Research Article

Method Development and Validation for Determination of Cefixime in Bulk Dosage Form by UV Spectrophotometry

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ABSTRACT

The objective of the current study was to develop simple, precise, accurate, and cost-effective UV visible spectrophotometry method and validation for determination of Cefixime in bulk dosage form by using UV spectrophotometry was developed according to ICH Q2 (R1) guidelines. The absorption maxima of Cefixime salt were found to be 287 nm. The method represents correlation coefficient (R2 = 0.9980) at concentration range of 1, 3, 5 and 7µg/ ml. The Validation of method was carried out using Linearity, accuracy, and precision value. The percent relative standard deviation of inter day and intraday precise range (3. 9 – 1.6) & (1.99 – 1.67) respectively shows the method was precise. The recovery of Cefixime salt was found to be 99.8% -100%. All material used in this project was purchased and used as such. All the data are analyzed by using UV- 1800. The final result was concluded and validated as precise UV –visible spectrometry method and suggested to research they can choose this method to analyze other antibiotic in same way.

Keywords: Cefixime; ICH guidelines; UV spectroscopy.

INTRODUCTION

Cefixime is third generation cephalosporin antibiotic. It is under the category of Beta Lactum Antibiotic/ Cell wall inhibitors. It acts by inhibiting an enzyme transpeptidase, involved in the building of bacterial cell walls. It is used in lower respiratory tract infection. It is helpful in acute urinary tract infection, bilary tract infection, sinusitis, acute otitis media, peptic ulcer and many more.

A simple and sensitive Spectrophotometric method has been developed for the determination of five cephalosporins namely cefpodoxime, ceftizoxime, ceftazidime, ceftriaxone, and Cefixime. Literature survey reveals that Cefixime is estimated in various combine dosage form like- azithromycin, ofloxacin, moxifloxacin, ornidazole, dicloxacillin, cefurodoxime axetile, cloxacillin by derivative spectroscopy method, Spectrophotometric method, simultaneous equation method and absorption ratio method, TLC densitometric method, RP-HPLC, HPLC, RP-HPLC, respectively. As per literature survey, no analytical methods have been reported for the estimation of Cefixime in pharmaceutical dosage forms. Therefore, the present research work, our aim is to develop a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Cefixime in routine analysis.

Cefixime as potent antibacterial activity against a wide range of bacteria, highly stable towards 3-lactamases and long duration of action. The chemical structure of Cefixime consists of the cephem nucleus, 3-lactum ring fused to a 6-membered dihydrothiazine ring. The cephem nucleus incorporates two importants groups: the vinyl group at the 3- position, which is responsible for the intestinal absorption of the intact molecules and the other groups are the aminothiazoles ring and the acetic acid oxy-imines group on the side chain at the 7-position, which are associated with the antibacterial activity.

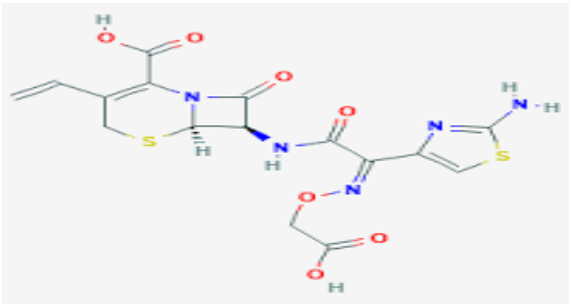

Figure 1: Structure of cefixime

Cefixime is active against a very wide spectrum of bacteria and is used in the treatment of otitis media, respiratory tract infection, and typhoid fever, complicated and uncomplicated urinary tract infection. It is officially in united states pharmacopoeia and British pharmacopoeia.

Both the pharmacopoeia describes HPLC method of analysis for Cefixime trihydrate. HPLC method is also official in Indian pharmacopoeia and japan pharmacopoeia. Literatures reports many analytical methods for the determination of Cefixime in single commercially dosage form and in combination with other drug, using UV spectroscopy, spectrofluorometry, HPLC, HPTLC.

The objective of the current study is to develop a easy, rapid, accurate, reliable, reproducible, validated and economical UV spectroscopy method for the determination of Cefixime in bulk dosage form.

MATERIALS AND METHODS

Analytical pure Cefixime was obtained as a gift sample from INNOVA Cap Tab Baddi, India. All chemical and reagent used were of analytical grade. Hydrochloric acid was obtained from central drug house (p) Ltd. Sodium hydroxide was obtained from central drug house (P) Ltd.

