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RESEARCH ARTICLE

Development and validation of UV Spectrometric Method for the Determination of Cefixime trihydrate in Bulk and Pharmaceutical Formulation

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ABSTRACT

The present study was undertaken to develop and validate a simple, accurate, precise, reproducible and cost effective UV-Visible spectrophotometric method for the estimation of cefixime trihydrate in bulk and pharmaceutical formulation. The solvent used throughout the experiment was the mixer of methanol and water. Absorption maximum (λ_{max}) of the drug was found to be 287 nm. The quantitative determination of the drug was carried out at 287 nm and Beer's law was obeyed in the range of 2-20µg/mL. The method was shown linear in the mentioned concentrations having line equation y = 0.025x + 0.078 with correlation coefficient of 0.999. The recovery values for cefixime trihydrate ranged from 99.57% - 100.86%. The relative standard deviation of six replicates of assay was less than 2%. The percent relative standard deviation (RSD%) of interday precision range was 0.059 – 0.546 % and intraday precision range was 0.102 – 0.299%. The limit of detection and limit of quantification was 0.053 µg/mL and 0.159 µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 0.258 - 0.365%. Hence, proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the bulk drug as well as dosage formulation.

Keywords: UV-Vis Spectrophotometer, Method Validation, Recovery studies.

1. INTRODUCTION:

Cefixime trihydrate is a third generation cephalosporin antibiotic. It acts by inhibiting the synthesis of cell wall of the bacteria. It is clinically used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections [1].

Literature survey reveals that HPLC [2-7], RP-HPLC [8-13], HPTLC [14-17], Voltametry [18-20], High Performance Capillary Electrophoresis methods [21] were reported for the estimation of cefixime trihydrate, although simultaneous UV-Visible Spectrophotometric [22-28] estimation had been reported in bulk and in pharmaceutical formulation. But single estimation of this drug with mixture of methanol and distilled water as solvent has not been reported in bulk and in pharmaceutical formulation.

Thus, the aim of the present work was to develop a simple, reproducible and economic analytical method to estimate cefixime trihydrate in routine analysis.

2. MATERIALS AND METHOD

2.1 Drug: Pure Standard of cefixime trihydrate was received as a kind gift from Renata Pharmaceuticals. Capsules of Cef -3 (cefixime trihydrate-200 mg) were purchased from the local market.

2.2 Reagents and Chemicals: Methanol (Merk, Germany) and distilled water were used as solvent. All other reagents were of analytical grade and were purchased from the local suppliers.

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2.3 Instruments: A Shimadzu UV-Visible spectrophotometer UV-1800 was used.

3. METHOD DEVELOPMENT

3.1 Solubility Test

Solubility test of cefixime trihydrate was performed by using various solvents. Water, methanol, ethanol, acetonitrile, 0.1N HCl, 0.1N NaOH were used as solvents. However, the drug is freely soluble in methanol. So, methanol was chosen and the further dilution was done by water.

3.2 Preparation of stock solution

The standard stock solution of 100 µg/mL of cefixime trihydrate was prepared by weighing 100 mg of the drug, taken in 100 mL volumetric flask and was dissolved in 50 mL methanol and then make up to the mark with distilled water. 10 mL of the stock solution was taken in 100 mL volumetric flask and was diluted with water up to 100 mL to produce a concentration of 100µg/mL which was used as standard stock solution. Further dilutions were made with distilled water to obtain concentrations ranging from 02-20 µg/mL.

3.3 Determination of λ_{max}

By appropriate dilution of standard solutions with distilled water, solutions containing 10 µg/mL of cefixime trihydrate were scanned in the range of 200-800 nm to determine the wavelength of maximum absorbance for the drugs. Cefixime trihydrate showed absorbance maxima at 287 nm (Fig. 1).

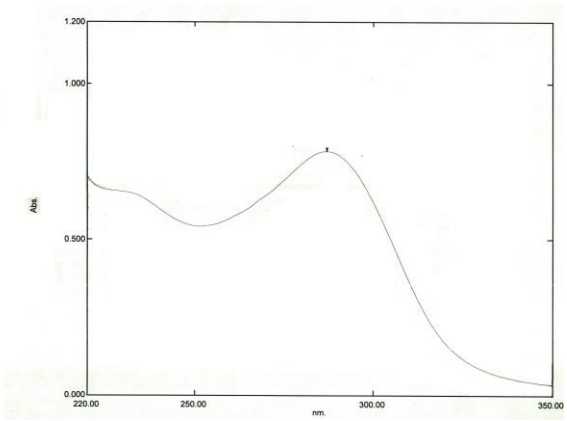

Figure 1. UV spectrum of cefixime trihydrate (λ_{max} determination)

4. METHOD VALIDATION

The proposed method was validated for different parameters like linearity, precision, accuracy, specificity, robustness, LOD, LOQ and assay.

