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ORIGINAL PAPER

Spectrofluorimetric Method for Determination and Validation of Cefixime in Pharmaceutical Preparations Through Derivatization with 2-Cyanoacetamide

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Abstract A simple, sensitive and accurate method has been developed for spectrofluorimetric determination of cefixime in pure form and pharmaceutical preparations. The method is based on the reaction of cefixime with 2-cyanoacetamide in the presence of 21% ammonia at 100 °C. The fluorescent reaction product showed maximum fluorescence intensity at λ 378 nm after excitation at λ 330 nm. The factors affecting the derivatization reaction were carefully studied and optimized. The fluorescence intensity versus concentration plot was rectilinear over the range of 0.02 to 4 $\mu g mL^{-1}$ with correlation coefficient of 0.99036. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 2.95 $ng mL^{-1}$ and 9.84 $ng mL^{-1}$, respectively. The proposed method was validated statistically and through recovery studies. The method was successfully applied for the determination of cefixime in pure and dosage form with percent recoveries from 98.117% to 100.38%. The results obtained from the proposed method have been compared with the official HPLC method and good agreement was found between them.

Keywords Cefixime · 2-Cyaonacetamide · Spectrofluorimetry · Derivatization

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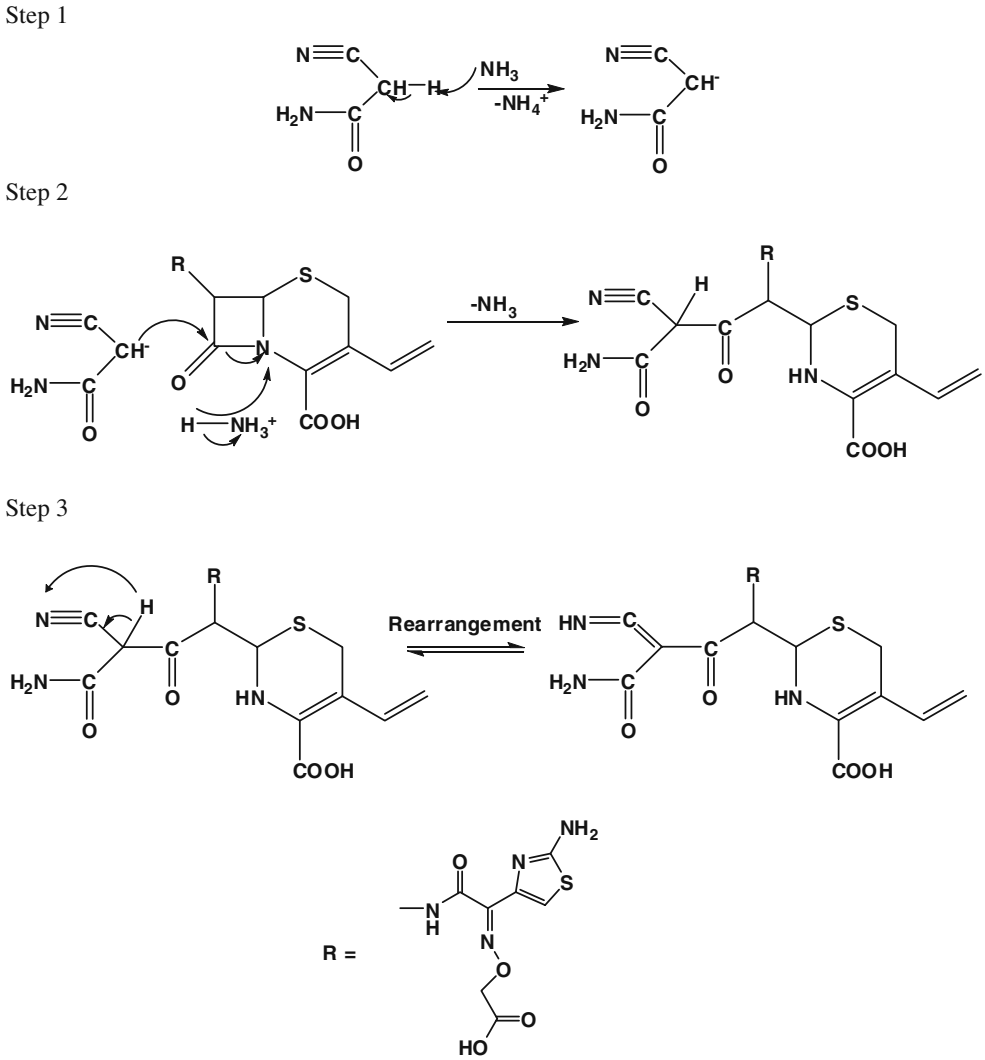
Introduction

