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Research Article

A Novel, Economical RP-HPLC Method for Quantification of Cefixime in Bulk and Pharmaceutical Solid Dosage form

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Abstract:
A new, precise, economical, and linear gradient reverse phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated for assay determination in Cefixime. The chromatographic separation was achieved with Hypersil C18(250*4.6)mmand 3 µm particle size column. The flow rate was 1.0 mL/min and eluents were detected at 220 nm using PDA detector. The retention time was found to be 7.3 min.The percentage recoveries for molecules were found to be in the range of 99–101%. The calibration curve demonstrated good linearity in the range of 20–100 gm/ml. for Cefixime. The approach has been validated in accordance with the International Conference on Harmonization's regulatory criteria. The evaluated parameters are precision, linearity, detection limit, quantification limit, specificity, accuracy, and robustness.The technique may be applied to stability investigations as well as routine analysis to identify and quantify Cefixime in pharmaceutical dosage form .
Keywords: Cefixim. Reversed Phase High Performance Liquid Chromatography. Assay substances. Methanol

1. Introduction

C efixime (C H N O S , 3H O) is chemically (6R, 7R)- 16 15 5 7 2 2 7 - [(Z) - 2 - (2 - a m i n o t h i a z o l - 4 - y l) - 2 - [(carboxymethoxy) imino] acetyl) amino]-3-ethenyl8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [7,8] trihydrate, is a third generation cephalosporin antibiotic . Cefixime (fig 1) has potent antibacterial activity against awide range of bacteria, highly stable towards β-lactamases and longer [11,12] duration of action. It is used to treatdifferent types of bacterial infections such as bronchitis,tonsillitis, ear and skin infections, gonorrhea, and urinary tractinfections.

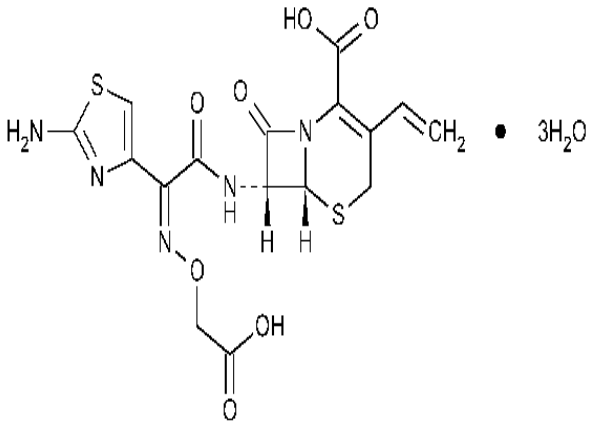

Figure 1: Structure of Cefixime

The principal goal of the current work is to develop and validate a new Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method to estimate Cefixim in bulk and pharmaceutical .Pharmaceutical parameter analysis is a crucial and important step in the entire drug development process. Thus, rapid and easy procedures for testing the quality of commercial formulations are required. In light of this, the authors have developed a new, accurate, and efficient technique for determining Cefixim in a Bulk dosage form.

2. Chemicals and Reagents:

The working standard of was received from stanford Biotech. & Cefixime (Cifix-200 mg) tablet was purchased from local pharmacy . Water, Methanol was HPLC grade and Potassium di hydrogen phosphate ,ortho phosphoric acid was also AR grade

3. Instrumentation:

The chromatographic separation was carried out using Shimadzu LC-Solution HPLC equipment coupled with a SPD M10 avp Detector., pH meter made by Lab India were employed. Cx220 citizen analytical balance &Frontline ultra sonic cleaner Fs-10 were used during analysis

4. Preparation of mobile phase :

Weigh Accurately about 119.31g of disodium hydrogen phosphate and dissolved in 1000 ml of water and pH was adjusted to 6.5 by 10% phosphoric acid and filtered with 0.45 micron

nylon filter, mixed 80ml of this buffer solution in 20ml of methanol and degassed before used.

