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SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF CEFIXIME IN BULK AND PHARMACEUTICAL PREPARATIONS USING FERROIN COMPLEX

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A method was developed for the quantitative determination of cefixime in bulk and pharmaceutical preparations using ferroin complex. The method is based on the oxidation of the cefixime with Fe(III) in acidic medium. The formed Fe(II) reacts with 1,10-phenanthroline, and the ferroin complex is measured spectrophotometrically at 510 nm against reagent blank. Beer's law was obeyed in the concentration range 0.2–10 μg/ml with a good correlation of 0.993. The molar absorptivity was calculated and was found to be 1.375×10^5 L/mol × cm. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.030 and 0.101 μg/ml respectively. The proposed method has reproducibility with a relative standard deviation of 5.28% (n = 6). The developed method was validated statistically by performing a recoveries study and successfully applied for the determination of cefixime in bulk powder and pharmaceutical formulations without interferences from common excipients. Percent recoveries were found to range from 98.00 to 102.05% for the pure form and 97.83 to 102.50% for pharmaceutical preparations.

Keywords: *cefixime detection, spectrophotometric method, pharmaceutical formulations, 1,10-phenanthroline complex.*

Introduction. Cefixime (CEF) is a semisynthetic and generally classified as a third-generation cephalosporin antibiotic for oral administration. It is used for the treatment of susceptible infections, including gonorrhea, otitis media, pharyngitis, and lower respiratory-tract infections such as bronchitis and urinary-tract infections [1–3]. These are antibiotics with the β-lactum ring having a broad spectrum of antimicrobial and antibacterial properties.

It acts by inhibiting the enzyme transpeptidase, involved in the building of bacterial cell walls. It is used in lower respiratory tract infections. It is not hydrolyzed by the common plasmid or by chromosomal β-lactamases that inactivate the oral penicillins and cephalosporins [4]. Cefixime is effective against a wide range of sensitive gram-positive, gram-negative, and anaerobic bacterial pathogens including β-lactamase producing strains.

Forty to fifty percent of the oral dose of CEF is absorbed from the gastrointestinal tract. CEF is better absorbed from oral suspension than from tablet dosage forms. The plasma half-life is usually about 3–4 h and may be prolonged when there is renal impairment. About 65% of CEF in the circulation is bound to plasma proteins [1].

In the literature a numbers of methods have been reported for the determination of cefixime. It has been determined by spectroflourimetric [5–8], high-performance liquid chromatography (HPLC) [9], high-performance thin layer chromatography (HPTLC) [10], voltammetry [11], and capillary electrophoresis [12].

Few spectrophotometric methods are available in the literature for the determination of cefixime [6, 13–16]. The first spectrophotometric method is based on oxidation of cefixime by Ce(IV) and measuring the absorbance at 317 nm. The other three methods are also spectrophotometric. These methods suffer from interferences from other compounds, require expensive reagents, or suffer from a narrow range of calibration curves.

The aim of this study was to apply redox reactions in developing simple, accurate, sensitive, and reproducible assays for determination of cefixime in pure form and pharmaceutical formulations by employing iron(III) with 1,10-phenanthroline. The method is more sensitive than the existing methods and is free from such experimental variables as heating or extraction

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steps. The method relies on the use of simple, cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC.

Experimental. A UV-Vis spectrophotometer SP-1800 (721 G) equipped with 1-cm matched glass cell was used for absorbance measurement. A digital analytical balance (OHAUS Corporation USA) and digital water bath, labacon, model LWB-104, single hexode was also used.

All reagents used were of analytical or a high grade purity. $FeCl_3 \times 6H_2O$ and H_2SO_4, 95–97% extrapure (Riedel-deHaën, Germany), 1,10-phenanthroline (Scharlau Chemi S. A, Barcelona Spain), and methanol (Merck, Darmstadt, Germany) were used in this work. Standard reference cefixime was provided by Cirin Pharmaceutical (Pvt) Ltd., Hattar, Pakistan. Commercial formulations of cefixime (Cefimax 200 mg/tablet manufactured by Ferroza International Pharmaceuticals (Pvt) Ltd., Lahore, Pakistan, and Medigate 400 mg/tablet manufactured by Medicon Pharmaceuticals Industries (Pvt) Ltd., Industrial State Hayatabad, Peshawar, Pakistan) were purchased from the local market.

