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Method Development and Validation for the Determination of Cefixime in Pure and Commercial Dosage Forms by Specrophotometry

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***Abstract*—**A simple, accurate and precise direct spectrophotometric method has been developed for the determination of cefixime in tablets and capsules. The method is based on the reaction of cefixime with a mixture of potassium iodide and potassium iodate to form yellow coloured product in ethanol-distilled water medium at room temperature which absorbed maximally at 352 nm. The factors affecting the reaction product were carefully studied and optimized. The validation parameters based on International Conference on Harmonisation (ICH, USA) guidelines were followed. The effect of common excipients used as additives has been tested and the tolerance limit was calculated for the determination of cefixime. Beer's law is obeyed in the concentration range of 4 – 24 μg mL^{-1} with apparent molar absorptivity of 1.52×10^4 L $mol^{-1}cm^{-1}$ and Sandell's sensitivity of 0.033 μg/cm^2/ 0.001 absorbance unit. The limits of detection and quantitation for the proposed method are 0.32 and 1.06 μg mL^{-1}, respectively. The proposed method has been successfully applied for the determination of cefixime in pharmaceutical formulations. The results obtained by the proposed method were statistically compared with the reference method using t- and F- values and found no significant difference between the two methods. The proposed method can be used as an alternate method for routine quality control analysis of cefixime in pharmaceutical formulations.

***Keywords*—**Spectrophotometry, cefixime, validation, pharmaceutical formulations.

I. Introduction

CEFIXIME trihydrate (CAS: 79350-37-1, M.W. 507.5) is chemically known as 7-{[2-(2-amino-1,3-thiazol-4-yl)-2(carboxymethoxyimino)acetyl]amino}-3-ethenyl-8-oxo5thia-1-azabicyclo oct-2-ene-2-carboxylic acid. It is an antibiotic which comes under third-generation cephalosporin like ceftriaxone and cefotaxime. It is highly stable in the presence of beta-lactamase enzymes produced by certain gram-negative bacteria. As a result, many organisms resistant to penicillin and some cephalosporin due to the presence of beta-lactamases, may be susceptible to cefixime. It is available in tablets/capsules (200 mg and 400 mg) and suspension (100 mg per 5-ml spoonful) and provided by mouth in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections especially bronchitis, and urinary-tract infections. At high dosage, gastrointestinal side effects, headaches, dizziness and rashes can appear. Therefore, the analysis of cefixime is important for obtaining optimum therapeutic concentration and for quality assurance in pharmaceutical formulations. The drug is officially listed in British Pharmacopoeia [1] which describes a liquid chromatographic method for the determination of drug in bulk and dosage forms. Several analytical methods such as liquid chromatography-mass spectrometry [2], high performance liquid chromatography [3]-[6], high performance thin layer chromatography [7], [8], derivative spectrophotometry [9], voltammetry [10], and capillary electrophoresis [11], [12] have been reported for its assay in bulk and dosage forms to ensure right quantity of drug in tablets, capsules and suspensions. Some spectrophotometric methods have been reported for the estimation of cefixime in pharmaceutical formulations were based on the reaction of cefixime with palladium [13], chloranilic acid [14], 7,7,8,8-tetracyanoquinodimethane [15], ferrihydroxamate [16], Folin-Ciocalteu reagent at 720 nm [17], a mixture of 3-methyl-2-benzothiazolinon hydrazone HCl and ferric chloride [18], ferric chloride and 2,2-bipyridyl [19]. Another method hydrolyzed β-lactum ring of cefixime with iodate to form iodine in acidic medium, then in turns, iodine oxidizes methylene blue to violet colored species of maximum absorption at 640 nm [19]. Potassium permanganate oxidizes cefixime in alkaline medium and itself reduces to manganate ion, which was measured at 598 nm [20] for the determination of cefixime in dosage forms. The determination of cefixime has been done by spectrofluorimetry utilizing the reaction of the drug with 2-cyanoacetamide**,** which exhibits maximum fluorescence intensity at wavelength 378 nm after excitation at 330 nm [21]. The main problem associated with these determinations is the more analysis time with a number of reagents and laborious cleanup procedure prior to analysis. The sample preparation of the drug included enrichment, separation techniques such as liquid-liquid or solid-liquid extraction, coprecipitation, electrodeposition to isolate and preconcentrate the drug. Therefore, there is a need for a rapid, simple, accurate and selective spectrophotometric method for the determination of cefixime in pharmaceutical formulations. Spectrophotometry is the best tool for determining drug in the laboratories of research, hospitals and pharmaceutical industries due to its low cost, inherent simplicity, versatility, adaptablity and affordability [22]. The proposed method is based on the formation of the yellow colored product because of the reaction of cefixime with a mixture of KI-KIO_3 in

