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Kinetic spectrophotometric method for the estimation of cefixime in pharmaceutical formulations

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ABSTRACT

The objective of the current study was to develop a simple, sensitive kinetic spectrophotometric method based on the oxidation of Cefixime using potassium permanganate in alkaline medium. The rate of change of absorbance was measured at 598 nm. The initial rate method and fixed time method (at 4 min) are utilised to construct calibration graphs for calculating the concentration of the drug. The results were validated through inter day and intraday precision assays according to the ICH guidelines and also through recovery studies. Statistical comparison of the proposed methods with that of reference method shows excellent agreement and indicates no significant difference in their accuracy and precision.

Keywords: Cefixime, initial rate method, fixed time method.

INTRODUCTION

Cefixime is a synthetic fluoroquinolone antibiotic [1] and is chemically 7-{[2-(2-amino-1,3-thiazol-4-yl)-2 (carboxymethoxyimino)acetyl]amino}-3-ethenyl-8-oxo-5thia-1- azabicyclo oct-2-ene-2-carboxylic acid. It is prescribed for urinary tract infection, bronchitis, pneumonia, prostatitis, syphilis and infections of reproductive organs.[2] Literature survey revealed the estimation of Cefixime has been determined along with other drugs by UV [3-4], HPLC [5-9], flow injection analysis [10] and HPTLC [11].

Kinetic spectrophotometric methods became of great interest in chemical and pharmaceutical analysis because of some specific advantages of the method, such as selectivity due to the measurements based on the absorbance with time of reaction, easily applicable, sensitive and the processing cost is low as the reagents used are commonly available in laboratories. There is no literature regarding estimation of cefixime using kinetic spectrophotometry. Therefore the objective of this study was to develop a sensitive method by employing the kinetic colorimetric

oxidation of Cefixime to increase selectivity and consequently determination of low concentration of the drug as possible.

MATERIALS AND METHODS

Apparatus:
A Shimadzu 1800 UV-Visible spectrophotometer with a pair of matched quartz cells was used to measure absorbance.

Reagents and standards:
All chemicals used were of analytical grade. Pure Cefixime was provided by Vinca Life Sciences, Baddi. A standard solution of Cefixime 1 mg/ml was prepared by dissolving in doubly distilled water and then making the volume with doubly distilled water up to 50 ml. A working test solution (0.1 mg/ml) of Cefixime was prepared by diluting 5 ml of the stock solution to 50 ml with doubly distilled water. The tablets containing Cefixime such as Cefi-200 (FDC) [Tablet A] and Zifi-200 (Piramal Healthcare) [Tablet B] were purchased from local market. Aqueous solutions of sodium hydroxide (1M) and potassium permanganate (0.01M) were prepared with doubly distilled water. Potassium permanganate should be prepared freshly.

Procedures for the determination of cefixime:
Initial rate method:
Aliquots of 0.5-1.5 ml of 0.01% Cefixime test solution were pipette out into a series of flasks and to these 1 ml of 1 M NaOH and 1.5 ml of 0.01 M $KMnO_4$ was added and then diluted them with doubly distilled water up to 10 ml. The contents of the mixture were mixed well and their absorbance was measured at 598 nm with a function of time. The initial rate of reaction at different concentrations was obtained from the slope of tangent to the absorbance time curve. The calibration graph was plotted between the logarithm of the initial rate of reaction and the logarithm of the molar concentration of Cefixime. The amount of drug was calculated either from the calibration graph or regression equation.

Fixed time method:
In this method, the absorbance of each sample solution at a preselected fixed time was accurately measured and plotted against the final concentrations of the drug. The content of the drug was calculated either from the calibration graph or regression equation.

Determination of Cefixime from dosage form:
An accurately weighed quantity of tablets, equivalent to 50 mg of the drug dissolved in doubly distilled water and volume made up to 50 ml of doubly distilled water and the residue was filtered using membrane filter. The stock solution was diluted according to need and analyzed by the recommended procedures.

Procedure for reference method:
Into a series of 10 ml standard volumetric flask, different volumes (0.5-1.5 ml) of 0.01% drug (0.1mg/ml) solution were pipette and diluted to volume with doubly distilled water. The absorbance was measured at absorption maximum of Cefixime that was found to be 287.5 nm. The amount of drug in a given sample was calculated from the calibration graph or regression

equation. Recovery was performed for studying the accuracy of this method and the results are shown in Table 8.

RESULTS AND DISCUSSION

Potassium permanganate acts as strong oxidising agent and has been used in oxidimetric analytical method for determination of many compounds. Basic principle involved during the due course of oxidation process is the alteration of valence of manganese. The heptavalent manganese ion (Mn VI) change to green colour in alkaline medium whereas in neutral and acidic medium the permanganate is further reduced to colourless (Mn II). This behaviour of permanganate is the primary basis for its uses in development of spectrophotometric methods.[12-13]

The absorption spectrum of aqueous potassium permanganate solution in the alkaline medium exhibited an absorption band peaking at 526 nm. The addition of Cefixime to this solution produced new characteristic band at 598 nm (Fig. 1). This band is primarily due to the formation of manganate ion, which resulted in the oxidation of Cefixime by potassium permanganate in alkaline medium. This could cause increase in intensity of the colour with time and this occurrence was utilized to develop a kinetics based analytical method for the determination of Cefixime. The different variables that affect the formation of manganate ion were studied and optimized.