Standard stock solution of cefixime (100 μg/ml) was prepared by dissolving 0.01 g authentic standard of cefixime in 5.0 ml of methanol with vigorous shaking and diluted up to 100 ml with distilled water. Working standard solutions of the required concentration were prepared daily by diluting an appropriate volume of the stock solution with distilled water.

Sample solution (100 μg/ml). The contents of three tablets of each sample, i.e., Cefimax (200 mg/tablet) and Medigate (400 mg/tablet), were weighed separately to get the average weight of one tablet. Powders of a drug sample claimed to contain 0.01 g of cefixime were dissolved in 5.0 ml methanol with vigorous shaking, filtered, transferred to 100 ml volumetric flasks, and made to the mark with distilled water. 1.0 ml of Fe^{3+} solution (0.002 M) and standard stock solution of cefixime in the concentration range 0.2–10 μg/ml were transferred to Erlenmeyer flasks followed by the addition of 3.0 ml of 1,10-phenanthroline solution (0.006 M). The solutions were heated on a water bath at 90°C for 20 min. The contents of the reaction flasks were transferred to 25 ml volumetric flask and diluted to the mark with distilled water. The absorbance was measured at 510 nm on a spectrophotometer against a reagent blank prepared in the same way except for the addition of the drug.

Results and Discussion. Ferric salts play a prominent role in the spectrophotometric determination of many pharmaceutical drugs. Acting as an oxidant, a ferric salt is reduced to a ferrous salt, and this amount corresponds to the drug concentration. The amount of Fe(II) can be determined by using 1,10-phenanthroline. This property has been utilized to develop a spectrophotometric method for the determination of cefixime in bulk powder and pharmaceutical preparations. 1,10-phenanthroline is an organic base similar to chemical structure contains the iron(II) specific group [17]. The method is based on the formation of tris(1,10-phenanthroline) iron(II) chelate upon the reaction of cefixime with an Fe(III) and 1,10-phenanthroline. The reaction proceeds through the reduction of Fe(III) to Fe(II) and the subsequent formation of the intensive orange-red color complex. The absorption spectra of the colored species show characteristic λ_{max} of 510 nm (Fig. 1). The experimental conditions were established by varying each parameter individually and observing its effect on the absorbance of colored species. In order to establish favorable experimental conditions for the proposed method, cefixime was allowed to react with Fe(III) in the presence of 1,10-phenanthroline.

Different experimental parameters affecting the complexation reaction were carefully studied and optimized. These parameters were changed individually while keeping the others constant. These factors include heating temperature, heating time, concentration and volume of Fe^{3+}, and concentration and volume of 1,10-phenanthroline.

The effect of temperature and heating time on the complexation reaction were studied. The effect of temperature was investigated in the range 60 to 100°C, and heating time was studied from 10 to 35 min. It was observed that maximum color formation occurred on heating the reaction mixture at 90°C for 20 min (Fig. 2).

The effect of Fe^{3+} concentration on the complexation reaction was also investigated. The effect of Fe^{3+} concentration was studied in the range 0.001 to 0.005 M (Fig. 3). It was found that maximum color formation was observed with 0.002 M Fe^{3+} solution. The effect of a volume of 0.002 M Fe^{3+} solution was also studied, and maximum absorbance was noted with 1.0 ml of 0.002 M Fe^{3+} solution.

The effect of concentration and volume of 1,10-phenanthroline solution on complex formation was also studied. The effect of 1,10-phenanthroline concentration was studied in the range 0.002 to 0.01 M. It was found that absorbance of the colored product increased with increase in concentration of 1,10-phenanthroline solution up to 0.006 M, after which it remained constant (Fig. 4). The effect of volume of 1,10-phenanthroline solution was also investigated by adding different volumes of 0.006 M of 1,10-phenanthroline while keeping the concentration of other reagents constant. It was found that maximum colored formation occurred with 3.0 ml of 0.006 M of 1,10-phenanthroline.