The instrument used for the present study was a UV-Vis double beam spectrophotometer (Shimadzu 1800), Hot plate (HICON), weighing Balance (Scale – Tec), Distilled water (In- house distilled).

UV Method Development

Solubility Test

Standard stock solution of Cefixime was performed by using various solvent. The solvent include water, methanol, ethanol, acetonitrile, 0.1 N hydrochloric acid (0.1 N HCL), 0.1N sodium hydroxide (0.1 N NaOH).

Determination of λ max

Preparation of stock solution

Standard stock solution of Cefixime was prepared by dissolving 10mg of Cefixime in 10ml of methanol which gives 1000µg/ml. 1 ml of the stock solution was taken and was diluted up to 10ml by using methanol to produce a concentration of 100 µg/ml solution.

Preparation of working solution

From the above stock solution 1ml was transferred in to 10ml volumetric flask and volume was made up to the mark with methanol to make 10µg/ml. Then the sample was scanned with UV – Visible spectrophotometer in the range 200-400 nm against 0.1Nand the wavelength corresponding to maximum absorbance was noted at 287nm, respectively.

Preparation of calibration curve

From the above stock solution (100 µg/ml) further dilution were made and the volume was make up to 10ml using methanol to produce 10 µg/ml, 20 µg/ml, 30 µg/ml ,40 µg/ml, 50 µg/ml solutions respectively. The correlation coefficient was found to be 0.999 & 0.99, respectively.

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The Developed method was validated according to ICH guidelines. The following parameters were considered: specificity, Linearity, Limit of detection (LOD), Limit of quantification (LOQ), accuracy and precision.

Accuracy

Accuracy was calculated as the percentage recoveries of blind sample of pure cefixime and its indicated the agreements between obtained results and those accepted as true, detailed result are presented in Table 5. To ascertain the accuracy of the suggested methods, recovery studies were carried out by the three different level (50%, 100%, 150%) The result are presented in Table 5.

Precision

Inter day and intraday precision studies were done by repeated measurements of the absorbance of standard mixed solution by the proposed assay method without changing the method procedure. Percentage RSD was calculated and result are presented in Table 2,3.

Robustness

Robustness study was carried out by changing the wavelength in +_ 0.2 nm from 3.90 – 210.00nm and the result are presented in Table 6.

Table 1: System Precision result of cefixime

S.NO	Wavelength (m)	Absorbance ($molL^{-1}$)
1	216.40	3.984
2	213.20	3.997
3	208.60	3.995
4	398.20	0.175
5	215.20	3.947
6	209.60	3.890
Average	243.53	3.33
SD	0.614	1.99
RSD%	0.252	1.67

Table 2: Intraday precision result of Cefixime

S.NO	% Assay
1	100.99
2	102.49
3	102.49
4	103.50
5	104.91
6	104.39
Average	102.8
SD	1.38
%SD	1.34

Table 3: Inter day precision results

Numbers	Day 1	Day 2	Day 3
1	3.984	3.89	2.75
2	3.997	3.79	2.98
3	3.995	3.59	2.99
4	0.175	0.09	0.08
5	3.947	2.99	1.98
6	3.890	3.05	2.99
Average	3.33	1.69	2.95
SD	1.99	0.282	1.10
%RSD	1.67	16.72	100

Table 4: Calibration data for cefixime

% Level	Conc. (µg/ml)	Abs.1	Abs.2	Abs.3
25	10	0.156	0.159	2.01
50	20	0.25	0.29	1.09
75	30	0.27	0.28	1.03
100	40	0.335	0.339	0.435
125	50	0.349	0.351	0.299
150	60	0.461	0.550	0.671
175	70	0.492	0.552	0.602
Regression equation		y=0.0051(x) +0.0207	y=0.0055(x) +0.0313	y= 0.0058(x) +0.039
Regression coefficient		0.999	0.99	0.997