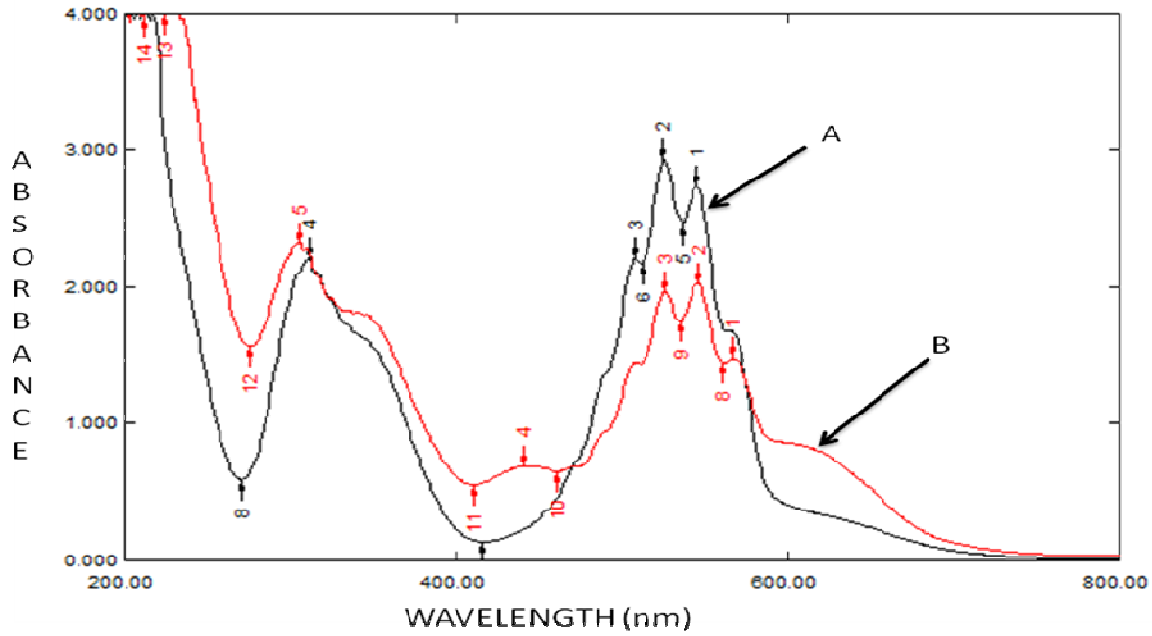


Figure 1: Absorption spectra of (A) 1.0 ml of 1 M NaOH + 1.5 ml 0.01 M $KMnO_4$; (B) 15 µg/ml of Cefixime + 1.0 ml of 1 M NaOH + 1.5 ml 0.01 M $KMnO_4$ in doubly distilled water.

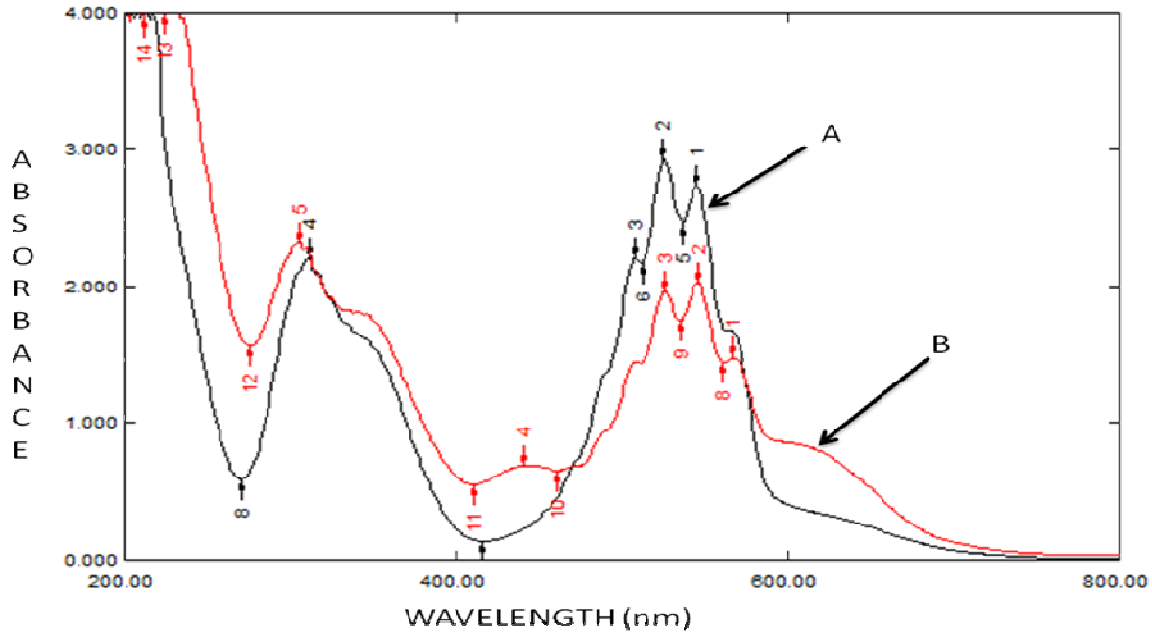


Figure 1: Absorption spectra of (A) 1.0 ml of 1 M NaOH + 1.5 ml 0.01 M $KMnO_4$; (B) 15 µg/ml of Cefixime + 1.0 ml of 1 M NaOH + 1.5 ml 0.01 M $KMnO_4$ in doubly distilled water.

