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Development and validation of spectrophotometric method for the estimation of cefepime in bulk and dosage form

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ABSTRACT

A simple, accurate, precise and sensitive spectrophotometric method for estimation of cefepime in bulk and dosage form was developed. The method is based on the formation of red coloured complex with 1, 10 phenonthroline in presence of Ferric nitrate in aqueous medium. The coloured species exhibiting maximum absorption at 515 nm. Beers law was obeyed in the concentration range of 0.28 – 7.26 µg /ml with correlation coefficient 0.998. The linear regression equation obtained by least square regression method were y = 0.094 x + 0.152, where y is the absorbance and x is the concentration of the pure drug solution. The method was validated for several parameters like accuracy and precision. The values of relative standard deviation and % recovery were found to be satisfactory, indicating that proposed method is precise and accurate and hence can be applied for the routine analysis and quality control of cefepime in bulk as well as dosage form.

Key words: Cefepime, 1, 10 Phenonthroline, Spectrophotometry.

INTRODUCTION

Cefepime is chemically 1 – [[(6R, 7R) -7 – [2 – (2 – amino – 4 – thiazyl) glyoxylamido] – 2 – carboxy – 8 – oxo – 5 thia – 1 – azabicyclo [4.2.0] oct – 2 – en – 3 – yl] methyl] – 1 – methyl pyrrolidinium chloride, 7^2 – (Z) – (0 – methyloxime) monohydrochloride , monohydrate. It is official in USP 29 / NF 24 [1]. It is a white to pale yellow powder highly soluble in water with molecular formula $C_{19}H_{25}Cl\ N_6O_5S_2HClH_2O$ **(Figure 1)** and a molecular weight of 571.5.

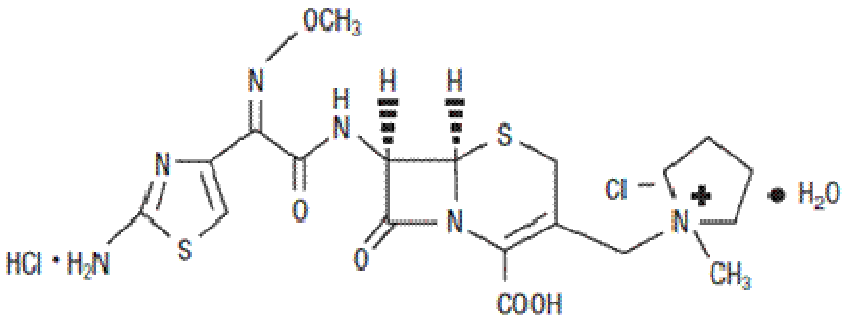

FIGURE 1 : Structure of cefepime

It is useful in lower respiratory tract infection caused by S. Aureus, S. Pneumonia, H. Influenzae, Urinary tract infections caused by E. Coli, skin and skin structure infections caused by S. epidermidis and intra abdominal infections.

Literature survey reveals that some analytical methods has been reported for the determination of cefepime in pharmaceutical formulation and in biological fluids which includes HPLC [2], [3] [4], [5], [6], voltammetry [7], [8], capillary zone electrophoresis [9] and few spectrophotometry [10], [11], [12]. The proposed method describes simple, sensitive, accurate and precise spectrophotometric method in the visible range of spectra for the estimation of cefepime in bulk and dosage form.

MATERIALS AND METHODS

Instrumentation

A Equiptronics visible spectrophotometer model EQ 822 with 1 cm matched quartz cells were used for spectral study.

Chemical and reagents

All chemicals used were of analytical grade and reagents were prepared using double glass - distilled water. The injectable formulations such as Cepime injection (Alembic) and Novapime injection (Lupin) were procured from local market.

Preparation of stock solution - Standard stock solution of Cefepime was prepared by dissolving accurately weighed 100 mg of pure Cefepime in water in a 100 ml volumetric flask. This stock solution was further diluted to get working standard of 40 µg/ml by transferring 4 ml of standard stock solution to 100 ml volumetric flask and volume was made up to the mark by water.

Preparation of sample solution of drug - Sample solutions of the Cepime injection and Novapime injection were prepared by dissolving 100 mg of powdered injection sample in 100 ml water by using separate 100 ml volumetric flask and filter using Whatman filter paper. These solutions were further diluted to get concentration equivalent to 40 µg/ml.

Preparation of reagents

1, 10 phenonthroline – 0.01 M solution of 1, 10 phenonthroline was prepared by dissolving accurately weighed 198.23 mg of powder in 100 ml water in volumetric flask.

Ferric Nitrate - 0.0033 M Ferric Nitrate solution was prepared by dissolving accurately weighed 133.32 mg of Ferric nitrate in volumetric flask in 100 ml water.

Phosphoric acid – 0.2 M solution of Phosphoric acid was prepared by dissolving 1.33 ml of phosphoric acid in 100 ml water.