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ethanol-distilled water medium at room temperature (25 ± 1ºC). The yellow coloured product of triiodide ions absorbed maximally at 290 and 352 nm and the reaction is utilized for the estimation of drug in pharmaceutical formulations by recording the absorbance of the reaction product at 352 nm. The reaction conditions are optimized and validated as per the International Conference on Harmonisation guidelines [23].

II. Materials and Methods

A. Apparatus

All spectral and absorbance measurements were made on a Helios Alpha UV-Vis Spectrophotometer (Thermo Electron Corporation, England, UK) with 1 cm matched quartz cells. IR spectra were recorded on an IRAffinity-1 spectrophotometer (Shimadzu, Kyoto, Japan) in wave number region 4000-400 cm^{-1} using KBr pellet technique. pH meter (Hanna, USA) was used to measure the pH of analyte solution.

B. Reagents and Standards

All reagents used were of analytical reagent grade. 0.04% cefixime trihydrate (CAS: 79350-37-1, M.W.: 507.5) solution was freshly prepared in methanol. The pure cefixime trihydrate (Batch No XMEO 110023) is gifted by National Pharmaceutical Industries Company, Oman. The solution was stable up to 12 h.

A mixture of 0.01M potassium iodide - 6.25×10^{-4} M potassium iodate solution was prepared by dissolving 0.332 g potassium iodide with 0.0535 g potassium iodate in distilled water and then transferred into a 100 mL volumetric flask and diluted up to the mark with distilled water. The pharmaceutical formulations of cefixime such as Cefrax 200 mg capsule (National Pharmaceutical Industries Company, Oman) and Suprax 200 mg tablet (Sanofi-aventis, UK) were purchased locally from Scientific Pharmacy (Muscat, Oman).

C. Recommended Procedure for the Determination of Cefixime

Aliquot of 0.1–0.6 mL of 0.04% cefixime solution corresponding to 2.0-24 µg mL^{-1} was pipetted into a series of 10 mL standard volumetric flask. To each flask, 1.0 mL of KI-KIO_3 mixture was added and diluted up to the mark with ethanol. The contents of the flask were mixed well and the absorbance was measured at 352 nm against the reagent blank prepared similarly except cefixime within the stability time period of 2 h. The amount of cefixime was obtained either from the calibration graph or the regression equation.

D. Determination of Cefixime in Pharmaceutical Formulations

The contents of commercially available cefrax capsule and suprax tablet (5 in number) of 200 mg strength of cefixime were weighed and finely grounded. The powder equivalent to 50 mg cefixime were taken in 60 mL methanol and kept for 10 min for complete dissolution of the drug. The mixture was filtered through Whatmann No. 42 filter paper (Whatmann International Limited, Kent, UK) in 100 mL standard volumetric flask. The residue was washed well with 3 × 10 mL portions of methanol for complete recovery of the drug and diluted up to the mark with methanol. The amount of cefixime was determined following the recommended procedure.

E. Procedure for Reference Method [19]

Aliquot of 0.1-1.0 mL of 0.01% cefixime was taken with 0.3 mL of 0.2% ferric chloride solution and 2 mL of 2% 2, 2 - bipyridyl into a 10 mL standard volumetric flask. The contents of the flask were heated at 70˚C for 15 min and then cooled. After cooling, the flask was diluted up to the mark with distilled water. The absorbance of the pink coloured complex was measured at 520 nm against the reagent blank prepared similarly except cefixime. The amount of cefixime was obtained either from the calibration graph or the regression equation.

F. Validation

The proposed method has been validated for linearity, sensitivity, precision, accuracy, robustness, specificity and evaluation of bias.

The linearity of the proposed method was investigated by taking 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL of 0.04% cefixime corresponding to 4.0, 8.0, 12.0, 16.0, 20.0 and 24 µg mL^{-1} cefixime. Each concentration was independently analyzed for 5 times. The absorbance obtained at each concentration was plotted against the concentration of cefixime in µg mL^{-1} and the linear regression equation was evaluated by least square treatment of the calibration data. The other statistical parameters of the proposed method were calculated using OriginPro 6.1 Software.