Cefixime,((6R,7R)-7-[(Z)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino) acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo-[4,2,0]-oct-2-ene-2-carboxylic acid), is a semisynthetic third generation oral cephalosporin antibiotic prescribed for the treatment of susceptible infections such as gonorrhea, otitis media, pharyngitis, lower respiratory tract infections such as bronchitis and urinary tract infections [1–4].

Relatively, limited number of methods has been published for the determination of cefixime. It has been determined by spectrophotometric [5–9], high performance liquid chromatography (HPLC) [10] and high performance thin layer chromatography (HPTLC) [11]. An LC-MS-MS method has been reported for the determination of cefixime in human plasma using cefetamet as internal standard [1]. Differential pulse voltammetry has also been employed for trace determination of cefixime in pharmaceutical formulation and urine samples [12].

Few methods are available in the literature for fluorimetric determination of cefixime [6, 13, 14]. The first method is based on oxidation of cefixime in the presence of Ce (IV) and indirect determination of cefixime through measurement of fluorescence active Ce (III) ion. The other two methods are also indirect methods based on quenching of fluorescent compound when reacted with cefixime. These methods are either suffers from interferences from other compounds, require expensive reagents or are suffer from narrow range of calibration curve.

Scheme 1 Proposed reaction mechanism for spectrofluorimetric determination of cefixime with 2-cyanoacetamide



2-Cyanoacetamide is a fluorogenic reagent used in post column derivatization in HPLC determination of catecholamines [15] and carbohydrates [16]. It has also been employed for fluorimetric determination of pharmaceutical compounds like prenalterol-HCl [17], oxamniquine [18], ascorbic acid [19], aminoglycosides [20], 3, 4-dihydroxyphenylalanine [21] and cephalexin [22].

The proposed method in the present work is based on direct determination of fluorescent compound formed by

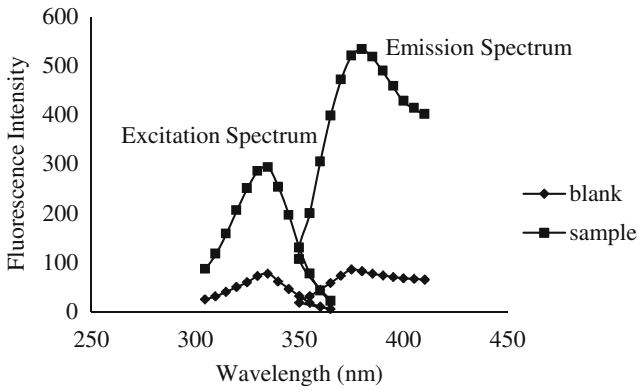


Fig. 1 Fluorescence spectra of the reaction product of cefixime with 2-cyanoacetamide

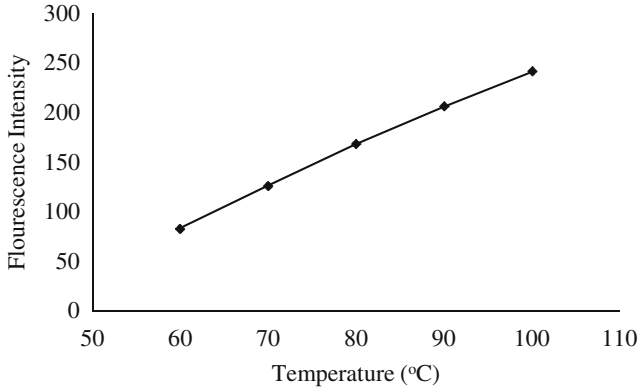


Fig. 2 Effect of temperature on the fluorescence intensity of the reaction product of cefixime with 2-cyanoacetamide

