EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 4
297-305
Year: 2021

DEVELOPMENT & VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF CEFUROXIME INJECTION DOSAGE FORM

B. Sucharitha[1]* and Prof. V. Anuradha[2]

[1*,2]Department of Chemistry, Vignan Degree College, Guntur, Andhra Pradesh, India.

***Corresponding Author: B. Sucharitha**
Department of Chemistry, Vignan Degree College, Guntur, Andhra Pradesh, India.

Article Received on 27/01/2021 **Article Revised on 16/02/2021** **Article Accepted on 08/03/2021**

ABSTRACT
A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Cefuroxime in injection formulations. The separation was achieved by using column Zodiac C8 (150 x 4.6mm, 5µm) mobile phase consisted of pH 3.4 acetate buffer and acetonitrile in the ratio of (10:1 v/v). The flow rate was 2.0mL.min-1. Cefuroxime was detected using UV detector at the wavelength of 254nm. The retention time of Cefuroxime was noted to be 3.08 min respectively. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

KEYWORDS: Liquid chromatography, Cefuroxime and Validation.

1.0 INTRODUCTION
Cefuroxime Axetil is a mixture of the 2diastereoisomers of (1RS)-1-(acetyloxy) ethyl (6R,7R)-3-[(carbamoyloxy)methyl]-7- [[(Z)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thial1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.[1]

Cefuroxime is a β-lactam type antibiotic. More specifically, it is a second-generation cephalosporin. Cefuroxime Axetil is the orally absorbed ester prodrug of the Cefuroxime sodium. Since Cefuroxime sodium is not absorbed orally, the 1-acetyloxyethyl ether was substituted for sodium on the Cefuroxime molecule to increase its lipid solubility and improve its gastrointestinal absorption.[2] The absorbed ester is hydrolyzed in the intestinal mucosa and in portal circulation.[3] Cefuroxime freely soluble in acetone, sparingly soluble in chloroform, ethyl acetate, methanol, slightly soluble in dehydrated alcohol. Insoluble in ether, water. Literature survey reveals that few analytical methods have been reported for the estimation of Cefuroxime in pharmaceutical dosage form including UV-Vis spectroscopy[3-5], high performance thin layer chromatography HPTLC[6-8] and HPLC.[9-18] The method was developed and validated as per ICH[19–21] and USP[22] guideline.

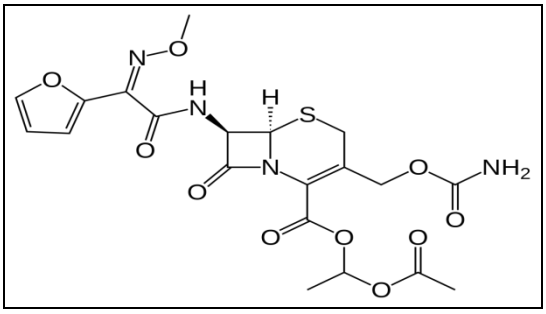

Figure 1.1: Structure of Cefuroxime.

2.0 Experimental
Chemicals and reagents: Analytical-grade ammonium acetate, glacial acetic acid and orcinol was from Merck chemicals Mumbai, India. Acetonitrile and water, both HPLC-grades, were from Merck chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, India.

Instrumentation: Agilent HPLC model:1260 with DAD, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were used.

Preparation of pH 3.4 Acetate buffer
Transferred 50 mL of 0.1 M ammonium acetate to a 1000 mL volumetric flask and dilute to volume with 0.1 N Acetic acid, adjusted the pH to 3.4.and mixed well.

Preparation of Mobile Phase
Prepared a mixture of pH 3.4 Acetate buffer and Acetonitrile in the ratio of volumes 10:1. Filtered through a 0.45 µm membrane and degas.

Diluent preparation: Use Milli-Q water.

Preparation of standard solution
Weighed and transferred 25 mg of Cefuroxime Sodium RS into 25 mL volumetric flask and diluted to volume with water. Immediately transferred 5.0 mL of the resulting solution to a 100 mL volumetric flask, added 20.0 mL of internal standard solution, diluted to volume with water, and mixed well.

Preparation of Internal standard solution
Weighed and transferred 75 mg of Orcinol into 50 mL volumetric flask and diluted to volume with water.

