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Novel RP-HPLC method development and validation of Cefixime in bulk and its dosage form by using hydrotropic solution as mobile phase

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ABSTRACT

High performance liquid chromatography of drugs is usually performed with the help of organic mobile phase like Methanol, Acetonitrile etc., which are toxic and expensive.In the present study, hydrotropic solution was employed as mobile phase for the estimation of poorly aqueous soluble drug Cefixime by HPLC.The analysis was carried out on Agilent 1220 Infinity LC (G4288C) model which consists of Agilent 1220 infinity LC pump, Rheodyne injector (20µL loop) and Variable wavelength detector. The analytical column used was Agilent eclipse plus C18 (4.6 x 100mm) with 3.5 µm particle size.An aqueous 6% sodium acetate (pH adjusted to 6.2 by acetic acid) at a flow rate of 1.0mL/minute was employed as mobile phase and the drug was detected at 290 nm at ambient temperature. The method was validated as per ICH guidelines. The retention time for Cefixime was found to be 2.34 minutes. Linearity was observed in the concentration range of 40-240 µg/mL with correlation coefficient 0.9999.The percentage of relative standard deviation of six replicate measurements was found to be 0.0055. This indicates the proposed method was precise. Recovery studies were conducted at three different concentration levels within the linearity limits and the average percentage in tablet dosage form was determined and found to be 99.54%. Therefore, the developed method employing hydrotropic solution as mobile phase was novel, simple, precise, cost-effective, eco-friendly, safe and can be successfully applied for the estimation of Cefixime in pharmaceutical dosage forms.

INTRODUCTION

Cefixime ($C_{16}H_{15}N_5O_7S_2$, $3H_2O$) is chemically (6R, 7R)-7-[(Z)-2-(2-amino thiazol -4-yl)-2-[(carboxymethoxy) imino] acetyl) amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trihydrate, is a third generation cephalosporin antibiotic [1,2]. Cefixime (fig 1)has potent antibacterial activity against awide range of bacteria, highly stable towards β-lactamases and longer duration of action. Literature survey revealed that a few HPLC [3-6] and spectrophotometric methods [7-9] were reported for the estimation of Cefixime. In all reported methods expensive and toxic organic solvents are used as mobile phase. But there was no method reported till date for any drugs in RP-HPLC by using hydrotropic solution as mobile phase. Maheshwari [10-12] has analysed various poorly water soluble drugs spectrophotometrically using hydrotropic agents. Also hydrotropy had applications in TLC and HPTLC [13,14]. Hydrotropy [15] refers to the ability of a concentrated solution of a chemical compound to increase the aqueous solubility of another compound (usually a sparingly soluble organic compound). Each hydrotropic agent is effective in increasing the water solubility of selected hydrophobic drugs. Examples for hydrotropic agents include Sodium acetate, Sodium salicylate, Sodium citrate, Sodium benzoate, Nicotinamide, Urea etc. Hence, the present study is to attempt and develop accurate, simple, sensitive, eco-friendly, cost effective method for the estimation of Cefixime by using hydrotropic solution as mobile phase.

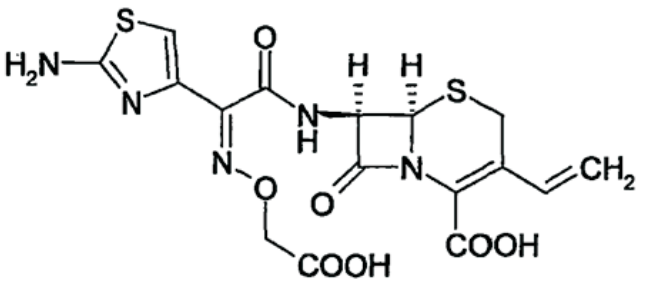

Figure 1 : Structure of Cefixime

MATERIALS AND METHODS

Instrumentation

Agilent 1220 infinityLC (G4288C) model consists of Agilent1220 infinity LC pump, Rheodyne injector (20µL loop) and Variable wavelength detector. The analytical column used was Agilent eclipse plus C_{18} (4.6 x 100mm) with 3.5 µm particle size.

HPLC Operating conditions

Column : Agilent eclipse Plus c18 (4.6 x 100 with 3.5 µm particle size)

Detector : Variable wavelength detector

Injection volume : 20µL

Flow rate : 1.0 mL/minute

Temperature : Ambient

Run time : 10 minutes

Mobile phase : 6% Sodium acetate (pH 6.2) solution in HPLC grade water

Chemicals and Reagents

Reference standard of Cefixime was obtained as gift sample from Kausikh Therapeutics Ltd, Chennai. The commercially available tablet SANIX-200, Sance Laboratories Pvt. Ltd, Kottayam, were procured from local market. HPLC grade water, Methanol, Sodium acetate and Acetic acid were purchased from Merck Pvt Ltd, Mumbai. All chemicals and reagents were of AR grade.