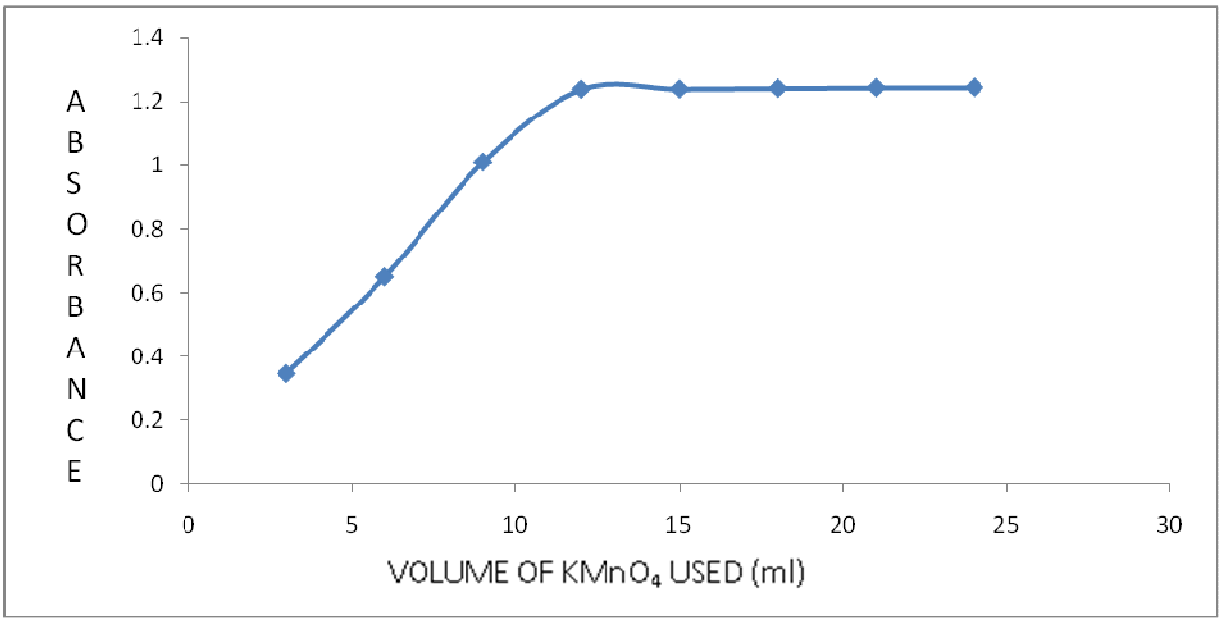


Figure 2: Effect of the volume of 0.01 M $KMnO_4$ on the intensity of color produced during the reaction (Cefixime 15 µg/ml; 1.0 ml of 1 M NaOH).

Effect of KMnO4 concentration:

To study the effect of the KMnO4 concentration, aliquots of Cefixime 100 µg/ml were transferred into a series of 10 ml volumetric flasks, followed by varying volumes of 0.01 M KMnO4 (0.2-2.0 ml) and 1 ml of 1 M NaOH solutions. The absorbance at 598 nm was measured at a fixed time of 16 min. It is apparent from Fig. 2 that the absorbance increased with increasing

volume of KMnO4 solution, and became more or less constant when the volume was 1.2 ml. Further, increase in the volume of KMnO4 resulted in no change in the absorbance (Fig. 2). This results suggest that the adoption of 1.5 ml of KMnO4 (0.01 M) in the final solution could be adequate for the highest concentration of Cefixime (15 µg/ml) used in its determination.

Effect of NaOH concentration:

The influence of NaOH concentration on the formation of manganate ions was examined. aliquots of Cefixime 100 µg/ml were transferred into a series of 10 ml volumetric flasks, followed by varying volumes of 0.01 M KMnO4 (1.5 ml) and (0.2-1.6 ml) of 1 M NaOH solutions. The absorbance at 598 nm was measured at a fixed time of 16 min. It is evident from the Fig. 3 that the highest absorbance of the reactant solution was obtained using 1.0 ml of 1M NaOH, in the current experimental condition. Hence, an optimum value of 1.2 ml of NaOH was selected and used in the further studies.