750 mg/vial Sample preparation
Reconstituted 1 vial with 10 mL of water and rinsed the vial thoroughly with water and transferred the contents into 200 mL volumetric flask. Repeat the procedure 2-3 times and added the rinsing to the volumetric flask, diluted the solution up to the mark with water and mixed well. Transferred 5 mL of this solution to 20 ml volumetric flask and diluted to the volume with water.

Further transferred 5 ml of the above solution to 100 ml volumetric flask containing 20 ml of internal standard solution, diluted with water to volume and mixed well.

1500 mg/vial Sample preparation
Reconstituted 1 vial with 10 mL of water and rinsed the vial thoroughly with water and transferred the contents into 200 mL volumetric flask. Repeated the procedure 2-3 times and added the rinsing to the volumetric flask, diluted the solution up to the mark with water and mix well. Transferred 2.5 mL of this solution to 20 ml volumetric flask and dilute to the volume with water. Further transferred 5 ml of the above solution to 100 ml volumetric flask containing 20 ml of internal standard solution, diluted with water to volume and mixed well.

Placebo solution
Reconstituted 1 placebo vial with 10 mL of water and rinsed the vial thoroughly with water and transferred the contents into 200 mL volumetric flask. Repeated the procedure 2-3 times and added the rinsing to the volumetric flask, diluted the solution up to the mark with water and mix well. Transferred 2.5 mL of this solution to 20 ml volumetric flask and diluted to the volume with water. Further transferred 5 ml of the above solution to 100 ml volumetric flask diluted with water to volume and mixed well.

Chromatographic conditions: Chromatographic analysis was performed on Zodiac C8 (150 x 4.6mm, 5µm) mobile phase consisted of pH 3.4 acetate buffer and acetonitrile in the ratio of (10:1 v/v). The flow rate was 2.0mL.min-1. The flow rate was 1.0mL/min, column oven temperature 25°C, the injection volume was 10µL, and detection was performed at 254 nm using a photodiode array detector (PDA).

3.0 RESULTS AND DISCUSSION

Method development: Spectroscopic analysis of compound Cefuroxime showed that maximum UV absorbance (λmax) at 254 nm respectively. To develop a suitable and robust LC method for the determination of Cefuroxime, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Inertsil ODS-3V, 150x4.6mm, 5µm with the following different mobile phase compositions like that 0.1% orthophosphoric acid buffer and acetonitrile in the ratio of 85:15 v/v. It was observed that when Cefuroxime was injected, higher retention time, Peak Tailing, not satisfactory. For next trial the mobile phase consisted of pH 3.4 acetate buffer and acetonitrile in the ratio of 10:1 v/v respectively, flow rate 2.0 mL/min. UV detection as performed at 254nm. The retention time of Cefuroxime is 3.05 minutes and the peak shape was good. The chromatogram of Cefuroxime standard using the proposed method is shown in **Figure: 1.2** system suitability results of the method are presented in **Table:1.2.**

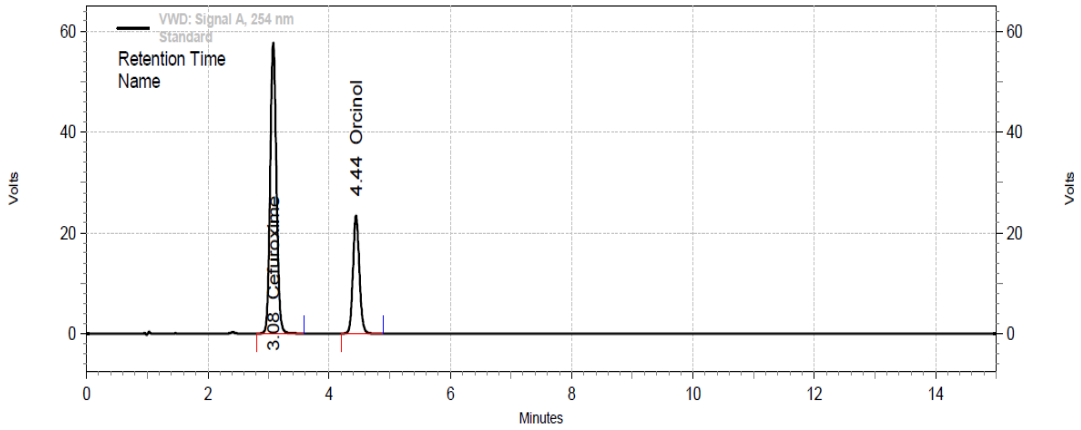

Figure 1.2: Chromatogram showing the peak of Cefuroxime.