Preparation of mobile phase

6%w/v solution of Sodium acetate was made in HPLC grade water and pH was adjusted to 6.2 by using Acetic acid. It was degassed by ultra-sonication for 20 minutes. The degassed mobile phase was filtered through 0.45 µ filter.

Preparation of standard solution

100 mg of Cefixime was weighed and dissolved in sufficient HPLC grade methanol and then the volume was made up to 100ml with methanol to get a concentration of 1mg/mL. Then the stock solution was diluted with methanol to obtain working standard solutions of concentration of 40 µg/mL to 240µg/mL

Preparation of sample solution

10 tablets of SANIX-200 were weighed accurately and average weight was determined. Then these tablets were powdered. The weight of powder equivalent to 100 mg of Cefixime was taken in 100 mL volumetric flask and 50 mL methanol was added in the flask. The flask was shaken for about 10 minutes and then sufficient methanol was added to make up the volume up to the mark. Then, the solution was filtered through membrane filter with pore size of 0.45µ. Then the filtrate was appropriately diluted with methanol to get suitable dilutions.

RESULTS

The detection wavelength of Cefixime was set at 290 nm. A volume 20µL was injected and the components eluted from the system were monitored for a run time of 10 minutes. A peak was found to be at 2.34 min. Typical chromatograms for the standard and sample were presented in figure 2 and 3.

METHOD VALIDATION

The developed method was validated as per ICH guidelines[16] for Linearity, Precision, Accuracy, Specificity, Robustness, Ruggedness, Limit of detection and Limit of quantitation by the following procedure.

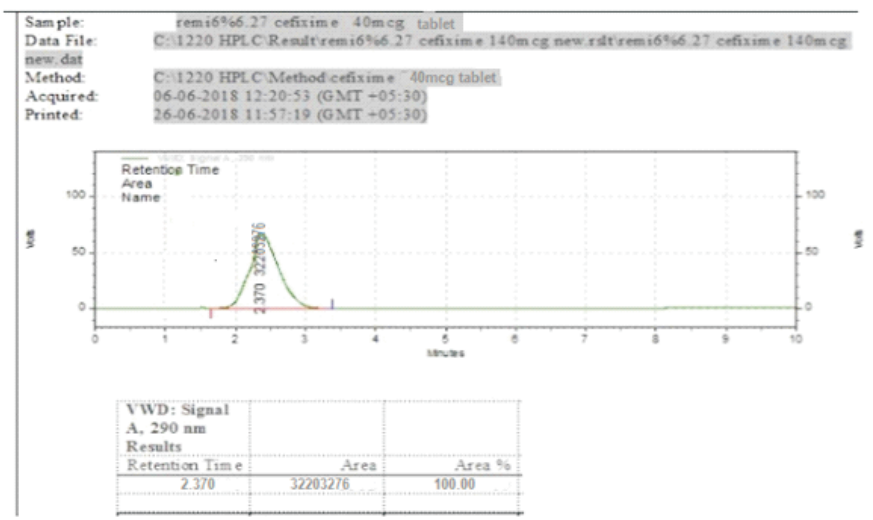

Figure 2 : Chromatogram of Cefixime tablet

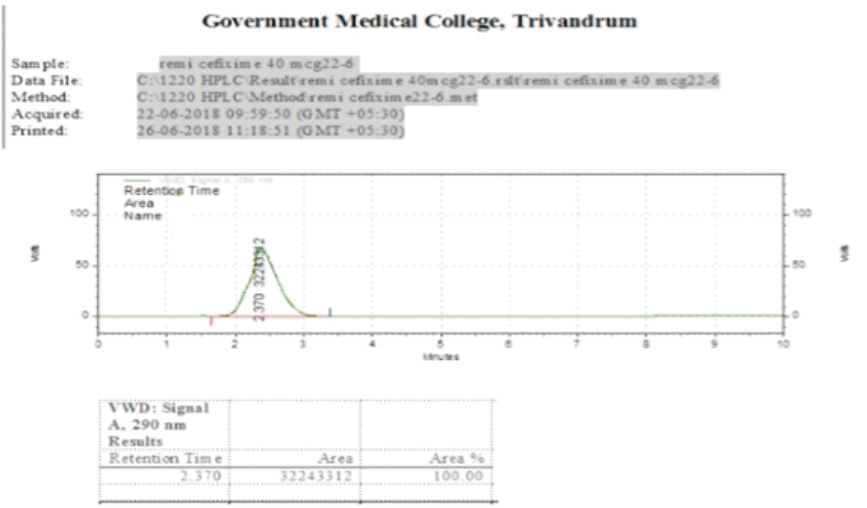

Figure 3 : Chromatogram of Cefixime standard

1. Linearity

Suitable dilutions were made from the standard stock solution containing 1000µg/mL of Cefixime to prepare standard solutions of six different concentrations ranging from 40-240µg/mL. Five replicates of each concentration were injected and chromatograms were recorded (table 1). The peak area was plotted against concentration to get calibration curve. The plots of peak area v/s respective concentration of Cefixime were found to be linear in the range of 40- 240 µg/mL with a correlation coefficient (r^2) of 0.9999 and shown in fig4.