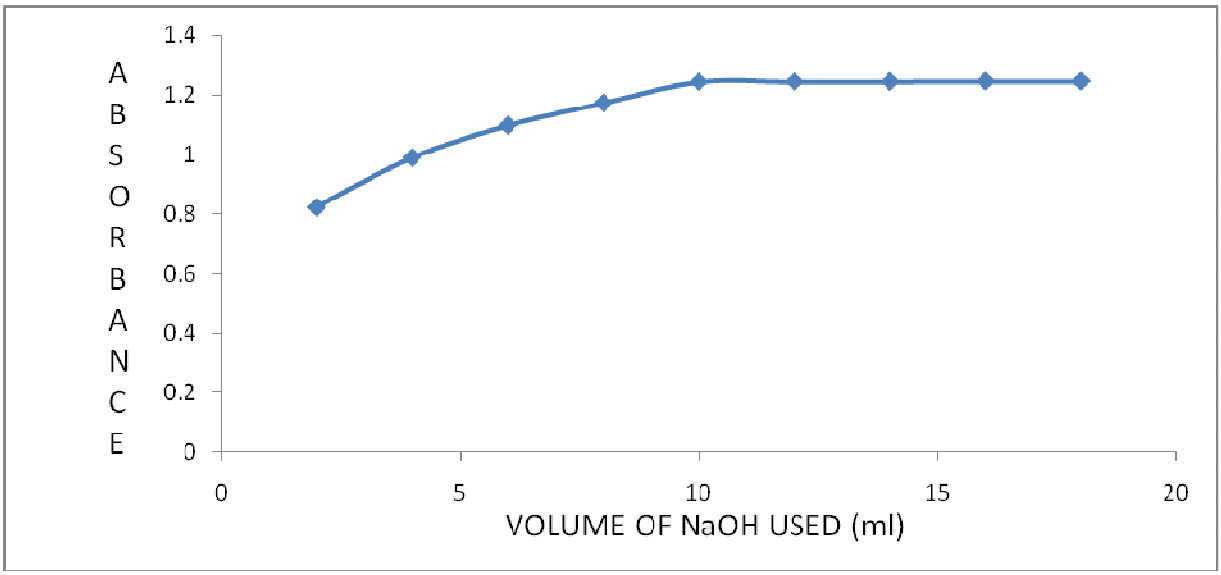
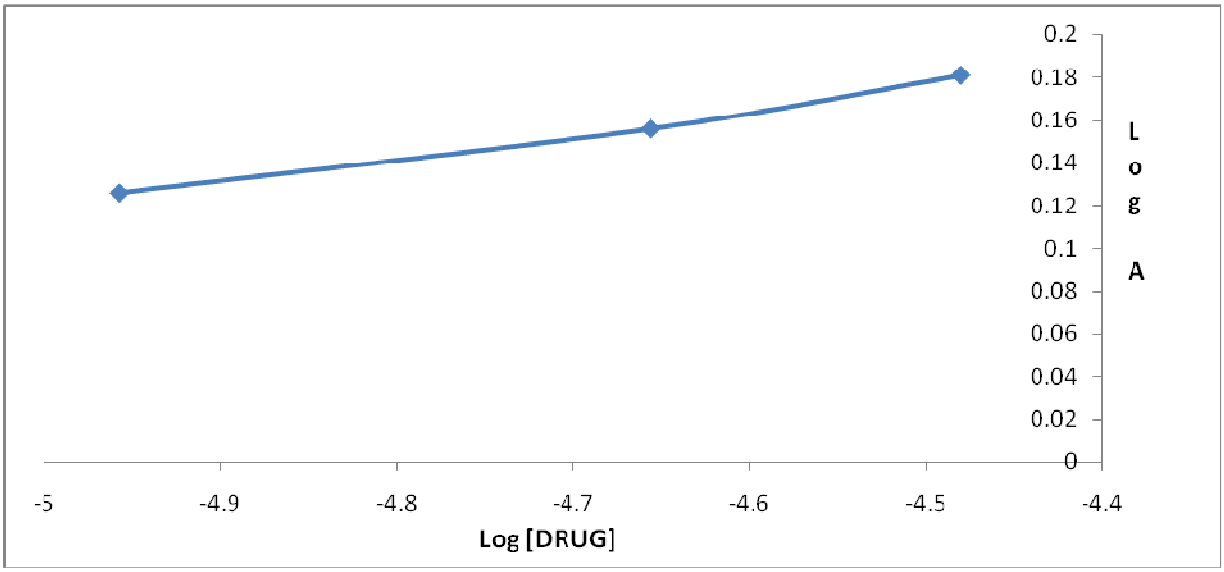


