery from these two pure samples was around 99%, which is a reasonable value when compared with other spectrometric methods that has been suggested for determination of this drug [31, 32].

Precision and accuracy

Table 1. shows the accuracy parameters of this method such as regression coefficient of standard curve, standard deviation, LOD and LQD. These values were compared with those found in other suggested spectrometric methods in table 4. The values of LOD and LQD for this method were 1.25 and 3.80 µg/ml respectively and occur as intermediated between those reported in the Table 4. Relative standard deviation (RSD) value in this method was found to be 2.80 that make this method among the accurate reported methods in determination of cefixime spectrophotometrically.

Interference study

The interference from other possible excipients and diluents was tested by carrying out the test for a solution of 9µg/ml of pure cefixime in presence of some of these materials in a ratio of 10:1 relative to cefixime. The resultant recoveries were presented in Table 5 that shows that the error from interference remains in the minimum and this method can be applied for determination of cefixime in its formulations.

Kinetic studies

The kinetic of the reaction has been investigated using pseudo-first order method. The concentration of the drug was assumed to be in excess over that of the reagent and its concentration can be considered unaltered during the reaction. The following equation is described for studying the kinetics of the reaction:

$$\ln(A_\infty - A_t) = \ln(A_\infty - A_o) - k_{obs}t \qquad (1)$$

where A_∞ , A_o and A_t are the absorbances at infinite time, initial time and time t.

Figure 9 shows graphitic representation of this equation in which $\ln(A_\infty - A_t)$ has been plotted against time for some selected volumes of the reagent. These curves show that pseudo-first order method is successfully applicable particularly at the early stages of the reaction. The observed rate constants, k_{obs}, were calculated from slopes of such curves in each experimental run. The same procedure has been repeated at different concentrations of cefixime, saffron, sodium hydroxide and potassium permanganate, calculated K_{obs}. The calculated of K_{obs} values are tabulated in Table 2. The observed rate constant was found to increase with increasing the concentration of $KMnO_4$ gradually and attained a maximum value after which k_{obs} was almost became independent of $KMnO_4$ concentration. Increasing the concentration of saffron extract also resulted in increase of k_{obs} linearly as shown in Figure 10 with intercept close to zero that confirms that the order of the reaction with respect to saffron is unity. The increase of NaOH concentration resulting in increasing K_{obs} value which reflects. The catalytic behaviour of oxidation by potassium permanganate in alkaline medium. The effect of temperature has been investigated by performing experimental runs at various temperatures in temperature range 283-323K. Figure 11 shows the graphitic representation of $\ln k_{obs}$ versus temperature inverse, which has been fitted to straight line satisfactorily. The activation parameters Ea, $\Delta H^{\#}$, $\Delta S^{\#}$ and $\Delta G^{\#}$ have been calculated from the slope of the straight line and found to be 6.6 $kJmol^{-1}$, 2.5 $kJmol^{-1}$, -45.5 $Jmol^{-1}K^{-1}$ and 15.9 $kJmol^{-1}$ respectively.