The limit of detection (LOD) and the limit of quantitation (LOQ) for the proposed method were calculated using the following equations:

$$LOD = 3 \times \frac{S_0}{b} \quad (1)$$

$$LOQ = 10 \times \frac{S_0}{b} \quad (2)$$

where S_0 is standard deviation of calibration line and b is the slope.

The precision of the proposed method was evaluated by intra-day and inter-day precisions at 3 concentration levels of 4.0, 12.0 and 24 µg mL^{-1}. Each concentration level was independently analyzed repeatedly for five times within a day (intra-day precision) and over five consecutive days (inter-day precision).

The accuracy of the proposed method was tested by analyzing freshly prepared tablet and capsule drug solutions in 5 replicate. The same drug solution was also tested by reference method. The percent recovery and standard deviations (S.D.) of the two methods were compared and tested for accuracy of the proposed method.

The accuracy of the proposed method was also checked by standard addition technique. In this technique, 0.3 mL of the 0.4 mg mL^{-1} of the formulated capsule (or tablet) sample solution was spiked separately with 0, 0.05, 0.1, 0.15 and 0.2

mL of the reference drug sample solution in 10 mL standard volumetric flask and diluted up to the mark with ethanol. Each level was independently analyzed repeatedly for five times. The nominal value of the cefixime concentration in tablet and capsule was determined by dividing the obtained intercept by slope.

The specificity of the proposed method was investigated by observing any interference encountered from common excipients of the pharmaceutical formulations such as glucose, fructose, lactose, starch, sucrose, fructose, povidone, methyl cellulose, crystalline cellulose and sodium benzoate at 20 µg mL^{-1} cefixime.

The robustness of the proposed method was evaluated by challenging the optimized parameter of the proposed method such as: 1 ± 0.2 mL of 0.02 KI M and 1 ± 0.3 mL of 0.000625 M KIO_3; working temperature, 25°C ± 1˚C, colour development time, immediately.

The point and interval hypothesis tests have been performed to compare the results of the proposed method with those of the reference method at 95% confidence level. The bias was evaluated by an interval hypothesis test based on the mean values of the proposed method and the reference method. The proposed method is considered acceptable when its true mean is within ±2.0% of that of the reference method. The lower (θ_L) and the upper (θ_U) acceptance limits can be calculated by the following quadratic equation [24]:

$$\theta^2\left(\bar{x}_1^2 - S_p^2 t_{tab}^2/n_1\right) + \theta \times -2\bar{x}_1^2\bar{x}_2^2 + \left(\bar{x}_2^2 - S_p^2 t_{tab}^2/n_2\right) \quad (3)$$

where $\overline{x_1}$ and $\overline{x_2}$ are mean values at n_1 and n_2 measurements, respectively. S_p is the pooled standard deviation and t_{tab} is the tabulated one-sided t-value at 95% confidence level.

III. Results and Discussion

The absorption spectrum of methanolic solution of cefixime is absorbed maximally at 210 and 290 nm whereas the absorption spectrum of aqueous solution of KI showed two absorption bands at 200 and 226 nm while the aqueous solution of KIO_3 showed no peak. When a mixture of KI-KIO_3 solution was mixed with the solution of cefixime, a red shift in the wavelength is observed due to the formation of the yellow coloured triiodide ions which exhibited two bands at 290 and 352 nm. Firstly in aqueous-ethanolic solution, the iodine was formed due to the reaction between cefixime and a mixture of iodide and iodate ions. Later on the formed iodine was reacted with iodide ions forming yellow triiodide ions. The same reaction has been reported in the literature [25] not with cefixime but with other inorganic and organic acids. The absorption spectra of cefixime, KI, KIO_3 and yellow coloured product are shown in Fig. 1. The absorbance measurement at 352 nm was measured at initial concentration of cefixime, thus exploited to develop a new, accurate and rapid spectrophotometric method for the determination of cefixime in pharmaceutical formulations. The reaction was carried out at room temperature (25˚C) and the yellow coloured product was stable up to 2 h.