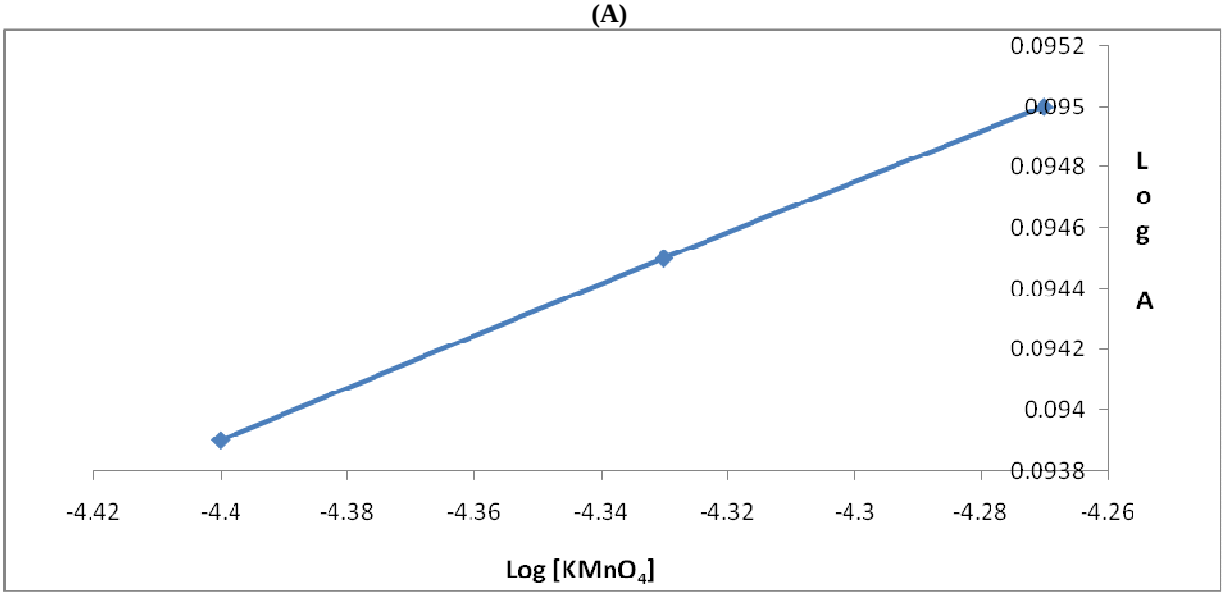
Figure 3: Effect of the volume of 1 M NaOH on the intensity of color produced during the reaction (Cefixime 15 µg/ml; 1.5 ml of 0.01 M $KMnO_4$).

Stoichiometry:

The stoichiometry ratio between potassium permanganate and Cefixime was determined by the limiting logarithmic method. It was established by performing two types of experiments. In the first type, KMnO4 concentration was kept constant and concentration of Cefixime was varied. In the second one KMnO4 concentration was varied while keeping concentration of Cefixime, constant. The logarithm of the absorbance was plotted against the logarithm of the corresponding concentration. The slopes of the two straight lines were calculated and found to be unity in each case. Thus the stoichiometric ratio between cefixime and potassium permanganate was found to be 1:1 (Fig. 4).



(A)

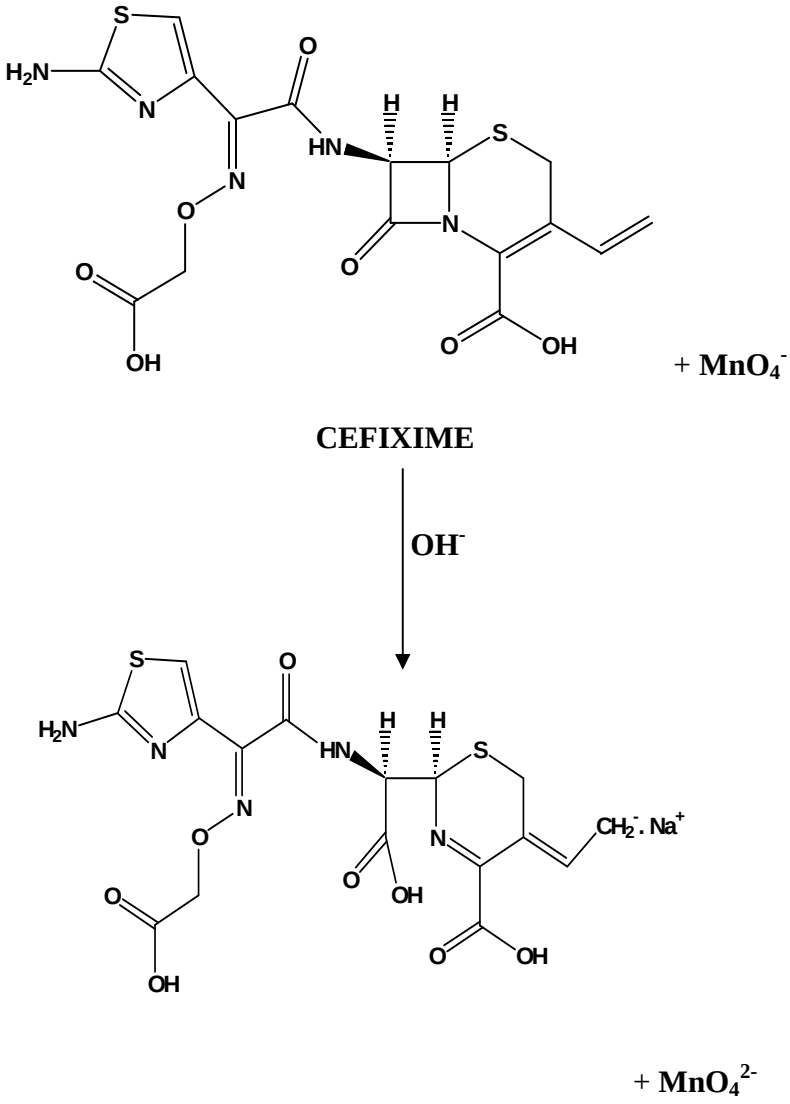


(B)

Figure 4: Limiting logarithmic plot for molar combining ratio between Cefixime and KMnO4: (A) log A vs. log [Drug]; (B) log A vs. log [KMnO4]

Reaction Mechanism

Literature revealed that beta-lactam antibiotics have a tendency to get oxidised by chloramine B, potassium permanganate in alkaline medium. [14-15] So, in this study we have used potassium permanganate (alkaline medium) as the oxidising agent for Cefixime. The reaction mechanism is given in Scheme 1.