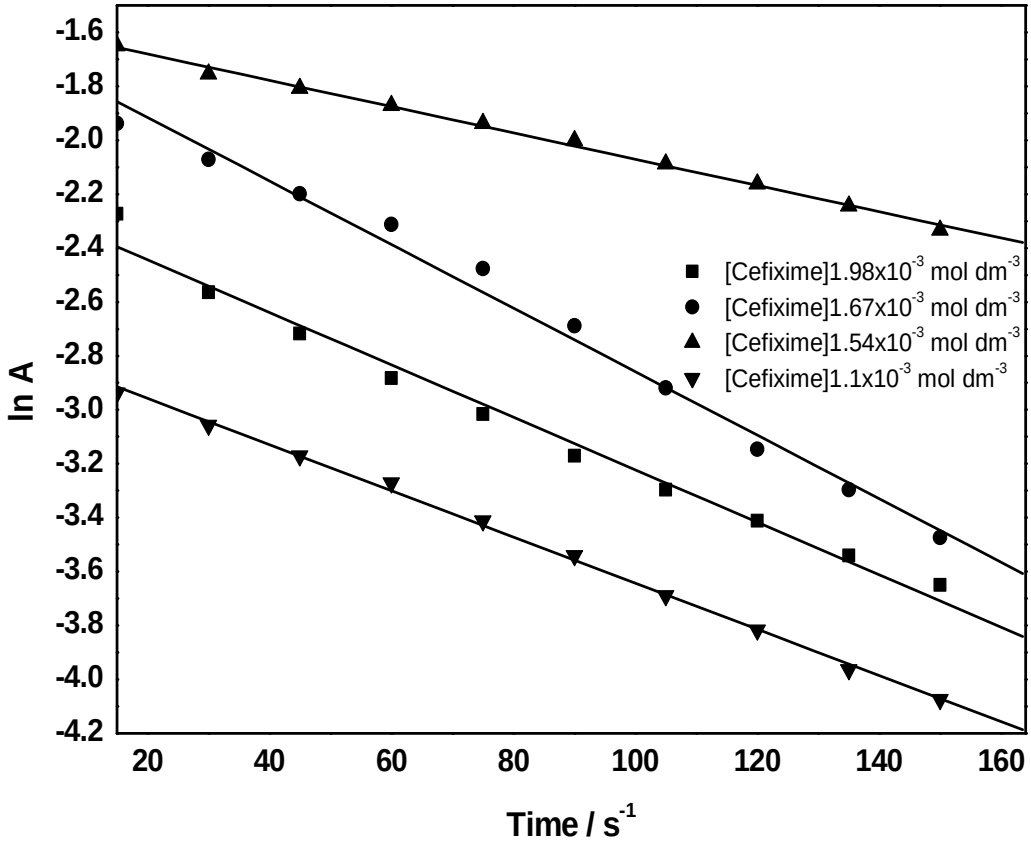


Fig 9. Pseudo first-order plots at following conditions: [cefixime] = 1.93×10^{-3} moldm^{-3} , [$KMnO_4$] = 2.63×10^{-3} moldm^{-3} , [NaOH] = 61.5×10^{-3} moldm^{-3}, [Saffron]= 0.0713 g