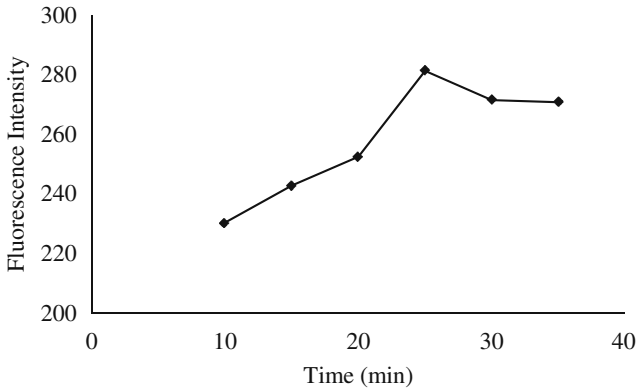


Fig. 3 Effect of heating time on the reaction product of cefixime with 2-cyanoacetamide

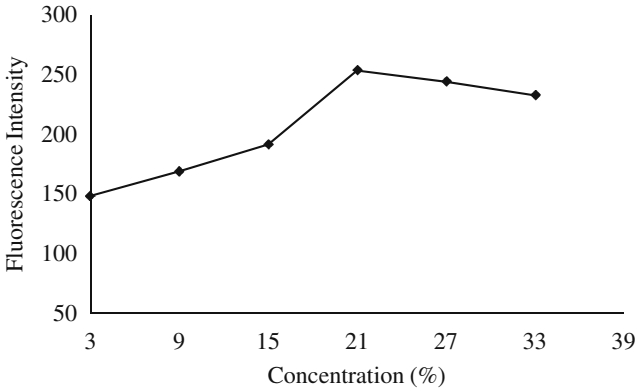


Fig. 5 Effect of concentration of ammonia on the fluorescent product formation of cefixime with 2-cyanoacetamide

reaction of cefixime with 2-cyanoacetamide in the presence of 21% ammonia.

Experimental

Instruments

RF-5301 PC Spectrofluorophotometer Shimadzu Japan, equipped with 150-watt Xenon discharge lamp, excitation, emission grating monochoromators and 1 × 1 cm quartz cell, was used for measurement of fluorescence intensities. The instrument was operated with excitation and emission slit width set at 5 nm. An electrical thermostatic water bath (YuJia china) with temperature range of 37–100 °C was used for heating purpose.

Materials and Reagents

All reagents used were of analytical reagent grade purity or of high grade purity. 2-Cyanoacetamide (Across organics, New Jersey, USA), ammonia (BDH, Laboratory suppliers Poole, England, 35%), ethanol (Merck, Darmstadt, Germany) was used in this work. Standard reference Cefixime was provided by Cirin Pharmaceutical (Pvt) Ltd., Hattar, Pakistan. Commercial formulations of cefixime (magnet caps 400 mg, manufactured by S J & G Fazal Ellahei (Pvt) Ltd, under license from Continental Pharmaceutical Karachi Pakistan, valdixime caps 400 mg and valdixime suspension 100 mg 5 mL^{-1} manufactured by WELMARK Pharmaceutical industrial Estate Hattar Pakistan for Valor Pharmaceuticals Industrial triangle kahuta road Islamabad), were purchased from local market. 2-Cyanoacetamide (2%) solution was prepared by dissolving 2 g of the reagent in distilled water and diluting up to 100 mL. Ammonia solution (21%) was prepared by diluting 63.7 mL of 35% ammonia to 100 mL with distilled.

Preparation of Standard Solution

Standard Cefixime stock solution (100 $\mu g mL^{-1}$) was prepared by dissolving 0.01 g of authentic standard Cefixime in 10 ml of distilled ethanol with vigorous shaking and diluted up to 100 mL with distilled water. Working standards were prepared daily by diluting appropriate quantity of the stock solution.

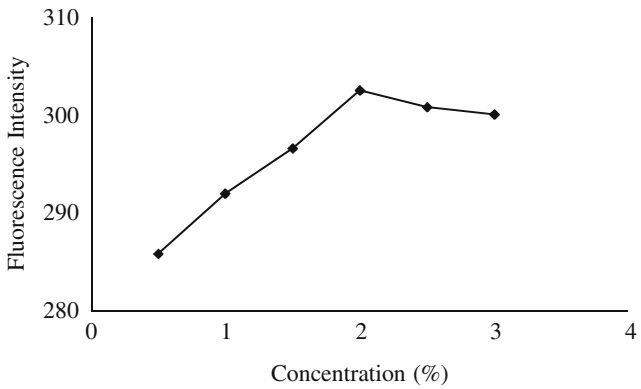


Fig. 4 Effect of concentration of 2-Cyanoacetamide on the fluorescence intensity of reaction product of cefixime