5. Preparation of standard solution :

20 mg of Cefixim as accurately weighed and transferred to a 100 ml volumetric flask and dissolved in small amount of mobile phase and then make up with mobile phase

6. Preparation of Sample solution:

About 10 tablets were weight (each tablet contain 200mg cefixime)) and powdered. Powered equivalent to average weight of 10 tablets was taken and dissolved in 100ml mobile phase and solution was filtered through whatman filter paper No.40. the solution make up to 1000 ml with mobile phase.

7. Chromatographic conditions:

Table 1: Optimized Chromatographic Conditions for RP-HPLC study

S.No.	Parameter	Chromatographic conditions
1.	Flow rate	1.0 ml per minute.
2.	Column	Hypersil C18(250*4.6)mm,
3.	Detector wave length	220 nm
4.	Oven temperature	Ambient
5.	Injection volume	20 µL
6.	Run time	60 min
7.	Diluent	Mobile Phase
8.	Mode of separation	Isocratic
9.	Mobile Phase	Buffer(pH6.5):MeOH(84:16)

8. Analytical Method Validation:

The suggested RP-HPLC technique is validated using the following factors: specificity, linearity, precision, accuracy, robustness experiments. The validation was performed in compliance with the International Conference on

Harmonization's requirements for validating analytical procedures (ICH) [19].

8.1 Specificity.

In order to confirm that there is no interference of Cefixime with placebo in standard samples or pharmaceutical formulations, the specificity and selectivity of the technique were assessed by injecting each of the System suitability solution, Standard Solution, Sample Solution.

8.2. System Suitability.

The system suitability test is a pre-use test to verify the compatibility and efficacy of a chromatographic system. Any chromatographic system's performance could fluctuate continually throughout routine operation, which could compromise the accuracy of the findings of analytical procedures. The system's suitability was tested by injecting the system suitability solution. The process was repeated every day during the validation of the method [20]. Tailing factor for Cefixime in system suitability solution should not be more than 2.0 and theoretical plate should not be less than 2000. RSD of six replicate injections for standard should not more than 5.0%.

8.3.Calibration Curve (Linearity)

Calibration curves were constructed by plotting peak area vs. concentration of Cefixime and regression equation were calculated (Figure-7).

The calibration curves were plotted over the concentration range of 20-100(µg/ml) µg/ml for Cefixime. Acceptance criteria squared correlation coefficient was not less than 0.99. A linear relationship was observed in the range of study.

8.4. Precision.

A method's precision is a measure of its ability to produce repeatable results. For system precision, six replicate injections of the standard preparation of both Cefixime was used, whereas for method precision, prepared six sample solution of from bulk at specification limit was prepared. With respect to intermediate precision, same procedure followed as method precision with variation of day and analyst.

8.5. Accuracy.

The recovery of the procedure was indicated by the percentage difference between the sample's measured concentration and its theoretical concentration. A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method into each volumetric flask for each spike level to get the concentration of drugs equivalent to 50%, 75%, 100%, 125% and 150% of the labeled amount as per the test method. The average % recovery was calculated.

8.6. Robustness.

The system suitability solution, standard solution, placebo, sample solution was injected under different chromatographic conditions.

9. Results and Discussion

Different mobile phases with different compositions and flow rates were tried to develop an accurate, selective, and precise stability indicating RP-HPLC method for estimating Cefixime in samples. After a number of compositions and combinations, the chromatographic conditions were devised and adjusted. With the gradient mobile phase and at a flow rate of 1.0 mL/min, reasonable estimate of cefixime with good peak symmetry and constant baseline was observered. The drug had one distinct peak with retention time (RT) of 7.3min and a distinct baseline at 220 nm. The detailed result for every parameter is described below. Each injection had a volume of 20 µl.

9.1. Specificity.

No interference observed from blank and placebo at the retention time of

Cefixime .(Table 2; **Figure.** 2-3).