OXIDISED PRODUCT

SCHEME 1

Initial rate method:

The initial rate of reaction was determined by measuring slopes of initial tangent to the absorbance time curves (Fig. 5). The order of reaction with respect to permanganate was determined from the reaction using different concentrations of KMnO4 with fixed Cefixime concentration. The order of reaction was found to be one, as the plot of initial rate against the initial absorbance was linear and was passing through the origin. Similarly, the order of Cefixime was determined by plotting the logarithm of the initial rate of the reaction with the logarithm of the molar concentration of Cefixime. Here too the order was found to be one.

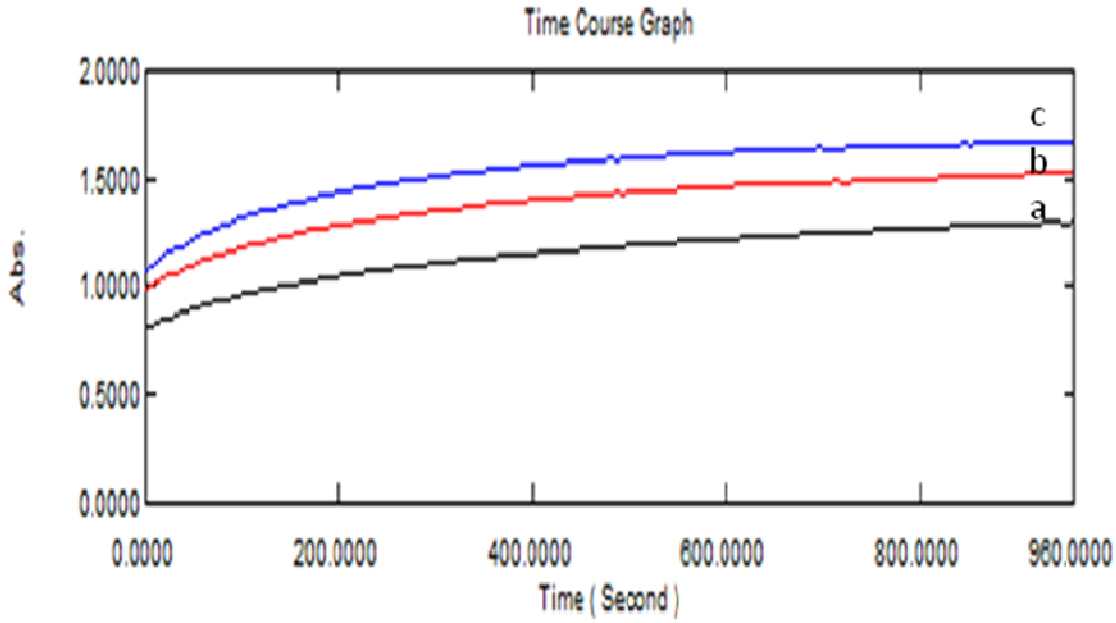


Figure 5: Absorbance versus time graphs for the reaction of Cefixime with potassium permanganate. $KMnO_4$ is 1.5×10^{-3} mol/ litre and Cefixime (a) 5 µg/ml (b) 10 µg/ml (c) 15 µg/ml for 16 min.

Under the optimized experimental conditions, the concentration of Cefixime was determined using an excess of KMnO4 and NaOH solution with respect to the initial concentration of Cefixime, under the optimized experimental condition. As a result, a pseudo zero order reaction with respect to their concentrations. However, the initial rate of the reaction would follow a pseudo first order and was found to obey the following equation;

Rate = $\Delta A/\Delta t$ = K1 Cn

Where K1 is the pseudo first order rate constant, C is the concentration of Cefixime, n is the order of the reaction. The logarithmic form of the above equation is written as,
Log (rate) = log K1 + n log C

The linear regression analysis using the method of least square was used to determine the slope, intercept and correlation coefficient. The calibration curves plotted between the log of initial rate versus log of molar concentration of Cefixime were linear over a range of 5-15 µg/ml with a correlation coefficient of 0.999.

Fixed time method:
In this method, the absorbance of coloured solutions with different amount of Cefixime was measured at a preselected fixed time (Fig. 6).