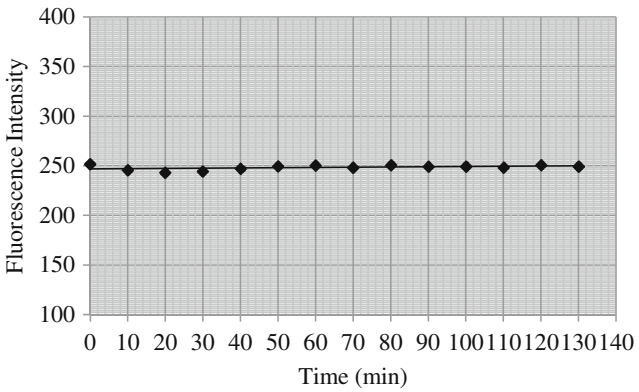


Fig. 6 Effect of time on stability of fluorescent reaction product

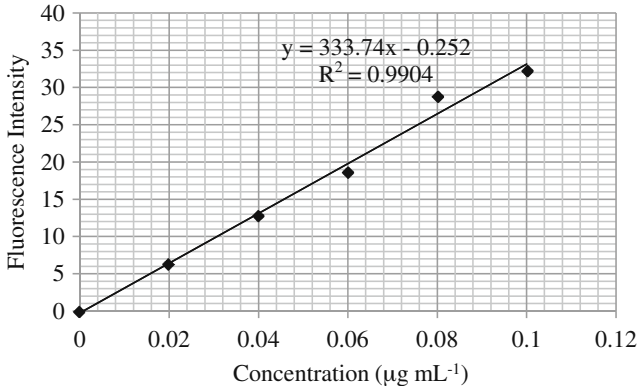


Fig. 7 Calibration curve of the fluorescent product of cefixime with 2-Cyanoacetamide

Sample Solution (100 $\mu g mL^{-1}$)

Contents of the five capsules were mixed, weighed and average mass of the powder in one capsule was calculated. The sample of the drug powder claimed to contain 10 mg were dissolved in 10 mL distilled ethanol, filtered, transferred to 100 mL volumetric flask and diluted up to the mark with distilled water. The required mass of the powder, from the suspension powder, was dissolved in 10 mL of ethanol, by vigorous shaking, filtered and then diluted to 100 mL with distilled water.

General Procedure

2.5 mL of 2-Cyanoacetamide (2%) and 2.5 mL of ammonia solution (21%) were transferred in Erlenmeyer flasks followed by the addition of standard solution of cefixime in the concentration range of 0.02–4 $\mu g mL^{-1}$. The solutions were heated on a boiling water bath for 25 min. The contents of the reaction flasks were transferred to 25 mL volumetric flask and diluted up to the mark with distilled water. The fluorescence Intensity of the resulting fluorescent product was measured at λ_{ex}330 nm and λ_{em}378 nm against a reagent blank prepared in the same way except the addition of the drug.

Table 1 Analytical parameter for spectrofluorimetric determination of cefixime

Parameter	Value
λ_{ex}(nm)	330
λ_{em} (nm)	378
Concentration range ($\mu g mL^{-1}$)	0.02–4.0
Limit of detection (ng mL^{-1})	2.95
Limit of quantification (ng mL^{-1})	9.84
Regression equation (y)	$Y = 333X - 0.252$
Slope (b)	333
Intercept (a)	−0.252
Correlation coefficient (r)	0.99036
RSD (%)	5.23

Table 2 Percent recovery of Cefixime (0.04 mgL^{-1}) in the presence of excipients

Excipients	Excipients added (mg/L)	Drug: Excipient	%Recovery ± RSD
Talc	0.02	1:1/2	101.71±3.85%
	0.04	1:1	98.29±3.01%
	0.08	1:2	95.73±4.09%
Magnesium stearate	0.02	1:1/2	101.8±2.55%
	0.04	1:1	104.4±2.49%
	0.08	1:2	100.26±4.5%
Sorbitol	0.02	1:1/2	97.5±5.12%
	0.04	1:1	95±2.63%
	0.08	1:2	97.5±2.56%
Starch	0.02	1:1/2	101.76±3.96%
	0.04	1:1	99.12±4.052%
	0.08	1:2	99.12±3.066%
Sucrose	0.02	1:1/2	99.02±3.089%
	0.04	1:1	100.88±3.98%
	0.08	1:2	101.66±2.99%