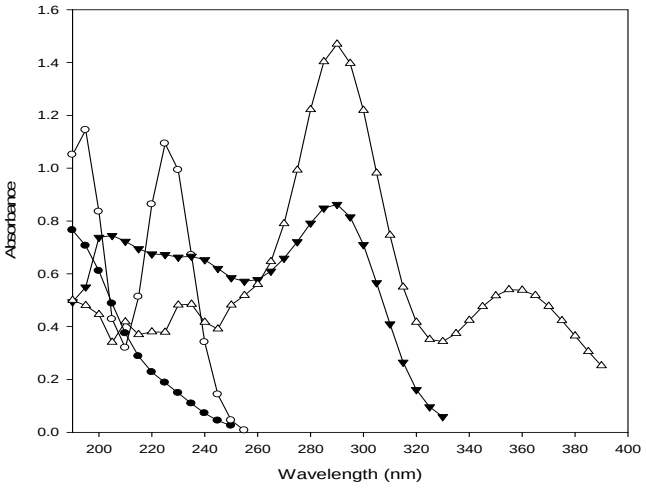
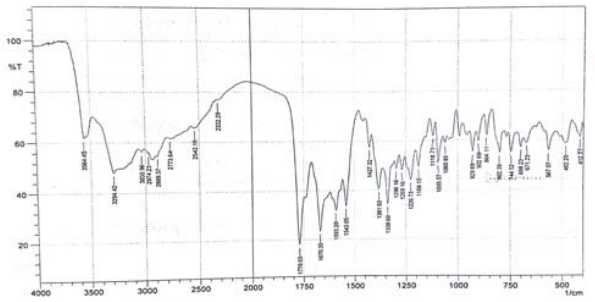
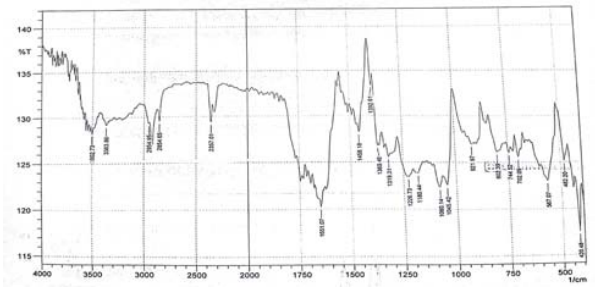


Fig. 1 Absorption spectra of (▼) 0.1 mL of 9.852×10^{-3} M cefixime (○) 0.1 mL of 0.01M KI in distilled water and (●) 1.2 mL of 6.25×10^{-4} M KIO_3 in distilled water (Δ) 0.45 mL of 0.04% M cefixime + 1.0 mL mixture of 0.01 M KI-6.25×10^{-4} M KIO_3 in 10 mL standard volumetric flask and diluted up to the mark with ethanol



(a)



(b)

Fig. 2 Infra red spectra of (a) pure cefixime and (b) reaction product of cefixime

The infra red spectra of pure cefixime and reaction product of cefixime are shown in Figs. 2 (a) and (b), respectively. Cefixime has O-H functional group because of the presence of carboxylic acid group in free cefixime. The O-H stretching band appeared at 3561 cm^{-1}. Comparison of IR spectra of the

solid residue obtained from the reaction product of cefixme with those of free cefixime indicated that the O-H group stretching band is missing in the reaction product. Hence it is evident that H^+ is ionized from COOH in the cefixime and reacted with a mixture of KI and KIO_3, produced triiodide ions which absorbed maximally at 352 nm. The reaction sequence of the reaction mixture is given in Fig. 3.

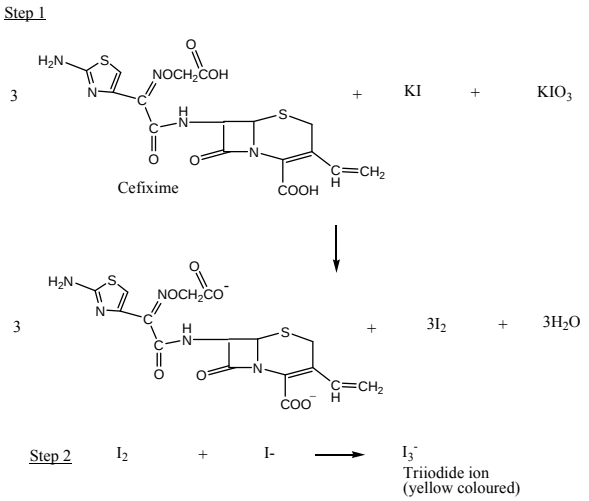


Fig. 3 Reaction sequence of the proposed method

A. Optimization of Variables

The optimization of variables was investigated by testing reaction time, concentration of potassium iodide, potassium iodate and solvents.