4.0 Method validation

The developed RP-HPLC method extensively validated for assay of Cefuroxime using the following parameters.

4.1 *Specificity & System suitability*

Blank and Placebo interference: A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **Figure:1.3** showed no peak at the retention time of Cefuroxime peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Cefuroxime in Cefuroxime injection. Similarly chromatogram of placebo solution **Figure: 1.4** showed no peaks at the retention time of Cefuroxime peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Cefuroxime in Cefuroxime injection.

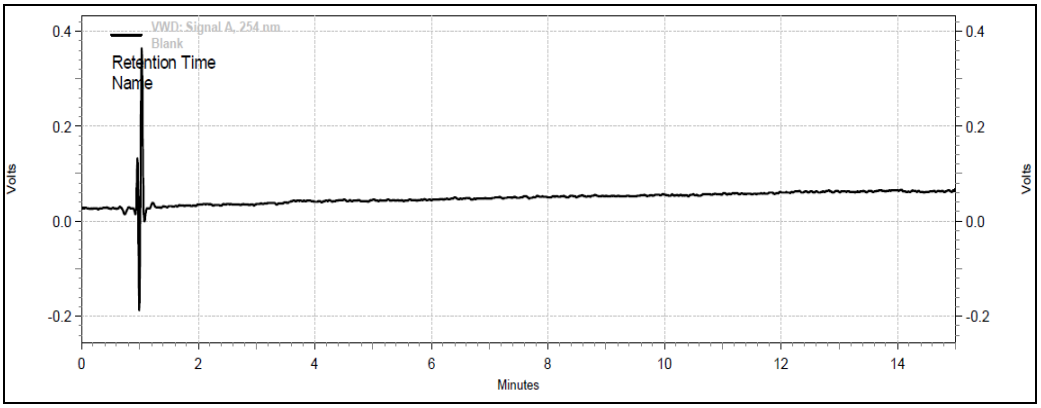

Figure 1.3: Chromatogram showing the no interference of diluent for Cefuroxime.

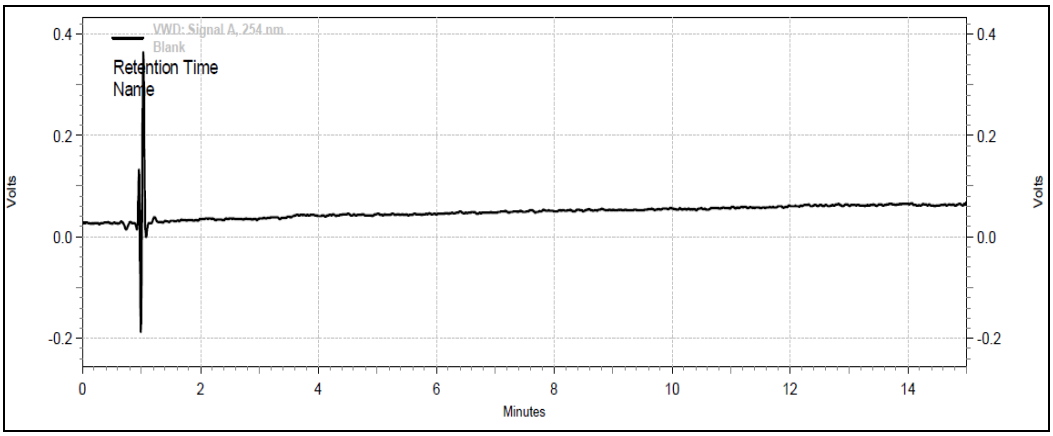

Figure: 1.4 Chromatogram showing the no interference of placebo for Cefuroxime.

Table 1.1: Specificity results for Cefuroxime.

S.No	Name	Retention Time (min)
1	Blank	ND
2	Placebo solution	ND
3	Standard solution	3.10
4	Sample solution	3.00

- The chromatogram of blank and placebo are not showing any peak at the retention time of Cefuroxime.

Table 1.2: System suitability parameters for Cefuroxime.