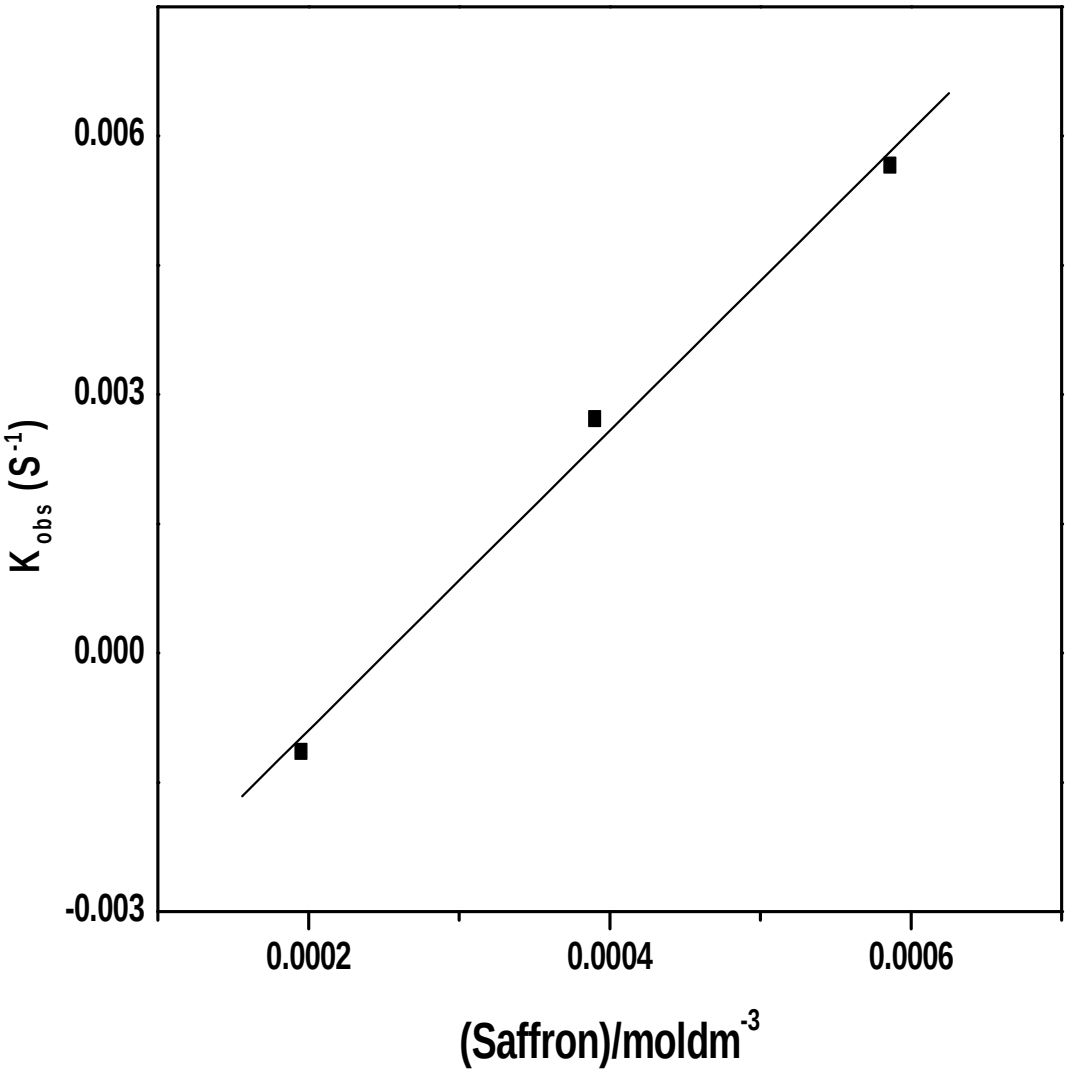


Fig 10. k_{obs} vs. concentration of saffron at 297K and the following conditions: [cefixime] = 1.93×10^{-3} moldm^{-3} , [$KMnO_4$] = 2.63×10^{-3} moldm^{-3} , [NaOH] = 61.5×10^{-3} moldm^{-3}.