Each result is the average of separate triplicate analysis

Result and Discussion

The ß-lactum ring of cefixime reacts with 2-cyanoacetamide in the presence of 21% ammonia producing fluorescent product. In the first step of the proposed mechanism, ammonia removes a proton (H^+) from the methylene group of 2-cyanoaceamide producing carbene. In the second step, carbene attack on the ß-lactum ring and ammonia is regenerated. The third step involves rearrangement producing fluorescent product (Scheme 1).

Table 3 Evaluation of precision of the proposed method for cefixime determination in pure form

µg taken	µg found	%Recovery	Confidence limit
0.02	0.0192	98.1±1.245%	98.1±3.039%
0.04	0.04015	100.38±3.79%	100.38±9.45%
0.06	0.06015	100.25±1.65%	100.25±3.91%
X'		99.58	
±SD		1.266	
±RSD		1.27%	
t-test		0.57(4.303)	

Each result is the average of separate triplicate analysis

Table 4 Evaluation of precision of the proposed method for cefixime determination in dosage form

Pharmaceutical preparation	Amount taken (μgmL^{-1})	Amount found (μgmL^{-1})	Recovery ± RSD	Confidence limit
Magnett Caps 400 mg	0.02	0.0193	96.6±5.93%	96.67±14.25%
	0.04	0.038	95±2.5%	95±6.22%
	0.06	0.057	95±3.5%	95±8.28
Valdixime suspension 100 mg/5 mL	0.02	0.0183	91.66±8.33%	91.66±18.97%
	0.04	0.0367	91.67±3.15%	91.67±7.19%
	0.06	0.0557	92.78±2.74%	92.78±6.32%
Valdixime Caps 400 mg	0.02	0.02013	100.65±3.46%	100.65±8.68%
	0.04	0.03977	99.42±2.74%	99.42±6.79%
	0.06	0.0599	99.92±1.64%	99.92±4.05%

Each result is the average of separate triplicate analysis

The excitation and emission spectrum of the fluorescent product showed maximum fluorescence intensity at λ_{em}378 nm and λ_{ex}330 nm (Fig. 1).

Effect of Temperature and Heating Time

The effect of temperature on the derivatization reaction of cefixime with 2-cyanoacetamide was studied in the range of 60–100 °C. It was observed that the fluorescence intensity increased linearly with increase in temperature. Similarly the effect of heating time at 100 °C on the derivatization reaction was studied. Maximum fluorescence intensity was exhibited when the reaction mixture was heated for 25 min (Figs. 2 and 3). Therefore, further analyses were performed at 100 °C for 25 min heating.

Effect of Different Bases

The effect of various bases, like NaOH, KOH and NH_3, on the derivatization reaction was studied. The ammonia, being strong base but relatively weaker nucleophile, was found to produce highest yield of fluorophore. Moreover, the NaOH and KOH may hydrolyze the cyano group instead of removing proton from the methylene group and inhibit the formation of the required fluorophore.

Table 5 Evaluation of recovery test of cefixime in tablets by the standard addition method

Pharmaceutical preparation	Amount added (μgmL^{-1})	Amount found (μgmL^{-1})	RE%	%Recovery ± RSD
Magnett Caps 400 mg	0.02	0.0194	3.0	97±5.15%
	0.04	0.0404	−1.0	101±2.56%
	0.06	0.0597	0.44	99.56±1.94%
Valdixime suspension 100 mg/5 mL	0.02	0.0207	−3.5	103.5±4.83%
	0.04	0.0414	−3.42	103.42±3.67%
	0.06	0.0617	−2.83	102.83±3.24%
Valdixime Caps 400 mg	0.02	0.01992	0.4	99.6±4.28%
	0.04	0.04014	−0.35	100.35±2.1%
	0.06	0.0595	0.83	99.17±1.39%

Each result is the average of separate triplicate analysis

Effect of Reagent Concentration

The effect of concentration of 2-Cyanoacetamide on the derivatization reaction of cefixime to form a fluorescent product was studied in the range of 0.5–3% (Fig. 4). It was observed that fluorescence intensity increased rapidly with increase in reagent concentration up to 2% beyond which no significant change was seen. Volume of 2% 2-Cyanoacetamide was also investigated for the reaction product formation and maximum signal was observed with 2.5 mL of 2% 2-Cyanoacetamide solution.