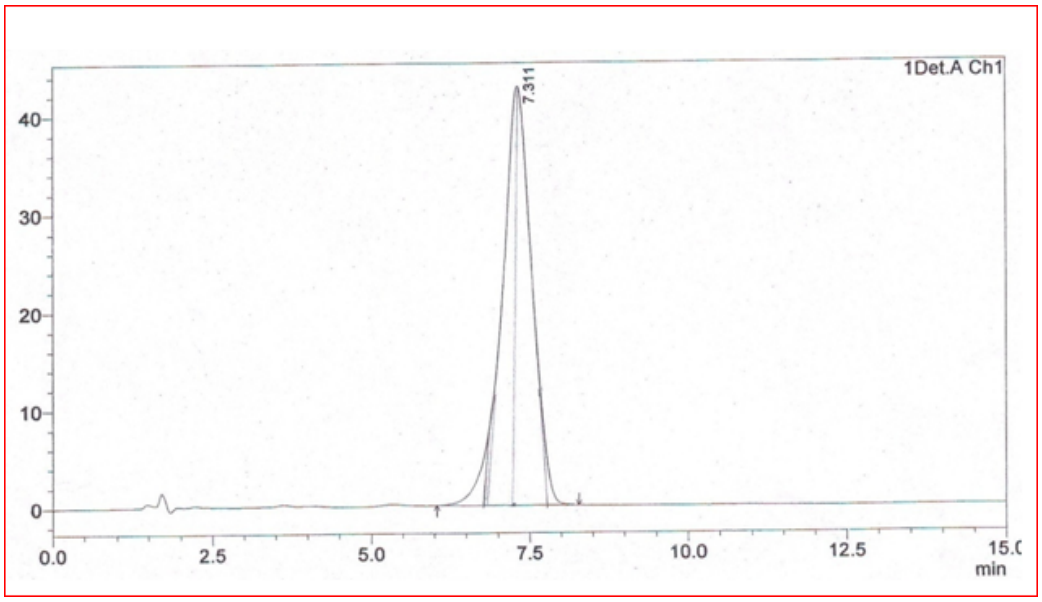

Figure 2: Chromatogram of Cefixime Standard

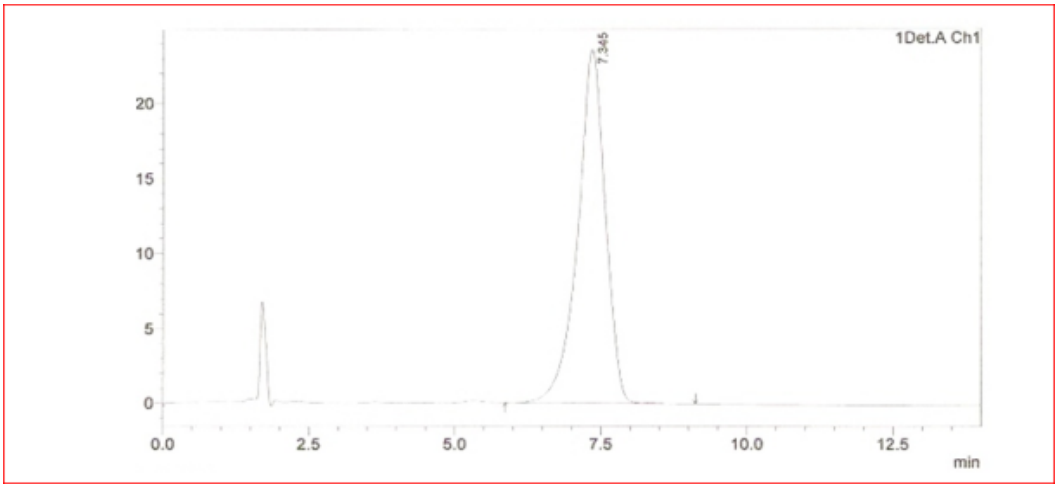

Figure 3: Chromatogram of cefixime sample solution.

9.2. System Suitability.

Separation variables were set and mobile phase 0.03M phosphate buffer(pH6.5):Methanol (80 : 20v/v) was allowed to saturate the column at flow rate 1.0 ml/min and a pH of buffer adjusted with 10% Phosphoric acid to got sharp base line. five replicates of reference standard of Cefixim were injected. so ,

1.The % RSD for the peak area responses of Cefixime tri hydrate was found to be 0.07% for 5 replicate injections .

2. The number of theoretical plates (N) for Cefixime was found not more than 2000.

4. The Tailing factor (T) was not more than 2.0

9..3. Linearity.