Determination of maximum wavelength (λmax)

From the working standard solution of Cefepime, pipette out 5 ml into 25 ml volumetric flask. 2.5 ml of ferric nitrate solution and 4.0 ml of 1, 10 phenonthroline solution were successively added to this flask. The flask was heated on water bath for 15 minutes and then cooled to room temperature and 3 ml of phosphoric acid was added and the rest volume was made by distilled water. A blood red coloured solution obtained was scanned in visible range of spectra against water as blank. The wavelength corresponding to maximum absorbance was found at 515 nm **(Figure 2).**

Preparation of calibration curve

For the preparation of standard calibration curve, aliquots of standard solution of cefepime ranging from 0.2 ml to 4.6 ml were transferred to a series of 25 ml volumetric flask. The solution in these flasks was treated in similar way as described under the head determination of maximum wavelength. The absorbance of each solution was measured at 515 nm against water as blank. Calibration curve of the drug was then plotted by taking absorbance obtained on y-axis and concentration of drug on x-axis **(Figure 3)**. The curve showed linearity in the range 0.28- 7.26 µg/ml with correlation coefficient 0.998.

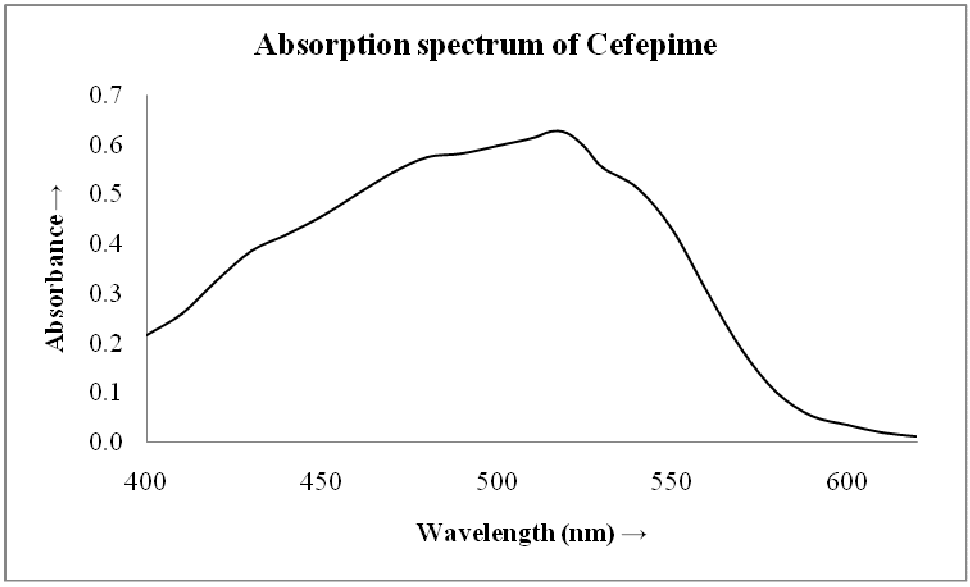

FIGURE 2 : Spectrum of cefepime showing maximum wavelength of absorption

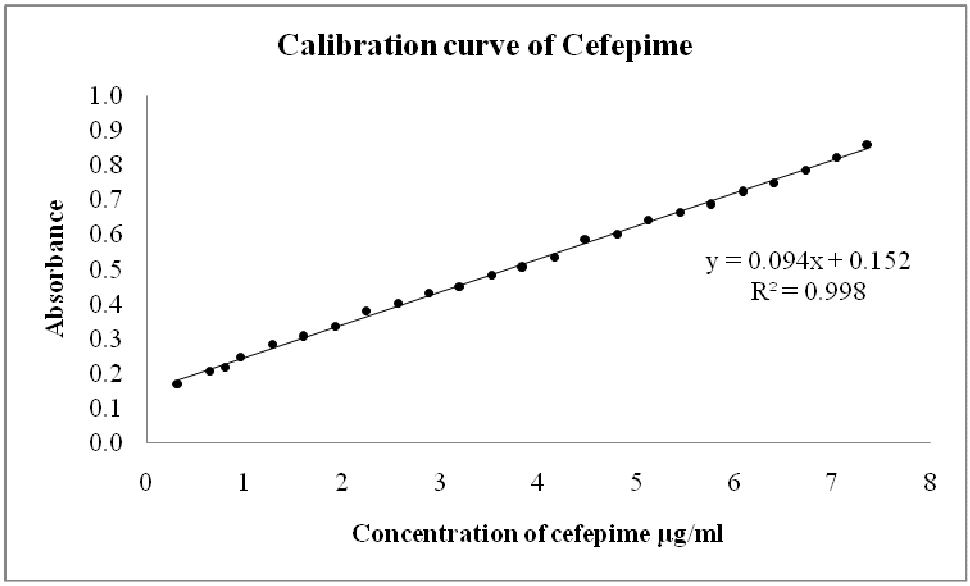

FIGURE 3 : Calibration curve of Cefepime

VALIDATION

The method was validated for several parameters like linearity, accuracy and precision.