The first step in the derivatization reaction involves the formation of carbene, which is strongly catalyzed by ammonia. The effect of concentration (3–33%) and volume (1–3.5 mL) of ammonia solution was investigated. Maximum fluorophore formation occurred when 2.5 mL of 21% of ammonia was used (Fig. 5).

Table 6 Determination of cefixime in commercial formulation and statistical comparison with reference method

S.NO	Name of commercial formulation	Labeled amount	Amount determined	
			Proposed method	Reference method [23]
1	Magnet Capsules	400 mg/Cap	384 mg	391.52 mg *F*-test = 2.01 (19) *t*-test = 3.36 (4.303)
2	Valdixime Capsules	400 mg/Cap	399.94 mg	404.16 mg *F*-test = 0.034 (19) *t*-test = 0.53 (4.303)
3	Valdixime Suspension	100 mg/5 mL	92.027 mg/5 mL	–

The stability of reaction product was studied for more than 2 h and no variation in the fluorescence intensity was observed, thereby, confirming the absence of any side reaction (Fig. 6).

Analytical Figures of Merit

The fluorescence intensity increased linearly with increase in concentration of cefixime. A linear relationship between concentration and fluorescence intensity was observed in the range of 0.02–4 $\mu g mL^{-1}$ under optimum experimental conditions of the proposed method (Fig. 7). The linear regression equation, slope, intercept, correlation coefficient and relative standard deviation of the response factors are given in Table 1. The limit of detection (LOD) was calculated with the concentration of cefixime leading to fluorescence intensity which is three times the blank standard deviation (3S/b). The limit of quantification (LOQ) was similarly calculated with concentration of cefixime leading to fluorescence intensity which is ten times the blank standard deviation (10S/b). The LOD and LOQ values were found to be 2.95 $ng mL^{-1}$ and 9.84 $ng mL^{-1}$ respectively.

Effect of Interference

To check the selectivity of the method the interferences effect from common excipients like talc, magnesium stearate, sorbitol, starch and sucrose were carefully studied. Solutions of synthetic mixtures containing cefixime and one of the excipients in ratio of 1:1/2, 1:1, 1:2 were analyzed by the proposed method. No interferences were observed in the determination of cefixime in the presence of the common excipients studied (Table 2). Average recoveries obtained were found in the range of 95.0–104.4%.

Precision and Accuracy

The precision of the proposed method was checked by determining cefixime in pure form and pharmaceutical preparations using three different concentrations in triplicate within the calibration curve range. The results are summarized in Table 3 for pure form and Table 4 for dosage form. The relative standard deviation (RSD) was found to be very satisfactory with excellent recoveries in the range of 98.117–100.38% (pure form), 95–100.65% (capsules 400 mg) and 91.66–92.78% (suspension 100 mg 5 mL^{-1}) indicating good reproducibility of the proposed method. The accuracy of the developed method was checked by standard addition method using two different brands of capsules (magnet 400 mg caps, valdixime 400 mg caps) and one brand of suspension (valdixime 100 mg 5 mL^{-1}). The recoveries obtained were in the range of 97–103.5%, which shows high accuracy of the developed method for cefixime in commercial pharmaceutical preparation (Table 5).

Application of the Proposed Method

The developed method was used for analysis of cefixime active ingredient in pharmaceutical preparations and the results were compared with the reference HPLC method [23] through statistical analysis with respect to precision using student's *t*-test and accuracy using variance ratio *F*-test. The results obtained from both methods shows no significant difference regarding the precision and accuracy of the proposed and reference HPLC method (Table 6).

Conclusion

Cefixime has been analyzed in pure and dosage forms using spectrofluorimetric method. The method is simple, sensitive, precise and accurate proved by statistical analysis. The developed method can be used as alternative to reference method (HPLC and other techniques) for determination of cefixime in pure and dosage forms in the industrial and research institutional laboratories.

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