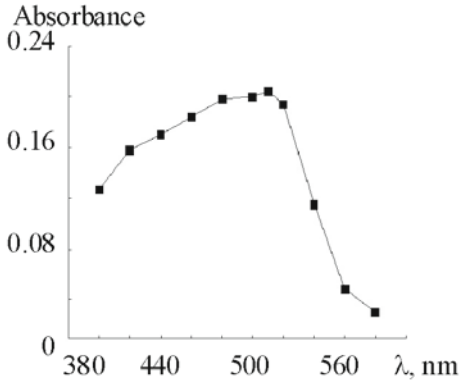


Fig. 1. Absorption spectra for the colored product conditions; 1.0 ml of Fe^{3+} (0.002 M), 10 μg/ml cefixime, 3.0 ml of 1,10-phenanthroline (0.006 M), heated at 90 °C for 20 min, diluted to 25 ml.

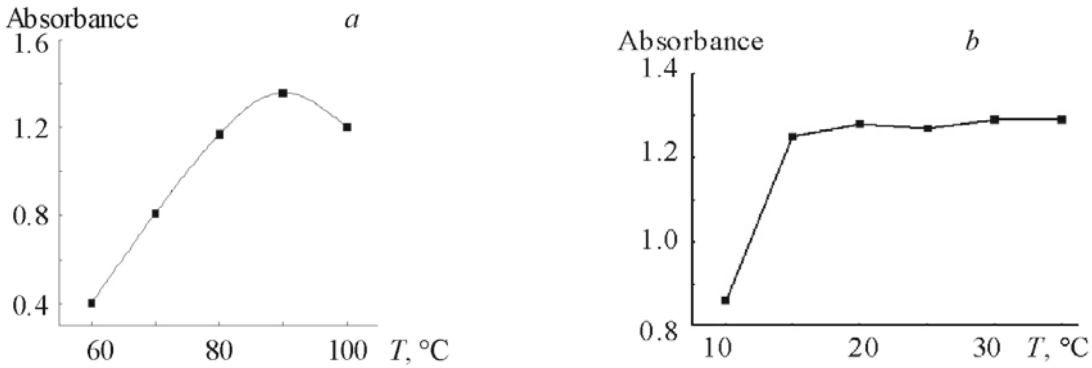


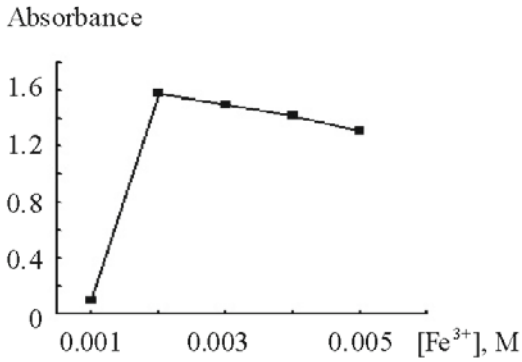
Fig. 2. Effect of temperature on absorbance conditions; 1.0 ml of Fe^{3+} (0.002 M), 10 μg/ml cefixime, 3.0 ml of 1,10-phenanthroline (0.006 M), heated at 60–90°C for 20 min (a), at 90°C for 10–35 min (b), diluted to 25 ml

The stability of the reaction product was studied by measuring the absorbance at regular intervals up to 2 h. No change in absorbance of the reaction product was observed. Thus the reaction product is stable and the result of analysis will not be affected even if the absorbance of the reaction product is measured after 2 h of dilution (Fig. 5).

The effect of interferences from commonly used excipients such as glucose, starch, and sucrose added in pharmaceutical preparations of cefixime was investigated (Fig. 6). Under optimum experimental condition, to a known amount of drug (cefixime 0.2 μg/ml), excipients in different concentration in the ratio of 1:1, 1:2, 1:6, 1:8, 1:10, and 1:50 were added and investigated by the proposed method. None of these common excipients was found to cause interference.

Under optimum experimental conditions of the proposed method, a linear correlation was found between absorbance and cefixime concentration. Beer's law was obeyed in the concentration range 0.2–10 μg/ml with a good correlation 0.993 (Fig. 7). The LOD was calculated as the minimum concentration at which cefixime can be detected reliably (3.3SD) using six repetitive determinations and was found to be 0.0305 μg/ml. The LOQ was similarly calculated as the lowest concentration of cefixime that can be measured with satisfactory precision and accuracy (10SD) and was found to be 0.1018 μg/ml. The optical characteristic such as the linear regression equation, intercept, slope, correlation coefficient, and relative standard deviation are given in Table 1. The molar absorptivity was calculated and was found to be 1.375×10^5 l/mol × cm.