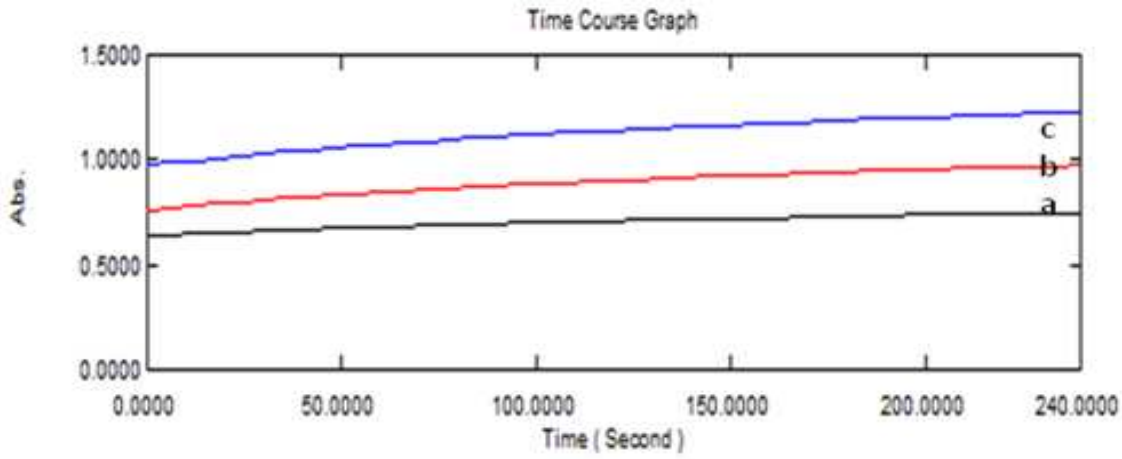


Figure 6: Absorbance versus time graphs for the reaction of Cefixime with potassium permanganate. $KMnO_4$ is 1.5 × 10^{-3} mol/ litre and Cefixime (a) 5 µg/ml (b) 10 µg/ml (c) 15 µg/ml at a fixed time of 4 min.

Calibration curve of absorbance versus initial concentration of Cefixime were plotted at a fixed time of 4, 8, 12 and 16 minutes. The regression equations, correlation coefficient, limit of detection, standard deviation and relative standard deviation are summarized in Table 1.

Table 1: Optical characteristics of fixed time method

Parameters	Fixed time method			
	4 min	8 min	12 min	16 min
Beer's law limit (µg/ml)	3-15	3-9	3-12	3-12
Regression equation	Y= m X + c			
Slope	0.063	0.093	0.064	0.066
Intercept	0.431	0.400	0.528	0.539
Correlation coefficient (R2)	0.998	0.998	0.998	0.998
LOD (µg/ml)	3.240	1.206	2.062	1.825
LOQ (µg/ml)	9.840	3.655	6.250	5.530

Table 2: Intra-day assay for the evaluation of accuracy and precision of the initial rate and fixed time method

Proposed method	Amount taken (µg/ml)	Amount found (µg/ml)	% Accuracy ± SD	%RSD
Initial rate method	05	05.040	100.80 ± 0.432	0.429
	10	09.830	098.30 ± 0.981	0.998
	15	15.230	101.53 ± 0.333	0.328
Fixed time method	05	05.030	100.73 ± 0.498	0.494
	10	10.063	100.63 ± 0.368	0.366
	15	15.090	100.60 ± 0.282	0.280

Values are mean ± SD for three determinations.

The lowest detection limit was obtained at 8 min where as the widest concentration range of quantification was obtained at a fixed time of 4 min. According to ICH guidelines [16] the detection limit is not required to be the part of the validation procedure for assays. Therefore on the basis of wider concentration range and less time of analysis, the fixed time of 4 min was selected for analysis.

The accuracy and precision of Cefixime was measured from the pure sample and tablet dosage form three times within a day by initial rate and fixed time method (Table 2 and 4). The daily precision was measured by analysing the pure sample and tablets on three consecutive days by both methods (Table 3 and 5). The standard deviation, relative standard deviation obtained in both intraday and inter-day assays was found to be satisfactory.

Table 3: Inter-day assay for the evaluation of accuracy and precision of the initial rate and fixed time method

Proposed method	Amount taken (µg/ml)	Amount found (µg/ml)	% Accuracy ± SD	%RSD
Initial rate method	05	04.961	099.22 ± 0.654	0.659
	10	09.967	099.67 ± 1.291	1.295
	15	15.160	101.11 ± 0.686	0.678
Fixed time method	05	05.010	100.20 ± 1.469	1.466
	10	10.114	101.14 ± 2.314	2.288
	15	15.080	100.53 ± 0.110	0.109

Values are mean ±SD for three determinations.

Table 4: Intra-day assay for the test of precision of proposed method in pharmaceutical formulations

Formulations	Initial rate method				Fixed time method			
	Amount taken (µg/ml)	Amount found (µg/ml)	% Accuracy ± SD	% RSD	Amount taken (µg/ml)	Amount found (µg/ml)	% Accuracy ± SD	% RSD
TABLET A	05	4.986	099.72±0.698	0.700	05	04.962	099.24 ± .741	1.754
	10	09.80	098.00±0.108	0.110	10	10.180	101.80 ±0.262	0.257
	15	15.09	100.64±0.749	0.744	15	15.006	100.04 ± .936	0.936
TABLET B	05	4.988	099.77±1.038	1.040	05	05.016	100.32 ± .188	1.184
	10	9.898	098.98±0.738	0.746	10	10.233	102.33 ± .247	1.219
	15	15.02	100.17±1.317	1.315	15	15.180	101.19 ± .524	1.506

Values are mean ±SD for three determinations.