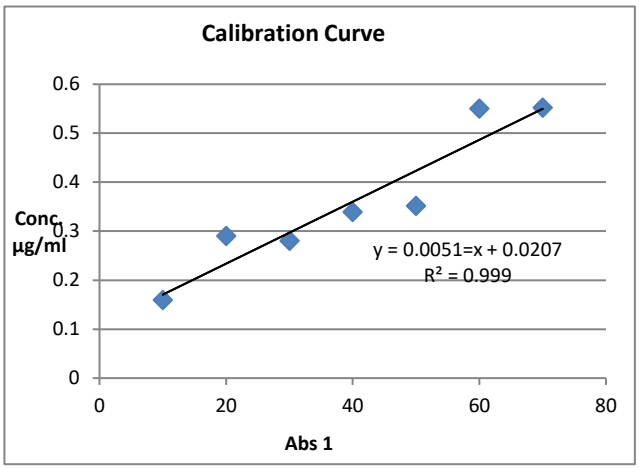

Figure 2: Calibration curve for Absorbance 1

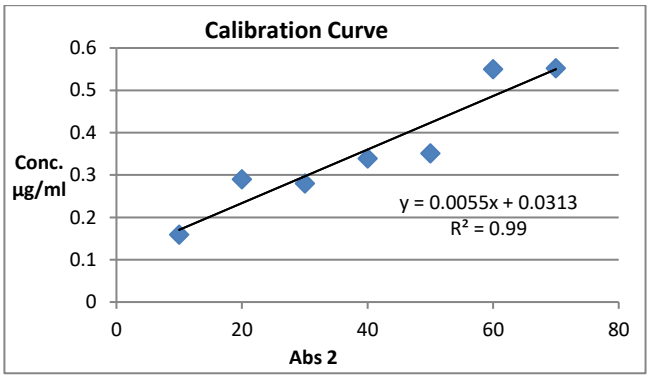

Figure 3: Calibration curve for Absorbance 2

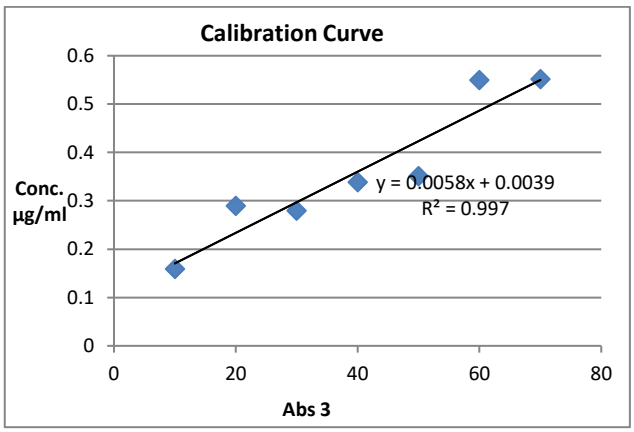

Figure 4: Calibration curve for Absorbance 3

Table 5: Results of Accuracy studies for Cefixime

Conc. Level	% Mean Recovery
50	98.9
100	99.7
150	100.05

Table 6: Robustness results of Cefixime Sample

Parameters	Variation	% RSD
pH (±0.2)	4.19	1.22
	4	1.53
	3.90	1.3
Wavelength(±5nm)	207.60	0.35
	394.20	1.53
	210.00	0.54

Table 7: Optical characteristics and validation parameters of Cefixime

Parameters	Results
Detection wavelength (nm)	287(nm)
Beer's Law limits (µg/ml)	10-80
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.3
Regression equation (y = mx+c)	y=0.0055(x)+0.0313
Correlation coefficient (r^2)	0.99
Slope (m)	-
Intercept (c)	-
% Relative Standard Deviation (% RSD) System precision	1.67
(% RSD) Intra-day precision	1.34
(% RSD) Inter-day precision	100
Accuracy (% Mean Recovery)	
50 % Level	98.9
100 % Level	99.7
150 % Level	100.05
LOD (µg/ml)	-
LOQ (µg/ml)	-
Robustness	
pH(± 0.2) (% RSD)	≤2
Wavelength (± 2nm) (% RSD)	≤2

RESULTS AND DISCUSSIONS

The proposed method was validated for precision, accuracy, specificity, linearity, and range, limit of detection (LOD) limit of quantitation (LOQ), robustness, ruggedness. Validation of the proposed method was carried out in accordance with the International Conference on Hormonization (ICH,) guidelines. The linearity of the calibration plots was confirmed by the high value of the correlation coefficient (r2 = 0.99). the recovery of added standard was calculated at different concentration levels. the value of standard deviation and % RSD were found to be < 2 % shows the high accuracy of the method. The assay

of cefixime was found to be 99.7% stability of cefixime in distilled water.

CONCLUSIONS

The UV method has been successfully applied for the determination of cefixime in sample solution. They were found to be accurate, simple, rapid and precise. Once the equation was constructed, analysis required only measuring the absorbance values of the sample solution at the selected wavelength followed by simple calculations. The proposed method was completely validated showing satisfactory data for all the method validation parameter tested.

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