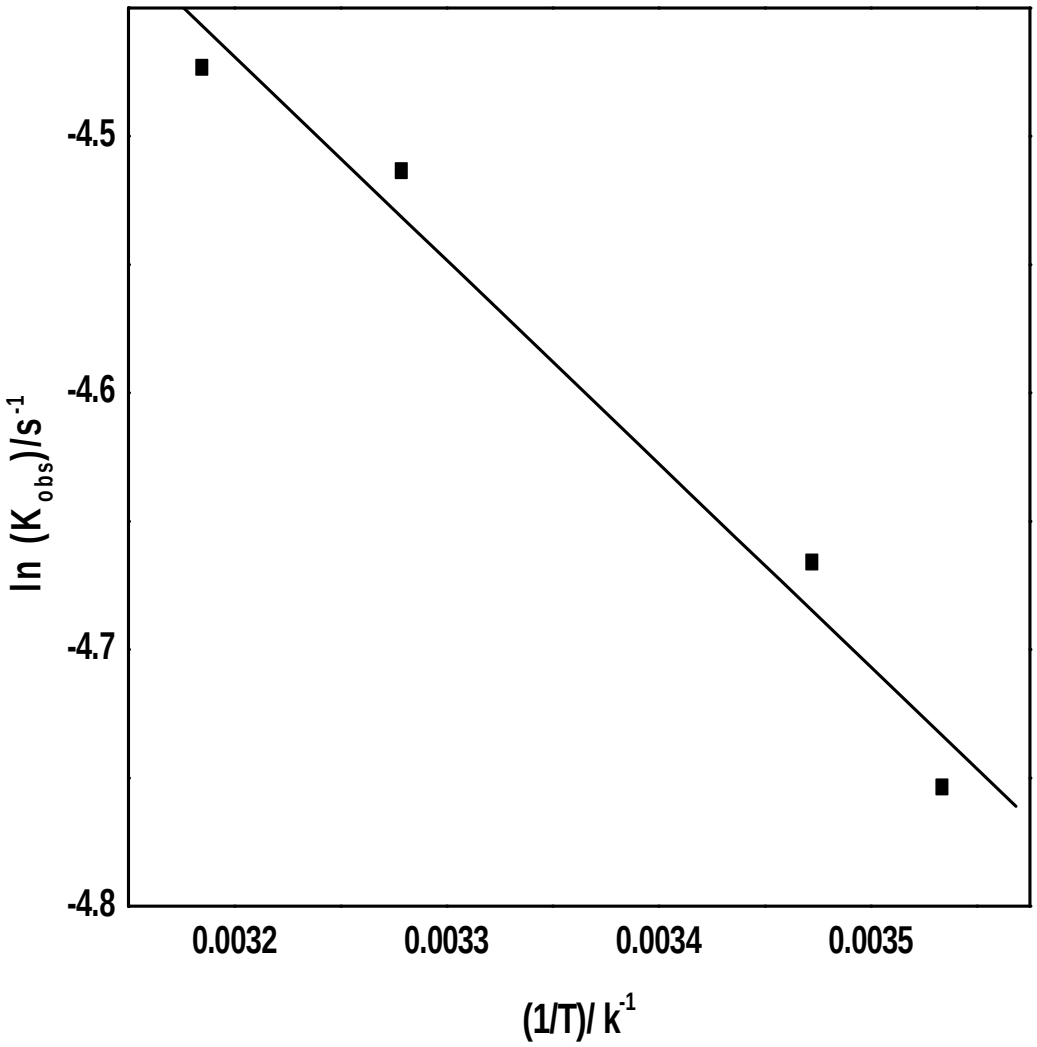


Fig 11. Plots of $\ln_{kobs}$ vs. temperature inverse at following conditions: [cefixime] = 1.93×10^{-3} $moldm^{-3}$, $6.25\times10^{-3}moldm^{-3}$, [$KMnO_4$]= 2.63×10^{-3} $moldm^{-3}$, [NaOH]= 61.5×10^{-3} $moldm^{-3}$, [Saffron]= 0.0713 g.

TABLE 1.

RESULTS OF OPTIMIZATION, PRECISION AND ACCURACY

S.No.	Parameters	Selected values
1	Wave length λmax (nm)	390 nm
2	Beer's law limits (µg mL^{-1})	0.6-10
3	Molar absorptivity (L mol^{-1} cm^{-1})	1.2×10^3
5	Regression equation	Y = 0.00595 + 0.0276 X
6	Coefficient of determination (R^2)	0.994
7	Standard deviation(S)	0.01049
8	LOD (µg/ml)	1.254
9	LOQ (µg/ml)	3.8
10	Time taken for maximum color development	12 min
11	Stability of the colored product	More than 8 hrs

TABLE 2.