No.of injections	Tailing factor	Theoretical plates	Area of Cefuroxime	Area of Orcinol	Ratio
Inj-1	1.1	4202	6956680	2866150	2.427
Inj-2	1.1	4194	6950549	2867425	2.424
Inj-3	1.1	4187	6947449	2868110	2.422
Inj-4	1.1	4182	6943535	2866312	2.422
Inj-5	1.1	4175	6929334	2867075	2.417
Inj-6	1.1	4180	6922998	2868520	2.413
		Average			**2.421**
		SD			**0.0050**
		%RSD			**0.21**

4.2 *Method precision:* The precision of test method was evaluated by doing assay for six samples of Cefuroxime injection as per test method. The content in mg and % label claim for Cefuroxime for each of the test preparation was calculated. The average content of the six preparations and %RSD for the six observations were calculated. The chromatogram was shown in **Figure: 1.5** and data were shown in **Table: 1.3.**

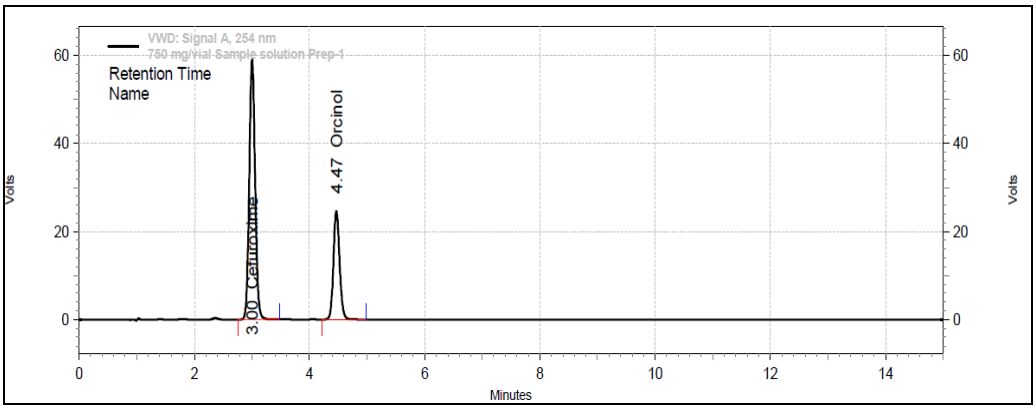

Figure 1.5: Method precision sample chromatogram.

Table 1.3: Method precision data for Cefuroxime.

No. of injections	Assay Cefuroxime
1	100.49
2	100.24
3	100.31
4	100.23
5	100.20
6	100.98
Average	**100.41**
%RSD	**0.30**

4.3 *Intermediate Precision*

The intermediate precision of test method was demonstrated by carrying out method precision study in six samples, representing a single batch by two different analysts on two different days, different column, different HPLC system and by different analyst. These samples were prepared as per the test method. The % assay was calculated for each of these samples. The precision of the method was evaluated by computing the % Relative standard deviation of % assay of Cefuroxime. The chromatogram was shown in **Figure: 1.6** and data were shown in **Table: 1.4.**

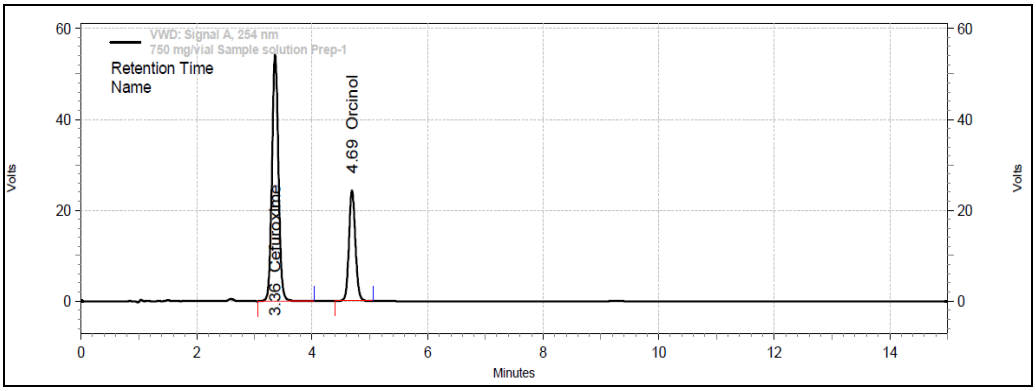

Figure 1.6: Intermediate precision sample chromatogram.