2. Precision

Precision was determined in two levels repeatability and intermediate precision

Repeatability of the method was checked by injecting replicate samples of 40µg/mL of the solution for six times on the

Table 1 : Linearity parameters for Cefixime

Sl.no	Concentration (µg/mL)	Peak area of Cefixime
1	40	32243312
2	80	64504396
3	120	96723869
4	160	128969482
5	200	161216113
6	240	193456231

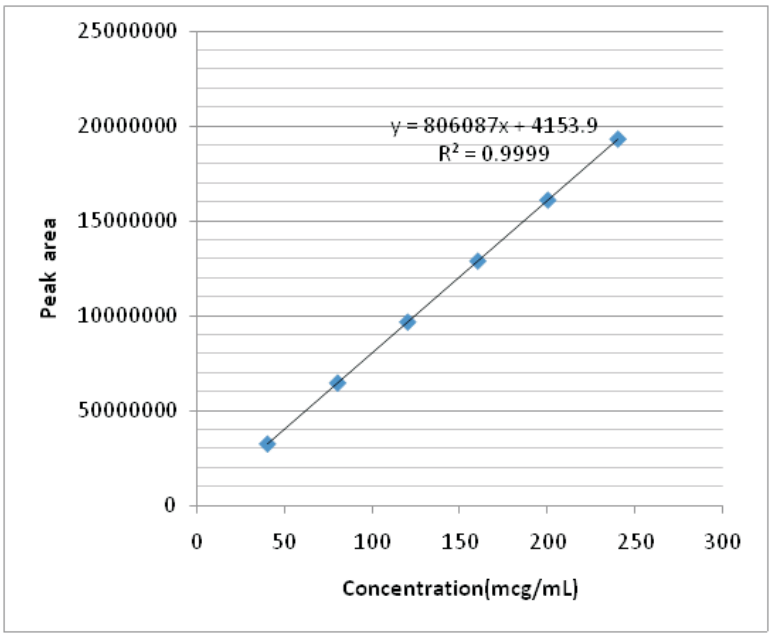

Figure 4 : Calibration curve of Cefixime

Table 2 : Repeatability study

Sl.no	Concentration (µg/mL)	Peak area	Percentage Label claim
1	40	32203276	99.85
2	40	32202936	99.87
3	40	32203498	99.88
4	40	32202749	99.88
5	40	32202498	99.90
6	40	32203094	99.90

same day as intraday precision (Table 2& 3). The interday precision was carried out by estimating the corresponding responses in triplicate for 3 days. (Table 4)

3. Accuracy

Accuracy of the method was determined by recovery studies. To the formulation (pre-analysed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery for Cefixime was found in the range of 98.70-100.02% indicates the accuracy of the proposed method. The results of studies along with its evaluation was shown in table 6.

Table 3 : Repeatability study - statistical validation

Mean of % Label claim	Standard deviation	% RSD	Coefficient of variation
99.88	0.0054	0.0055	0.000054

Table 4 : Inter day precision data

	Peak area	Average % label claim	Standard deviation	% RSD
DAY 1	32202913	99.87	0.00111	0.00111
	32203436			
	32202924			
DAY 2	32203498	99.86	0.0033	0.0033
	32203084			
	32205271			
DAY 3	32206102	99.86	0.0033	0.0033
	32208185			
	32208208			

Table 5 : Assay result of formulation

Formulation	Label claim (mg)	Amount found (mg)*	% Label claim
Tablet SANIX 200	200	199.71	99.86%

* Average of six determinations

Table 6 : Accuracy of proposed method at three different concentrations

Spike level	Concentration (µg/mL)	% Recovery	Average % recovery	Standard deviation	Relative standard deviation
80%	144µg/mL	99.93 99.75 100.02	99.90	0.0577	0.0578
100%	160µg/mL	99.99 100.00 99.95	99.98	0.0116	0.0115
120%	176µg/mL	98.80 98.76 98.70	98.75	0.0212	0.0212