Reliability of the Method. The precision of the developed method was studied by determining cefixime in the pure form and pharmaceuticals preparations using three different concentrations within the calibration curve range, in triplicate. Results are listed in Table 2 for the standard and in Table 3 for pharmaceutical preparations. The percent recoveries obtained ranged from 98.00 to 102.05% for the standard and 99.70 to 102.50% for pharmaceutical preparations with narrow relative standard deviations, indicating that the proposed method has good reproducibility. The accuracy of the present method was



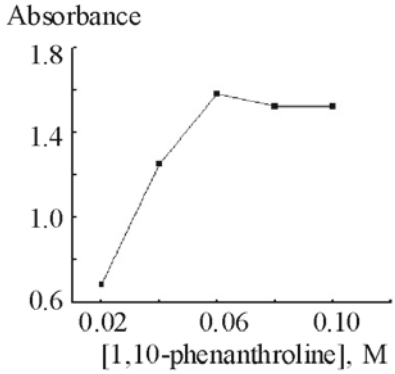


Fig. 3. Effect of Fe^{3+} concentration on absorbance conditions; 1.0 ml of Fe^{3+} 0.001–0.005 M, 10 μg/ml cefixime, 3.0 ml of 1,10-phenanthroline (0.006 M), heated at 90°C for 20 min, diluted to 25 ml.

Fig. 4. Effect of concentration of 1,10-phenanthroline on absorbance conditions; 1.0 ml of Fe^{3+} 0.002 M, 10 μg/ml cefixime, 3.0 ml of 0.002–0.01 M 1,10-phenanthroline, heated at 90°C for 20 min, diluted to 25 ml.

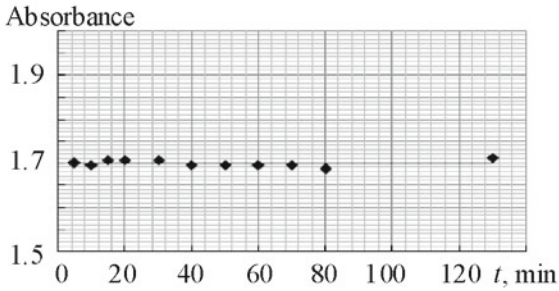


Fig. 5. Effect of time on stability of reaction product after dilution conditions; 1.0 ml of Fe^{3+} 0.002 M, 10 μg/ml cefixime, 3.0 ml of 0.06 M 1,10-phenanthroline, heated at 90°C for 20 min, diluted to 25 ml.

TABLE 1. Analytical Parameters for the Spectrophotometric Determination of Cefixime

Parameter	Value	Regression equation (y)	$y = 0.395x + -0.0204$
λ_{max}, nm	510	Slope, b	0.395
Linear range, μg/ml	0.2–10	Intercept, a	–0.020
Molar absorptivity, l/mol × cm	1.375×10^5	Correlation coefficient, r^2	0.993
Limit of detection 3 × SD, μg/ml	0.030	Standard deviation, μg/ml	0.01
Limit of quantification 10 × SD, μg/ml	0.101	Relative standard deviation, %	5.28

investigated by the standard addition method using two different brands of tablets Cefimax and Medigate (containing 200 and 400 mg of cefixime respectively). Certain amounts of standard cefixime solution were added to tablet solutions and analyzed by the proposed method. Recoveries were calculated by comparing the results obtained before and after adding standard cefixime solution, and percent recoveries ranged from 97.83 to 101.21% (Table 4).