The effect of reaction time on the absorbance of yellow coloured reaction product and its stability was investigated. The yellow coloured product got stabilized immediately at 25 ± 1˚C and remained stable for 2 h.

The effect of the volume of 0.02 M potassium iodide on the absorbance of yellow coloured product was investigated in the range 0.2-1.2 mL. It is evident from Fig. 4 that the maximum absorbance was obtained with 0.8 mL of 0.02 M potassium iodide. Above this volume up to 1.2 mL of 0.02 M potassium iodide, the absorbance remained unchanged. Therefore, 1.0 mL of 0.02 M potassium iodide was used in the determination process of cefixime.

The effect of the volume of 6.25×10^{-4} M potassium iodate on the absorbance of yellow coloured product was investigated in the range 0.1-1.3 mL. It is clear from Fig. 5 that the maximum absorbance was obtained with 0.7 mL of 6.25×10^{-4} M potassium iodate. Above this volume up to 1.3 mL of 6.25×10^{-4} M potassium iodate, the absorbance remained unchanged. Therefore, 1.0 mL of 6.25×10^{-4} M potassium iodate was selected as the optimum volume for the determination of cefixime.

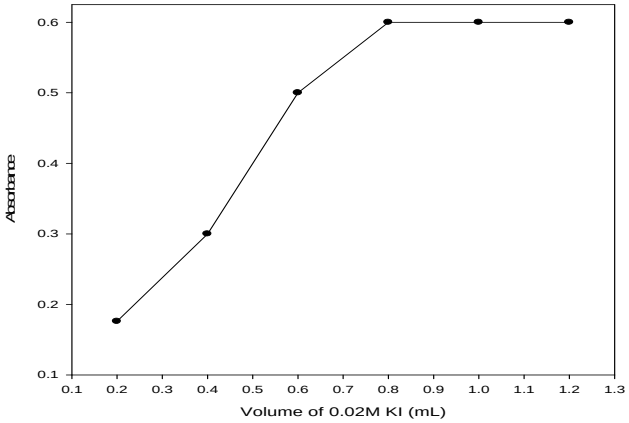


Fig. 4 Effect of the volume of 0.02M KI on the yellow coloured reaction product

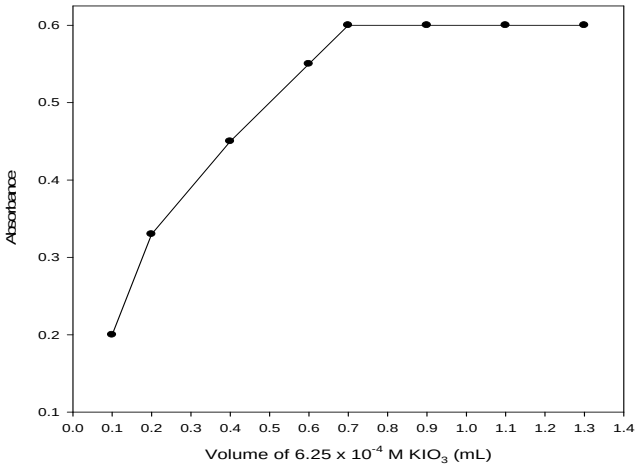


Fig. 5 Effect of the volume of 6.25×10^{-4}M KIO_3 on the yellow coloured reaction product