Table 1.4: Intermediate precision data for Cefuroxime.

S.No.	Assay of Cefuroxime
1	100.73
2	100.59
3	100.47
4	100.31
5	100.21
6	100.05
Average	**100.39**
%RSD	**0.25**

- Overall and individual % of Assay are complies as per test method specification.
- The relative standard deviation of six assay preparations is 0.26.
- The overall relative standard deviation of six assay preparations of precision study and six assay preparations of intermediate precision study is 0.43.

4.4 *Linearity of detector response*

The standard curve was obtained in the concentration range of 26-156 ppm for Cefuroxime. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r] of standard curve were calculated and given in **Figure: 1.7** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.5** the method was found to be linear within the proposed range.

Table 1.5: Linearity studies for Cefuroxime by proposed method.

%Level	Concentration (ppm)	Area
50	0.026	1.123
75	0.039	1.669
100	0.052	2.256
125	0.065	2.729
150	0.078	3.321
300	0.156	6.549
correlation coefficient		0.9999
Slope		41.6829
Intercept		0.0511
%Y-intercept		0.001

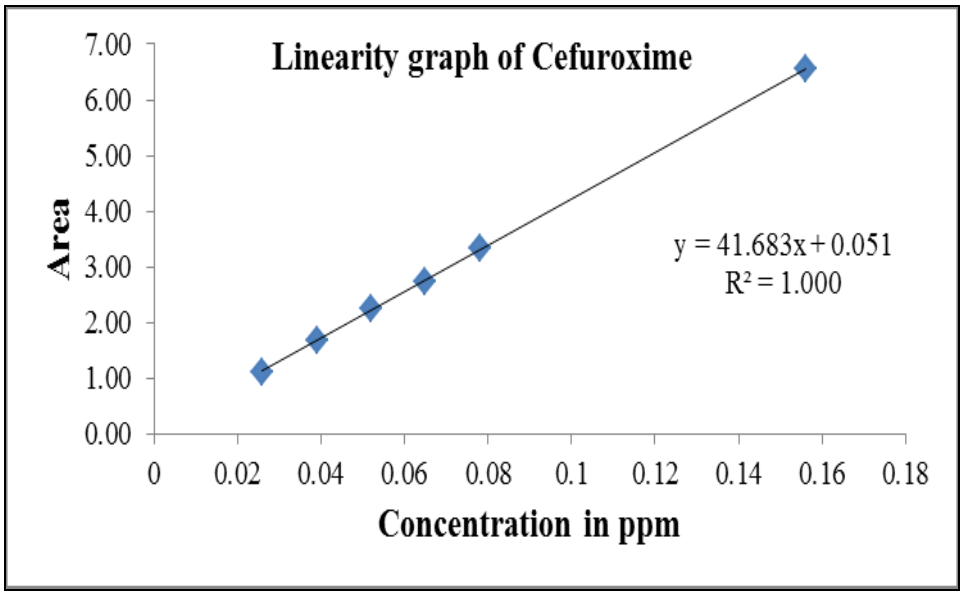

Figure 1.7: Calibration curve for Cefuroxime.

4.4 *Accuracy*

The accuracy of the test method was demonstrated by preparing recovery samples of Cefuroxime at 50%, 100%, 150% and 300% of the target concentration level. The recovery samples were prepared in triplicate for each concentration level except 50% and 300 % (50% and 300% are six preparations). The above samples were chromatographed and the percentage recovery of each sample was calculated for the amount added. Evaluated the precision of the recovery at each level by computing the Relative Standard Deviation of six preparations for 50% and 300% level recovery samples results. The percentage recoveries with found in the range of 100.7 to 101.8 for Cefuroxime. The chromatogram was shown in **Figure: 1.8 to 2.1** the data obtained which given in **Table: 1.6** the method was found to be accurate.