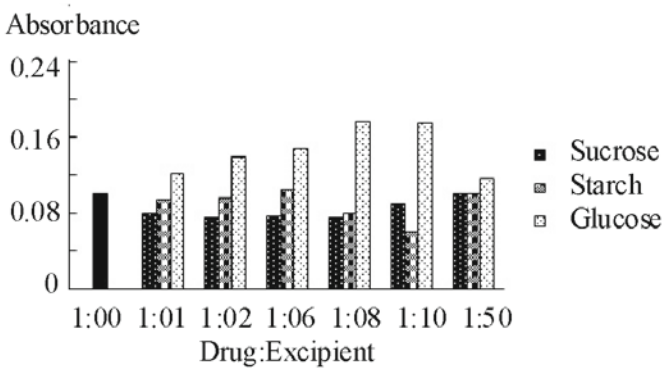


Fig. 6. Effect of common excipients on determination of cefixime by the proposed method.

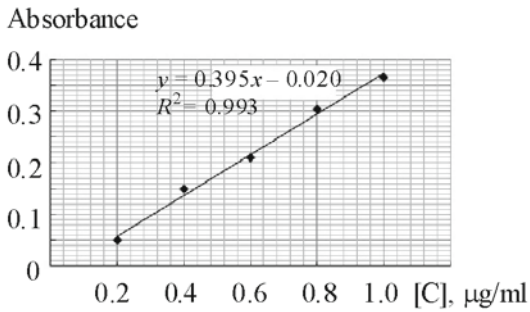


Fig. 7. Effect of concentration of cefixime on absorbance.

TABLE 2. Accuracy and Precision of the Present Method Using Solutions of Pure Cefixime

Amount taken, µg/ml	Amount found, µg/ml	% Recovery ± RSD
0.2	0.196	98.02 ± 4.64
0.4	0.392	98.00 ± 0.73
0.6	0.612	102.05 ± 0.96
Mean ± SD = 99.35 ± 2.33, *t*-test = –0.483 (4.303)		

Results are the averages of three separate analyses; RSD = Relative standard deviation.

TABLE 3 Evaluation of Accuracy and Precision of the Present Method for Determination of Cefixime in Pharmaceutical Preparations

Pharmaceutical preparation	Amount taken, µg/ml	Amount found, µg/ml	% Recovery ± RSD
Cefimax, 200 mg/tablet	0.2	0.2022	101.14 ± 0.54
	0.4	0.4101	102.50 ± 1.20
	0.6	0.603	100.50 ± 1.72
Medigate, 400 mg/tablet	0.2	0.2031	101.56 ± 3.93
	0.4	0.4071	101.75 ± 0.43
	0.6	0.5982	99.70 ± 1.26

Results are the averages of three separate analyses; RSD = Relative standard deviation.

TABLE 4 Evaluation of Percent Recovery of Cefixime in Commercial Formulations (tablets) Using the Standard Addition Method

Pharmaceutical preparations	Amount added, μg/ml	Amount found, μg/ml	% Recovery ± RSD
Cefimax, 200 mg/tablet	0.2	0.199	99.50 ± 2.29
	0.4	0.399	99.92 ± 1.55
	0.6	0.587	97.83 ± 1.79
Medigate, 400 mg/tablet	0.2	0.1998	99.99 ± 2.29
	0.4	0.4039	100.97 ± 0.26
	0.6	0.6073	101.21 ± 1.44

Results are the averages of three separate analyses; RSD = Relative standard deviation.

TABLE 5. Determination of Cefixime in Pharmaceutical Preparations (tablets)

Brand name	Active ingredient, mg/tablet		
	Label value	Found ± SD	% Recovery ± RSD
Cefimax, 200 mg/tablet	200	202.55 ± 0.462	101.27 ± 1.23
Medigate, 400 mg/tablet	400	402.80 ± 0.231	100.19 ± 2.21

Results are the averages of three separate analyses; RSD = Relative standard deviation.

The devised method was successfully applied to the determination of cefixime in the two pharmaceutical preparations. The results obtained were in close agreement with label quantities (Table 5), which shows that the proposed method can be used to determine cefixime quantities in pharmaceutical formulations.

Conclusions. A fast, simple, sensitive, selective, and inexpensive spectrophotometric method was developed for quantification of cefixime that are widely available in the market and manufactured by the indigenous units. The developed method was found to have a wider linear range and lower limits of detection and quantification than other reported methods. Furthermore, the developed method was successfully used to quantify cefixime levels in commercial tablet formulations with good recovery and excellent reproducibility. Finally, excipients commonly found in pharmaceutical preparations did not interfere with the analysis.

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