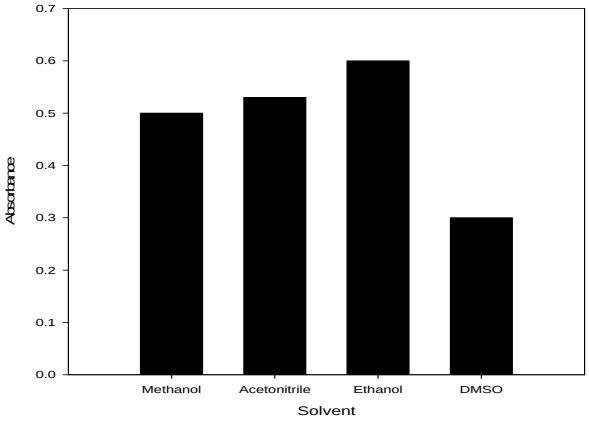


Fig. 6 Effect of solvent on the absorbance of yellow coloured product

The effect of solvents such as methanol, acetone, acetonitrile, ethanol, dimethylsulphoxide (DMSO) and distilled water were investigated on the absorbance of the

yellow coloured product. The absorbance of yellow coloured product using 20.0 μg mL^{-1} cefixime diluted with various solvents are recorded at 352 nm and shown in Fig. 6. The dilution of the reaction product with distilled water is decreasing the absorbance and finally the absorbance is declined and reaching to zero. It is clear from the figure that the highest absorbance was obtained in ethanol. Therefore, ethanol was selected as the best solvent for the determination of cefixime in pharmaceutical formulations.

B. Validation

Under the optimized experimental conditions, the calibration graph was constructed for assessing the limit of linearity by plotting the absorbance against initial concentration of cefixime at 6 independent concentration levels. Beer's law is obeyed in the concentration ranges of 4 - 24 μg mL^{-1} with apparent molar absorptivity of 1.52×10^4 L $mol^{-1}cm^{-1}$ and Sandell's sensitivity of 0.033 μg/cm^2 /0.001 absorbance unit. The linear regression equation is obtained by statistical treatment of the calibration data which is fitted with the straight line equation in the form of A = a + bC, where A is absorbance at 352 nm, C is concentration in μg mL^{-1}, b is slope and a is intercept of calibration line. The high value of correlation coefficient (0.9999) indicated excellent linearity (Table I). The experimental intercept of the calibration line was investigated for significance of deviation from the theoretical intercept of zero. t-value was calculated with this relation, $t = a / S_a$ [26] to justify the above fact and found to be 1.496, which is less than the tabulated t-value (2.447, ν=6) at 95% confidence level. Hence, it is clear from the above findings that the intercept in the calibration equation of the proposed method is not significantly different from zero. Thus, the proposed method is free from procedural error.

The precision of the proposed method was studied by considering 3 lower, middle and upper concentration levels from the Beer's law range. The calculated relative standard deviation values were found to be less than ± 2% indicating good repeatability and reproducibility of the proposed method. The results and their statistical analysis were summarized in Table II. It is clear from the table that percentage recovery and relative standard deviation (RSD) for intra day and inter day precisions were in the ranges of 99.82-100.22% and 0.12-1.65%, respectively. It can be seen from the table that percentage recovery and RSD values were precise and can be used to determine cefixime in pharmaceutical formulations.

The accuracy of the proposed method was investigated in pharmaceutical formulations by performing recovery experiments through standard addition technique. The absorbance for all standard addition solutions was recorded and the results of analyses are summarized in Table III. In both cases, the coefficient of correlation is 0.999, indicated excellent linearity of the regression lines for tablet and capsule samples. The concentration of cefixime in pharmaceutical formulations was calculated either by taking the ratio of the intercept and slope or by extrapolation (Fig. 7) The concentration of cefixime is subjected to some error (S_{xE}) and calculated by the following expression [27]:

$$S_{x_E} = \frac{S_{y/x}}{b}\left[\frac{1}{n} + \frac{\bar{y}^2}{b^2 \sum_i (x_i - \bar{x})^2}\right]^{1/2} \quad (4)$$

and found to be 0.05 and 0.03 μg mL^{-1}, respectively for tablet and capsule samples. The confidence limit for the concentration of cefixime in pharmaceutical formulations is calculated by $x_E \pm tS_{xE}$ at n - 2 degrees of freedom and found to be 12.07 ± 0.15 and 19.94 ± 0.09, μg mL^{-1}, respectively. The most attractive feature of the proposed method using standard addition method is its relative freedom from various excipients found in drug formulations.

The robustness of the proposed method was established by deliberately changing the volumes of 0.01M KI, 1.0 mL (± 0.2 mL) and 6.25×10^{-4} M KIO_3, 1.0 mL (± 0.3 mL) for the determination of cefixime. The tablet and capsule formulated sample solutions containing 10 μg mL^{-1} cefixime was analyzed five times repeatedly by the proposed method. Percentage recovery and RSD were found to be 100.23% and 0.38% and 100.17% and 0.28% for tablet and capsule, respectively indicating the robustness of the proposed method.