4.1 Linearity

Standard stock solutions, 100µg/mL were further diluted with water to obtain 2µg/mL, 4µg/mL, 6µg/mL, 8µg/mL, 10µg/mL, 12µg/mL, 14µg/mL, 16µg/mL, 18µg/mL, 20µg/mL solutions. The absorbances of the spectra were measured at 287 nm (Fig.2). The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

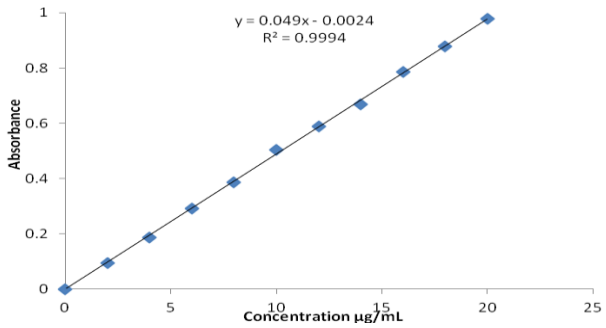

Figure 2. Calibration curve of cefixime trihydrate.

4.2 Specificity

Various aliquots were prepared from the stock solution (100µg/mL) ranging from 2-20 µg/mL**.** The solutions was scanned in UV-Visible spectrophotometer in the range 200-800 nm to determine the wavelength of maximum absorbance for the drugs. The result is shown in figure 3.

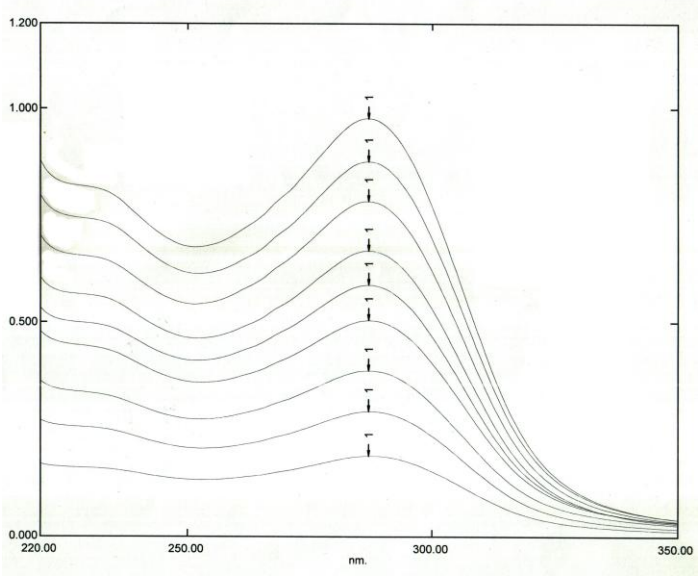

Figure 3. Specificity of cefixime trihydrate

4.3 Precision

The system precision is a measure of the method variability. It was determined by performing three replicate analyses of the same working solution. Precision of the method was demonstrated by intraday and interday variation studies. The intraday precision of the developed UV method was determined by preparing the samples of the same batch in nine determinations with three concentrations (5, 10, 20, µg/mL) and three replicate (n=3) each on same day i.e. zero hour, fourth hour and eighth hour. The percentage RSD of the results was used to evaluate the method precision. The interday precision was

determined by assaying the samples in triplicate (n=3) per day for consecutive 3 days.

4.4 Accuracy

Accuracy of the method was calculated by recovery studies at three levels (80%, 100% and 120%) by standard addition method. Capsule filling powders from twenty capsules were weighed and average weight was calculated. The segregated powders were crushed to obtain fine powder. Capsule powder equivalent to 10mg cefixime trihydrate was transferred to 100 mL volumetric flask. 25 mL methanol was added to dissolve the drugs and then volume was made up to the mark with distilled water and sonicated for 10 minutes. The solution was then filtered through a Whatmann filter paper (No. 41). From the filtrate 1mL was transferred to three 10 mL volumetric flasks and add 0.8 mL (Flask 1), 1 mL (Flask 2), and 1.2 mL (Flask 3) of stock solution of API and then made up to the mark with distilled water to made them 80%, 100% and 120% spiking.

4.5 Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was determined by carrying out the analysis by two analysts at two different temperatures i.e. at 20^0C and 30^0C. The absorbance was measured and assay was calculated for six times. The result was expressed in percent RSD.

4.6 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the ten replicate determinations of same conc. (10µg/mL), standard deviation (SD) of the responses was calculated. From these values, the limit of detection and limit of quantitation were determined on the basis of standard deviation and slope of the regression equation.

4.7 Assay

Twenty capsules were dissembled and weight of content of each capsule was taken. The contents were grinded to make fine powder. From the fine powder equivalent to the weight of 10 mg of cefixime trihydrate was accurately weighed and taken into 100 mL volumetric flask and 25 mL methanol was added. The volumetric flask was sonicated for 20 min to effect complete dissolution of cefixime trihydrate, the solution was then made up to volume with distilled water and was filtered. The aliquot of the filtrate was further diluted to get final concentration of 10µg/mL of cefixime trihydrate. The % assay of the drug was calculated.