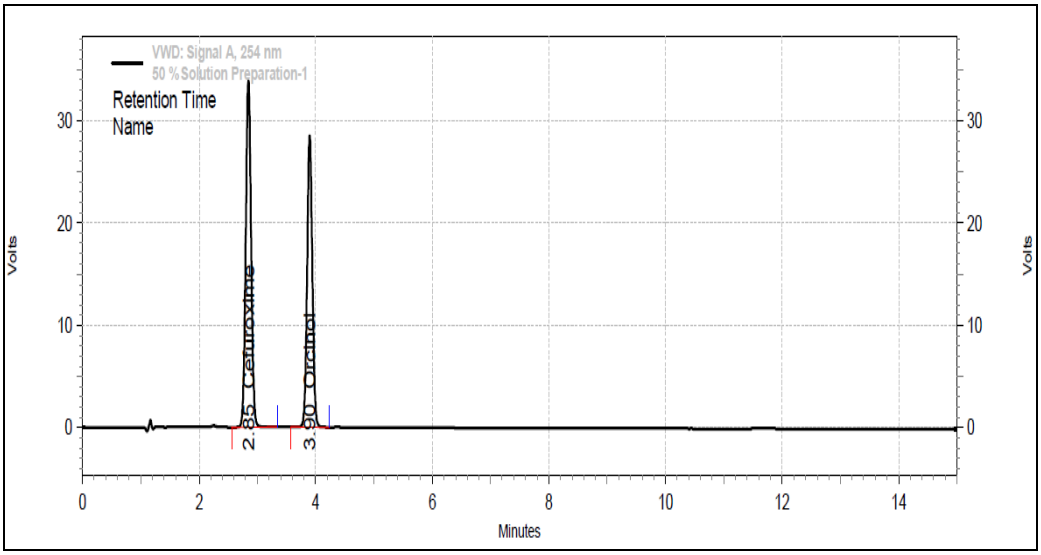

Figure 1.8: Accuracy (Spike level 50%) chromatogram.

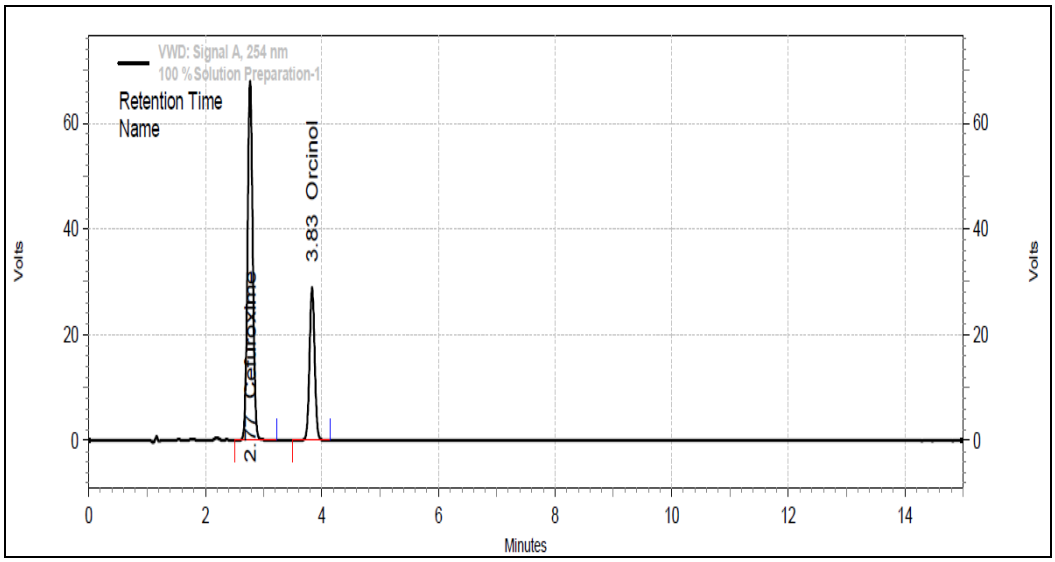

Figure 1.9: Accuracy (Spike level 100%) chromatogram.