TABLE I
OPTICAL AND REGRESSION CHARACTERISTICS OF THE PROPOSED METHOD

Parameters	Analytical data
λ_{max} (nm)	352 nm
Beer's law limit (μg mL^{-1})	4- 24
Molar absorptivity (L $mol^{-1}cm^{-1}$)	1.52×10^4
Sandell's sensitivity	0.033 μg/cm^2/ 0.001 Abs. unit
Linear regression equation[1]	$A = 4.4 \times 10^{-3} + 2.983 \times 10^{-2}$ C
S_a	2.94×10^{-3}
$\pm tS_a$	8.16×10^{-3}
S_b	2.98×10^{-3}
$\pm tS_b$	5.23×10^{-4}
Correlation coefficient (r)	0.9999
Variance (S_o^2)	9.92×10^{-6}
Standard deviation of calibration line (S_o)	2.98×10^{-3}
LOD (μg ml^{-1})	0.317
LOQ (μg ml^{-1})	1.055

[1]With respect to A= a + bC, where C is the concentration in μg mL^{-1} and A is absorbance.
$\pm tS_a$ and $\pm tS_b$ are the confidence limits for intercept and slope, respectively.

TABLE II
PRECISION OF THE PROPOSED METHOD

	Intra day assay: Measured Concentration ± SD (μg mL^{-1}); RSD (%)[1]	Inter day assay: Measured Concentration ± SD (μg mL^{-1}); RSD (%)[1]
4.0	3.995 ± 0.045; 1.13	4.01 ± 0.05; 1.33
12.0	12.01 ± 0.05; 0.46	12.03 ± 0.05; 0.42
20.0	19.99 ± 0.14; 0.72	20.05 ± 0.21; 1.06

[1]Mean for five independent analysis.

TABLE III
TEST OF ACCURACY IN SUPRAX TABLET AND CEFRAX CAPSULE BY STANDARD ADDITION TECHNIQUE

	Concentration (μg mL^{-1})			Linear regression parameters			Recovery (%)[2]
Sample	Standard	Nominal	Error (X_e)	Intercept	slope	r^1	
Suprax-12	0, 2, 4, 6, 8	12.07	0.05	0.3622	0.0299	0.9999	100.57
Cefrax-12	0, 2, 4, 6, 8	11.94	0.03	0.3594	0.0301	0.9999	99.50

[1]Coefficient of correlation
[2]Mean for five independent analyses

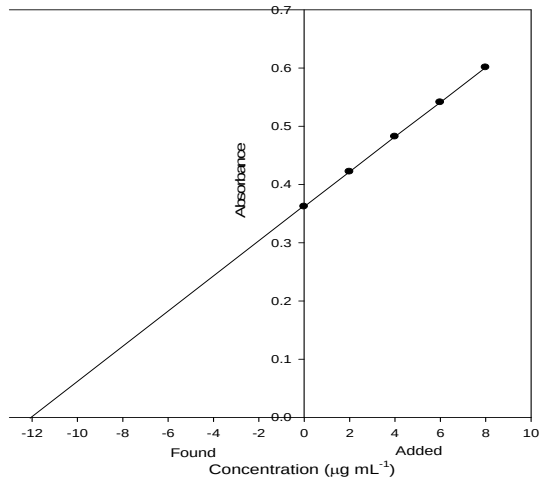


Fig. 7 Standard addition technique for the accuracy of the proposed method in tablet formulation (12.07 μg mL^{-1})

The effect of excipients added as additives on the determination of 20 μg mL^{-1} cefixime was studied. For this purpose, the varying concentrations of excipients such as glucose, fructose, lactose, starch, sucrose, fructose, povidone, methyl cellulose, crystalline cellulose and sodium benzoate with 20 μg mL^{-1} cefixime were taken and the absorbance was recorded to know the concentration of cefixime. Table IV shows the maximum tolerance value of the studied excipients. The maximum tolerance value was taken, when the absorbance value did not exceed ±2% on addition of excipients.