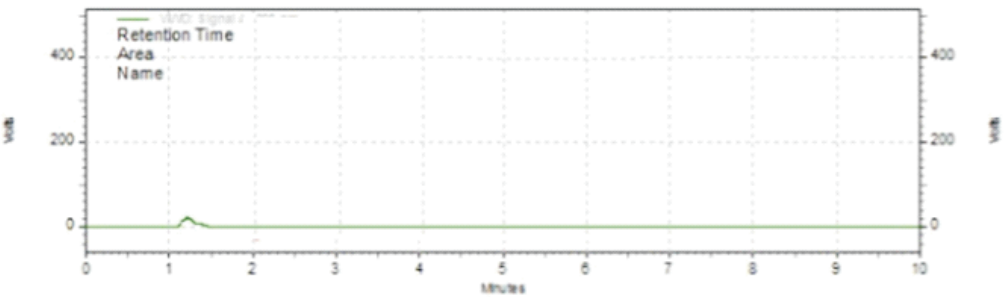

Figure 5 : Placebo chromatogram

4. Specificity

Specificity was performed by injecting samples of mobile phase, placebo, sample solution and spiked sample. The results showed no interference at the retention time of Cefixime. The representative chromatogram of placebo was shown in fig 5.

5. Robustness

Robustness is performed by making slight variations in the flow rate and concentration of mobile phase. The changes and results were tabulated in table 7.

6. Ruggedness

Interday variations were performed by using six replicate injections of sample solutions which were prepared and analysed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst (Table 8).

Table 7 : Results of Robustness study

Parameters	Adjusted to	Average area	Retention time	% Label claim	Standard deviation	% RSD
Flow rate	0.9mL /minute	31479374	2.370	97.66	0.6786	0.6786
	1 mL /minute	32202749	2.372	99.88	0.0055	0.0054
	1.1 mL /minute	31260185	2.401	96.98	0.2796	0.2796
Mobile phase composition	5.8% sodium acetate	32053133	2.289	99.44	0.1456	0.1456
	6% sodium acetate	32202749	2.370	99.88	0.0055	0.0055
	6.2% sodium acetate	32037016	2.290	99.39	0.1049	0.1049

Table 8 : Results of Ruggedness study

Parameter	Peak area	Average % label claim	Standard deviation	% RSD
Analyst I	32203276	99.85	0.0054	0.0054
	32202936			
	32202498			
Analyst II	32202913	99.88	0.0033	0.0033
	32203436			
	32206102			

7. Limit of Detection and Quantitation (LOD and LOQ)

LOD and LOQ were determined by linearity curve method and by using the equations.

LOD = 3.3 (σ/S)

LOQ = 10 (σ/S)

Where is the standard deviation of the response and 'S' is the slope of the linearity curve. The LOD and LOQ values of Cefixime were 0.0258 and 0.0781 respectively.

DISCUSSION

Conventional RP-HPLC method uses volatile, costlier and toxic organic solvents as mobile phase. The proposed method describes a novel RP-HPLC method for the determination of Cefixime employing a special mobile phase called hydrotropic solution comprising of 6% sodium acetate(pH adjusted to 6.2 with acetic acid) in water was found to be satisfactory and give sharp peak for Cefixime. The hydrotropic mobile phase is eco-friendly, non-volatile, nontoxic and non-expensive in nature. The developed method was found to be specific and validated as per ICH guidelines. The linearity for detector response was observed in 40- 240 µg/mL for Cefixime and found to be linear with r^2 of 0.9999. The calibration plot was given in fig 4. The results of linear regression analysis were presented in table 1. Percentage recovery for Cefixime was found in range of 98.75 % - 100.01% indicates accuracy of the proposed method and the results were presented in table 6.The percentage RSD for both the tablet analysis and recovery studies were less than 2% indicates high degree of precision. The LOD and LOQ for Cefixime were 0.0258 and 0.0781 respectively. The LOD and LOQ values showed that the proposed method is sensitive. The results of robustness study indicate that the method is robust and is unaffected by small variation in chromatographic conditions. It was observed that the excipients present in the formulation did not interfere with peak of

Table 8 : Summary of Results

Parameters	Result
UV detection wavelength (nm)	290
Linearity range (µg/mL)	40-240
Regression equation	Y= 806087 +4153.9
Correlation coefficient	0.9999
Accuracy 80% 100% 120%	 0.0578 0.0115 0.0212
Precision (% RSD)	
Interday	0.00257
Repeatability	0.0055
% Recovery	98.70 %- 100.02 %
LOD	0.0258
LOQ	0.0781
Ruggedness (% RSD)	
Analyst I	0.0054
Analyst II	0.0033

Cefixime. The proposed method was applied for the determination of Cefixime in tablet formulation and the result was comparable with the corresponding labelled amount present in table 5. A typical chromatogram showing the separation of Cefixime is shown in fig 3.

CONCLUSION

The present study employs hydrotropic solution as mobile phase which preclude the usage of costlier and corrosive organic solvent as mobile phase. So the proposed method is novel, simple, precise, cost-effective, eco-friendly, safe and can be successfully applied for the routine analysis of Cefixime in pharmaceutical dosage forms.

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