K_{OBS} VALUES AT VARIOUS CONCENTRATIONS OF CEFIXIME, $KMNO_4$, SAFFRON AND NaOH

10^{-3}Cefixime ($moldm^{-3}$)	10^{-4} Saffron ($moldm^{-3}$)	10^{-5} KMnO4 ($moldm^{-3}$)	10^{-3} NaOH ($moldm^{-3}$)	k_{obs} (s^{-1})
1.54	21.7	154	61.5	0.00908
1.32	21.7	154	61.5	0.00891
1.1	21.7	154	61.5	0.00748
0.88	21.7	154	61.5	0.0128
0.6	21.7	154	61.5	0.01268
2.2	1.95	154	61.5	0.00114
2.2	3.9	154	61.5	0.00272
2.2	5.86	154	61.5	0.00566
2.2	7.8	154	61.5	0.06786
2.2	9.76	154	61.5	0.0223
2.2	21.7	1.54	61.5	0.00483
2.2	21.7	4.62	61.5	0.00772
2.2	21.7	6.16	61.5	0.00799
2.2	21.7	7.7	61.5	0.00795
2.2	21.7	154	1.23	0.00601
2.2	21.7	154	1.84	0.00524
2.2	21.7	154	2.46	0.00518
2.2	21.7	154	3.07	0.0041
2.2	21.7	154	3.69	0.00388

TABLE 3.

ANALYSIS OF CEFIXIME FROM PHARMACEUTICAL PREPARATIONS

S.No.	Name of drug	Labeled amount	Amount found	% Recovery ± %RSD*
1	Cefix; PHARMA INTERNATIONAL, AMMAN - JORDAN	200mg/Cap	190.08	95.04 ±2.93
2	Fixoral; ALPHA- ALEPPO PHARMACEUTICAL INDUSTRIES ALEPPO- SYRIA	200mg/Cap	197.45	98.725 ±8.93
3	Zimasafe ; , MODERN PHARMA, YEMEN	200mg/Cap	187.51	93.755 ±0
4	Zimasafe ; , MODERN PHARMA, YEMEN	400mg/Cap	381.655	95.41 ±0.46
5	Cefim; OMAN PHARMACEUTICAL, SALALAH, SULTANATE OF OMAN	400mg/Cap	358.05	89.51 ±0.99
6	Cefim; OMAN PHARMACEUTICAL, SALALAH, SULTANATE OF OMAN	200mg/Cap	166.75	83.375 ±2.48
7	Magnacef ; RAM PHARMACEUTICAL INDUAMMAN- JORDAN	200mg/Cap	191.83	95.915 ±2.2
8	Lxime; NEW INDUSTRIAL, RAISEN (M.P.) INDIA0020	400mg/Cap	523.66	130.9 ±1.02
9	Topcef; TORRENT PHARMACEUTICALS, MEHSANA, INDIA	200mg/Tab	183.46	91.173 ±1.1

TABLE 4.

COMPARING OF SOME SPECTROPHOTOMETRIC METHODS USED IN ANALYSIS OF CEFIXIME

Parameters	Method I	Method II	method III	method IV	Current method
Reagent	Complexation With Cu(II) Using Acetate–NaOH Buffer in Water: Methanol	Schiff's Base System Using Vanillin	iodate/iodide mixture	1, 2 naphthoquinone-4-sulfonic (NQS) in alkaline medium	Saffron Extract + $KMNO_4$
Wave length (nm)	410	414	352	520/600	390
Beer's law limits (µg mL-1)	0.2267 – 22.671	2-20	5-25	10-35	0.6-10
Coefficient of determination (R2)	0.9995	0.998	0.9988	0.99871	0.994
LOD (µg/ml)	0.030	-	0.24	2.02	1.254
LOQ (µg/ml)	0.091	-	0.74	6.114	3.8
%RSD	4.0	< 1.5	1.66	0.53	2.8
References	Ref. [33]	Ref. [34]	Ref. [35]	Ref. [36]	Proposed method

TABLE 5.

INTERFERENCE STUDY

Interfering substances	Amount added of interfering (mg L-1)	Amount of drug found (µg ml-1)	% Recovery ± %RSD
Starch	2	9.26	102.9 ±1.1
Cellulose	2	8.73	97.04 ±0.99
L-Arabinose	2	10.0	110.01 ±1.01
D- Ribose	2	9.89	109.9 ±0.45

CONCLUSION

The major advantage of this method is to gives a new kinetic spectrophotometric for determination of cefixime by natural reagent as well as, provide simple, rapid, accurate, precise, economical, and applicable method for the determination of cefixime samples.

ACKNOWLEDGEMENT

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