The linearity method was performed and evaluated by preparing 5 concentrated solution. and cefixime conatained in the range of 20–100 gm/ml.Each concentration was prepared and analyzed in triplicate. Good linearity was observed over the above-mentioned ranges with linear regression equationsY = 40986X -21991 for Cefixime. The correlationcoefficients were found to be 0.9999 Cefixime(**Figure**.7-8.)

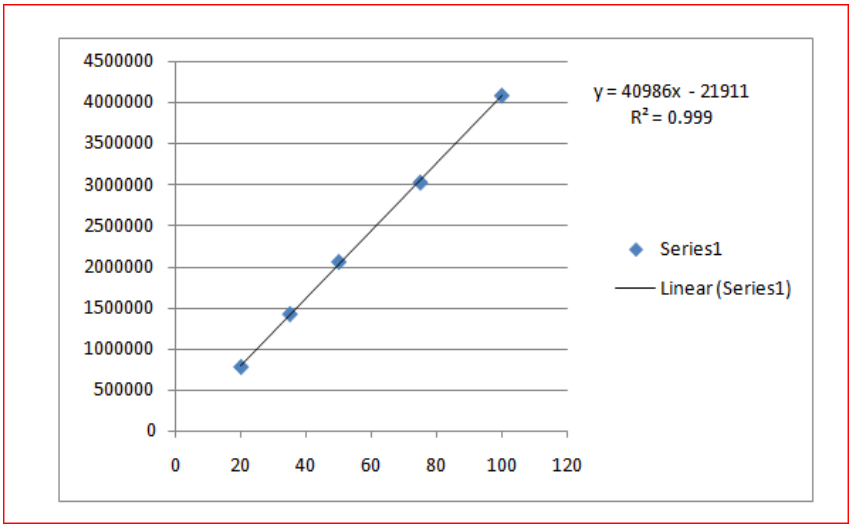

Figure 4: Calibration curve of Cefixime

9.4.Precision.

The precision of method was determined by repeatability and intermediate precision. Repeatability was examined by performing six determinations of the same concentration of Cefixime on the same day, intra-day and inter-day under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis intra-day and inter-day for Cefixime (**Table** 2).The overall mean of intermediate precision is 99.04.The % RSD of system precision is 0.02%.Therefore, the HPLC method for the determination of Assay of Cefiximes is precise. (**Table** 2).

Table 2: Precision and Intermediate precison for Cefixime

Injection	Precison		Inermediate precision	
	System Precision	Method Precision	System Precision	Method Precision
	Area	% Assay	Area	% Assay
1	3554121	99.2	4055109	100.1
2	3564132	98.1	4056180	99.6
3	3555241	98.6	4045124	98.7
4	3553132	97.8	4054138	99.6
5	3545639	98.2	4156162	98.4
6	3554654	98.1	4100214	99.5
Mean (n=6)	3067939.83	98.33	**4077821.17**	99.32
SD	**529.37**	0.497	**43018.38**	0.637
%RSD	**0.02**	0.505	**1.05**	0.641

Note: SD referred to Standard Deviation; RSD referred to Relative Standard Deviation.

9.5.Accuracy.

The results were analyzed and they were found to be within the limits.The mean recovery is 100.09% and RSD is 0.608% for Potassium clavulanate. and for Cefixime mean recovery is 100.032 % and RSD is 0.658.

9.6.Robustness

The HPLC method for the determination of related substances of Cefixime was robust for small changes in pH, Column temperature, Flow rate Temperature and wavelength.

Table 3: Robustness studies for Cefixime

Parameter	%RSD (Cefixime)
pH +0.2 units	0.209
pH -0.2 units	0.286
Flow -0.1 mL/min.	0.375
Flow +0.1 mL/min.	0.422
Wavelength +5nm	0.485
Wavelength -5nm	0.154
Temp. + 5°C	1.890
Temp. - 5°C	1.879

10. Conclusions

A novel gradient HPLC approach has been developed for the estimation of the Cefixime in pharmaceutical dosage form . The technique has been proven to be precise,accurate and suitable for assaying the drug It is simple, sensitive, linear, and exact. The technique may be applied to the routine and stability examination of dosage forms and medicinal substances.

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