The applicability of the proposed method for the determination of cefixime in pharmaceutical formulation samples has been studied. Results of the proposed method were statistically compared with those of reference method using point and interval hypothesis tests. The paired t- and the F-values at 95% confidence level were calculated and found to be less than the tabulated t- (2.036 at υ = 8) and the F-values (6.39 at υ = 4,4) at 95% confidence level [28], thus confirming no significant difference between the performance of proposed method and the reference method (Table V). The bias calculated by interval hypothesis test in the form of lower limit (θ_L) and upper limit (θ_U) were in the range of 0.98 - 1.02. Thus, the proposed method is suitable for routine analysis of cefixime in dosage forms. The speed of analysis and less number of reagents utilized in the proposed method are the main advantages of the proposed method as compared to reference method.

TABLE IV
EFFECT OF FOREIGN SPECIES ON THE DETERMINATION OF CEFIXIME

Sample number	Foreign species	Maximum tolerance limit (μg mL^{-1})
1	Glucose	225.2
2	Fructose	56.30
3	Lactose	450.4
4	Starch	25.0
5	Sucrose	427.9
6	Sodium benzoate	27.02
7	Methyl cellulose	50.00
8	Povidone	50.00
9	Crystalline cellulose	25.00

The performance and utility of proposed method are compared with other methods for determining cefixime in pharmaceutical formulations (Table VI). It can be seen that the sensitivity of the proposed method is higher than most of the compared methods. Only methods with sample preconcentration [12], [21] yielded higher sensitivity but the instrumental set-up is much more complex and expensive.

IV. CONCLUSIONS

The proposed method is a simple, accurate and precise direct spectrophotometric method for the determination of cefixime in tablet and capsule formulations. The method has advantage of using very a common and frequently available solvent i.e. ethanol with the use of less expensive reagents such as KI and KIO_3 at room temperature. The proposed method has avoided the use of heating the reaction mixture and buffer solution. The proposed method was successfully utilized in determining the active drug in tablets and capsules and can be used as an alternate method for routine quality control analysis of cefixime in biological samples too.

TABLE V
SIGNIFICANCE OF TESTING: POINT AND INTERVAL HYPOTHESIS TESTS FOR THE DETERMINATION OF CEFIXIME IN PHARMACEUTICAL FORMULATIONS AT 95% CONFIDENCE LEVEL

Formulations	Proposed method		Reference method		t- & F values[2]	θ_L[3]	θ_U[3]
	Recovery (%)	RSD (%)[1]	Recovery (%)	RSD (%)[1]			
Suprax tablet 200 mg	100.23	0.38	99.94	0.397	t = 0.119, F= 1.085	0.996	1.01
Cefrax capsule 200 mg	100.17	0.277	99.89	0.305	t = 0.149, F= 1.234	0.997	1.01

[1]Mean for 5 independent analyses.
[2]Theoretical t (ν = 8) and F-values (ν = 4, 4) at 95% confidence level are 2.306 and 6.39, respectively.
[3]A bias, based on recovery experiments, of ± 2% is acceptable.

TABLE VI
COMPARISON OF THE PROPOSED SPECTROPHOTOMETRIC METHODS WITH EXISTING RELATED TECHNIQUES FOR THE ASSAY OF CEFIXIME IN PHARMACEUTICAL FORMULATIONS

Reagents	$\lambda_{max, nm}$	Molar absorptivity, L mol^{-1} cm^{-1}	Linear range, µg mL^{-1}	RSD, %	References
Capillary electrophoresis: sodium tetraborate buffer	UV detector 214	-	0.5-5	0.3-1.9	[12]
Spectrophotometry: $PdCl_2$	352	1.015×10^4	2.5-35	0.12-1.65	[13]
Chloranilic acid	520	2.8×10^2	100-1200	0.87-1.3	[14]
7,7,8,8-tetracyanoquinodimethane	842	2.3×10^3	10-240	0.02-0.12	[15]
I_2	360	1.32×10^4	0.25-17.5	0.06-1.19	
$KMnO_4$ in alkaline medium.	598	-	3-15	0.28-0.998	[20]
Fluorimetry: 2-cyanoacetamide	λ_{ex} = 230 nm λ_{em} = 280 nm	-	0.02-4	0.3-1.27%	[21]
HPLC: H_2O, acetonitrile and acetic acid (72: 25: 3) (v/v/v)	UV detector 273	-	1-500	0.669	[15]
Spectrophotometry: KI and KIO_3	352	1.52×10^4	4-24	0.42-1.33	This work

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