Table 5: Inter-day assay for the test of precision of proposed method in pharmaceutical formulations

Formulations	Initial rate method				Fixed time method			
	Amount taken (µg/ml)	Amount found (µg/ml)	% Accuracy ± SD	% RSD	Amount taken (µg/ml)	Amount found (µg/ml)	% Accuracy ± SD	% RSD
TABLET A	05	05.01	100.50±0.527	0.524	05	04.971	099.42± 0.539	0.542
	10	09.74	097.40±0.555	0.570	10	10.180	101.80± 0.883	0.867
	15	15.18	101.22±0.686	0.678	15	15.030	100.22± 0.565	0.564
TABLET B	05	4.997	099.94±1.495	1.496	05	05.070	101.41± 1.433	1.413
	10	09.86	098.60±0.701	0.711	10	10.076	100.76± 0.205	0.203
	15	15.09	100.62±1.935	1.923	15	15.060	100.39± 0.662	0.659

Values are mean ±SD for 3 determinations.

The accuracy of the methods was determined by performing recovery studies. For this a known amount of pure drug was added to the preanalysed tablet dosage form and then reanalysed by the recommended procedure. The results obtained are shown in Table 6 and 7. However, there was no interference by the formulations excipients.

Table 6: Recovery studies for the determination of Cefixime in tablet dosage form by initial rate method

Formulations	Initial rate method				
	Amount taken (µg/ml)	Amount of pure drug added (µg/ml)	Total Amount found (µg/ml) ± SD	% Recovery	% RSD
TABLET A	5	4	09.02±0.024	100.29	0.0266
		5	10.05±0.036	100.50	0.0358
		6	11.03±0.029	100.27	0.0263
TABLET B	5	4	09.03±0.009	100.36	0.0100
		5	10.05±0.020	100.50	0.0199
		6	11.04±0.014	100.36	0.0127

Values are mean ±SD for three determinations.

Table 7: Recovery studies for the determination of Cefixime in tablet dosage form by fixed time method.

Formulations	Fixed time method				
	Amount taken (µg/ml)	Amount of pure drug added (µg/ml)	Total Amount found (µg/ml) ± SD	% Recovery	% RSD
TABLET A	5	4	09.050 ± 0.021	100.51	0.0232
		5	10.023 ± 0.079	100.23	0.0788
		6	11.060 ± 0.026	100.55	0.0286
TABLET B	5	4	09.080 ± 0.028	100.88	0.0308
		5	10.034 ± 0.049	100.34	0.0488
		6	11.070 ± 0.024	100.65	0.0217

Values are mean ±SD for three determinations.

Table 8: Recovery studies of the reference method

Formulations	Reference method				
	Amount taken (µg/ml)	Amount of pure drug added (µg/ml)	Total Amount found (µg/ml) ± SD	% Recovery	% RSD
TABLET A	5	4	09.05±0.037	100.53	0.0409
		5	10.03±0.024	100.30	0.0239
		6	11.06±0.047	100.60	0.0425
TABLET B	5	4	09.06±0.008	100.60	0.0088
		5	10.03±0.012	100.36	0.0120
		6	11.07±0.009	100.63	0.0081

Values are mean ±SD for three determinations.

Table 9: Point hypothesis: comparison of the proposed method with reference method using paired student t-test

Formulations	Initial rate method			Fixed time method			Reference method	
	%Recovery	%RSD	t-value	%Recovery	% RSD	t-value	% Recovery	% RSD
TABLET A	100.29	0.0266	0.51	100.51	0.0232	0.93	100.53	0.0409
	100.50	0.0358	0.59	100.23	0.0788	0.94	100.30	0.0239
	100.27	0.0263	0.40	100.55	0.0286	0.83	100.60	0.0425
TABLET B	100.36	0.0100	0.12	100.88	0.0308	0.40	100.60	0.0088
	100.50	0.0199	0.12	100.34	0.0488	0.94	100.36	0.0120
	100.36	0.0127	0.18	100.65	0.0217	0.92	100.63	0.0081

Degree of freedom = 2

Theoretical t-value (n = 3) at 95% confidence level is 1.943

Application of the proposed method to pharmaceutical dosage form:
The initial rate and fixed time methods of the proposed kinetic spectrophotometric method for the determination of cefixime have been tested on commercial pharmaceutical dosage forms (tablets) and concentration of cefixime was computed from its corresponding regression equation. The results of the proposed method were then compared with the results of reference method (Table 8) using paired student t-test. The values of the t-test obtained are given in Table 9 which were less than the theoretical values and thus confirms that there was no difference in the performance of the proposed method and the reference method.

Advantages of the proposed method:
The proposed method involves measurements in the visible region and is more selective than the reported UV based methods. Further, the proposed method utilizes doubly distilled water as solvent which is easily available, safe and economical as well. In contrast, the other methods reported used costly organic solvents which cause health and environmental hazards and they require special care for their sanitary disposal. Moreover, the current method is very simple as it is a single step reaction and is not tedious.

From economical point of view, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. The process used in the proposed method need not require any tedious liquid-liquid extraction for the drug base or extraction of the chromophores.

CONCLUSION

The initial rate and fixed time method can be easily applied to Cefixime in its determination in pure and tablet dosage form. These methods do not require any elaborate treatment of the analyte. The proposed methods are sensitive enough for the determination of lowest amount of drug. These advantages encourage the application of the proposed method in routine analysis of Cefixime in laboratories.

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