4.8 Statistical analysis: The results were expressed as mean±SD. Some results were expressed as %RSD.

5. RESULTS and DISCUSSION

The method discussed in the present work provides a convenient and accurate way for analysis of cefixime trihydrate. The drug obeys the Beer's law with the concentration range 2– 20 µg/mL with R^2 value 0.999 (Fig.2, Table 1).

Concentration (µg/mL)	Absorbance (nm)
2	0.094
4	0.188
6	0.291
8	0.386
10	0.504
12	0.588
14	0.668
16	0.785
18	0.878
20	0.978

Table 1: Linearity of cefixime trihydrate by UV-Visible spectrophotometer

The percent RSD was found in the range of 0.046 – 0.058 % for accuracy (Table 2);

% Recovery	Concentration (µg/mL) Formulation	Drug added	Drug found	% Recovery	Avg. Recovery	% RSD
80	10	8	7.97	99.6		
80	10	8	7.97	99.6	99.57%	0.058
80	10	8	7.96	99.5		
100	10	10	10.09	100.9		
100	10	10	10.09	100.9	100.86%	0.057
100	10	10	10.08	100.8		
120	10	12	11.98	99.83		
120	10	12	11.98	99.83	99.80%	0.046
120	10	12	11.97	99.75		

Table 2. Determination of accuracy of cefixime trihydrate by UV-Visible spectrophotometer (n=3)

0.102 – 0.299% for intraday precision (Table 3) and 0.059 – 0.546 % for interday precision (Table 3).

Concentration (µg/mL)	Absorbance 0 hour	4 hour	8 hour	SD	% RSD
Inter-day precision					
5	0.281 ± 0.16	0.278 ± 0.23	0.280 ± 0.19	0.00153	0.546
10	0.504 ± 0.21	0.506 ± 0.27	0.505 ± 0.22	0.001	0.198
20	0.978 ± 0.25	0.977 ± 0.18	0.977 ± 0.23	0.00058	0.059
Intra-day precision					
05	0.278 ± 0.19	0.277 ± 0.24	0.278 ± 0.18	0.00058	0.208
10	0.510 ± 0.18	0.511 ± 0.26	0.513 ± 0.32	0.00153	0.299
20	0.980 ± 0.23	0.979 ± 0.34	0.978 ± 0.26	0.001	0.102

And 0.258 - 0.365% for robustness and ruggedness (Table 4).

Table 3: Intra-day and inter-day precision of assay UV-Visible spectrophotometer (n= 3)

	% Assay (300C)	% Assay (20^0 C)		% Assay (300C)	% Assay (20^0C)
Analyst 1	99.45	99.37	Analyst 2	99.70	99.64
	99.67	99.56		99.63	99.53
	99.58	99.65		99.47	99.71
	99.19	99.41		100.18	99.33
	100.11	100.01		100.12	99.98
	99.23	99.98		99.25	99.99
Mean	99.54%	99.66%	Mean	99.72%	99.70%
% RSD	0.339	0.277	% RSD	0.365	0.258

Table 3: Intra-day and inter-day precision of assay UV-Visible spectrophotometer (n= 3)

The mean limit of detection (LOD) and limit of quantitation (LOQ) value were found to be 0.053 µg/mL and 0.159 µg/mL respectively, (Table 5).

Sr. No	Concentration (µg/mL)	Absorbance	SD	LOD (µg/mL)	LOQ (µg/mL)
1	10	0.504	0.00078	0.053	0.159
2		0.504			
3		0.503			
4		0.503			
5		0.503			
6		0.502			
7		0.502			
8		0.503			
9		0.502			
10		0.504			

Table 5: LOD and LOQ

The mean % assay was found to be 99.58% (Table 6).

Concentration	Absorbance	% Assay	Mean	% RSD
10	0.515	99.97%	99.58%	0.392
10	0.511	99.19%		
10	0.513	99.58%		

Table 6: Assay (n=3)

The overall results of various validation parameters were summarized in Table 7.

Sr. No	Parameter	Result
1	Linearity	R2 = 0.9994
2	Accuracy	99.57% - 100.08%
3	Precision (% RSD)	
	(a) Intraday	0.102 - 0.299%
	(b) Interday	0.059 - 0.546%
4	Robustness and ruggedness (% RSD)	0.258 - 0.365%
5	Limit of detection (LOD)	0.053 µg/mL
6	Limit of Quantitation (LOQ)	0.159 µg/mL
7	Assay	99.58%

Table 6: Assay (n=3)

6. CONCLUSION:

This UV-spectrophotometric technique was quite simple, accurate, precise, reproducible and sensitive. The UV method has been developed for quantification of cefixime trihydrate in pharmaceutical dosage forms. The validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations.

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