Linearity

The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of standard solution of the drug were analysed. The drug showed linearity in the range of 0.28 – 7.26 µg/ml with correction coefficient 0.998. The linearity data are shown in **Table 1.**

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amount (10%, 20%, 40%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results are shown in **(Table 2)**

TABLE 1 : Linearity table of Cefepime

Concentration (µg/ml)	Absorbance
0.32	0.172
0.64	0.208
0.80	0.220
0.96	0.248
1.28	0.284
1.60	0.305
1.92	0.336
2.24	0.381
2.56	0.402
2.88	0.432
3.20	0.452
3.52	0.485
3.84	0.504
4.16	0.535
4.48	0.586
4.80	0.602
5.12	0.644
5.44	0.666
5.76	0.685
6.08	0.722
6.40	0.749
6.72	0.787
7.04	0.824
7.36	0.861

TABLE 2 : Accuracy reading of Cefepime

Labeled claim (mg)	Level of Addition (%)	Amount of pure drug added (mg)	% Recovery	Statistical Analysis	
				MEAN	SD
250	10	25	100.7	100.27	0.404
250	10	25	99.9		
250	10	25	100.2		
250	20	50	99.8	99.9	0.361
250	20	50	100.3		
250	20	50	99.6		
250	40	100	101.1	100.37	0.751
250	40	100	100.4		
250	40	100	99.6		

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability was determined by preparing five replicates of same concentration of the sample and the absorbance were measured. Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day.

TABLE 3 : Precision results showing repeatability

Concentration (µg/ml)	Absorbance	Statistical Analysis
4	0.519	Mean = 0.518 SD = 0.000837 % RSD = 0.167
4	0.520	
4	0.521	
4	0.520	
4	0.519	

TABLE 4 : Intraday Precision

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Average % RSD
4	0.520	0.519	0.519	0.2109
4	0.519	0.520	0.518	
4	0.519	0.518	0.519	
4	0.518	0.520	0.516	
4	0.517	0.519	0.517	
% RSD	0.2198	0.1611	0.2518	

The same procedure was followed for three different days to determine inter day precision. The results were reported as % RSD. The precision results showed a good reproducibility **(Table 3)** with very low relative standard deviation value. The results of intraday and interday precision studies are shown in **(Table 4)** and **(Table 5)**.

TABLE 5 : Interday Precision

Concentration (µg/ml)	% RSD			Average % RSD
	DAY 1	DAY 2	DAY 3	
4	0.26	0.39	0.54	0.397

The results of various parameters of the developed method are shown in **Table 6.**

TABLE 6 : Summary of the spectral parameters of the method developed

Parameter	Result
Wavelength of maximum absorbance (nm)	515
Beer's law limit (µg/ml)	0.28 - 7.26
Sandell's Sensitivity (µg/cm^2 per 0.001 Absorbance unit)	0.004499
Molar extinction Coefficient (ε)	5.2558 x 10^4
Regression equation (1 Mol^{-1} cm^{-1})	0.94 x + 0.152
Slope (m)	0.094
Intercept (c)	0.152
Correlation coefficient (r)	0.998
Accuracy	99.6 to 101.1%
Precision	Intraday (0.2109) Inter day (0.397)

Estimation in dosage form

To analyse the concentration of drug in dosage form, powder sample taken from vial procured form local market equivalent to 100 mg of pure cefepime was accurately weighed and transferred to 100 ml volumetric flask and dissolved in water. Final volume was made by water. The solution was filtered using whatman filter paper no. 1 to remove any excipients. The solution was suitably diluted to get about 40 µg/ml concentration. The suitable aliquots were taken for estimation in linearity range and the same treatment was given as in determination of maximum wavelength. (515 nm). The results of analysis are shown in (**Table 7**).

TABLE 7 : Statistical analysis of quantitative determination of Cefepime in pharmaceutical formulation.

Formulation	Labeled claim	Amount found	% Recovery	% Error	SD*	RSD
Inj. Cepime	250 mg	249.821 mg	99.9285	-0.0715	± 0.1692	0.1693
Inj. Novapime	1. 0 g	0.99985 g	99.9845	- 0.0155	± 0.05555	0.0556

** mean of five determination*

RESULTS AND DISCUSSION

Cefepime reduces Fe (III) to Fe (II) at elevated temperature. The blood red coloured complex was formed due to reaction of Fe (II) with 1, 10 phenonthroline. It show maximum wavelength of absorbtion at λmax 515 nm. The optical and spectral data are as summarized in Table 6. The Beer's law limit was found to be satisfactory linear over the range of concentration 0.28 µg/ml to 7.26 µg/ml. The complex was intensly red coloured and have large value of molar extinction coefficient 5.2558 x 10^4 l mol^{-1} cm^{-1} so that method is sensitive and useful for determination of even very low concentration of cefepime.

Accuracy of the proposed method was determined by the recovery studies, and good % recovery (99 % to 101 %) of the drug obtained indicate that the method is accurate. The method was found to be precise as interday (0.397) and intraday (0.2109) was found to be low values. The results of the assay obtained were found to be in good agreement with the labeled claim, indicating the absence of interference of the excipients.

CONCLUSION

The developed method is simple, sensitive, accurate, precise and reproducible. The proposed method is specific without an interference of excipients and hence can be used for the routine analysis of cefepime in bulk as well as in pharmaceutical formulations.

Acknowledgements

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