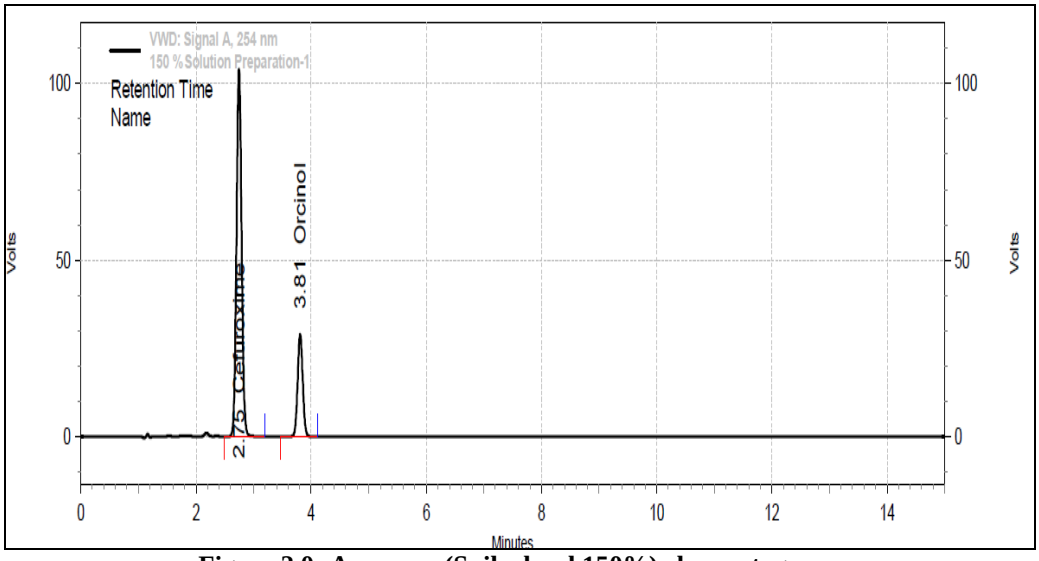

Figure 2.0: Accuracy (Spike level 150%) chromatogram.

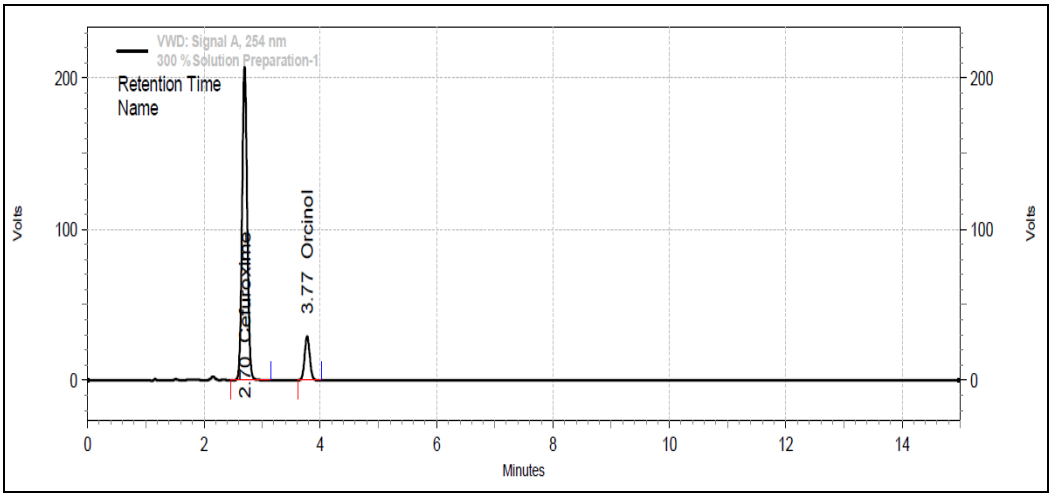

Figure: 2.1 Accuracy (Spike level 300%) chromatogram.

Table 1.6: Recovery studies for Cefuroxime by proposed method.

Recovery of Cefuroxime			
Sample	**% Recovery**	**Mean % Recovery**	**% RSD**
50% sample-1	100.9	101.0	0.27
50% sample-2	100.8		
50% sample-3	101.2		
50% sample-4	101.3		
50% sample-5	101.2		
50% sample-6	100.6		
100% sample-1	101.8	101.8	0.25
100% sample-2	102.0		
100% sample-3	101.5		
150% sample-1	100.9	100.7	0.25
150% sample-2	100.7		
150% sample-3	100.4		
300% sample-1	101.5	101.4	0.28
300% sample-2	101.7		
300% sample-3	101.1		
300% sample-4	101.2		
300% sample-5	101.7		
300% sample-6	101.1		

4.6 *Robustness studies*

To validate the method robustness the chromatographic performance at changed conditions was evaluated compared to nominal conditions of the method. Standard solution was injected at each of the following changed conditions.

Table 1.7: Robustness studies Results.

Parameter		**Theoretical plates**	**Tailing factor**	**%RSD of peak area**
Flow variation ±10%	10%	3811	1.1	0.14
	-10%	4583	1.1	0.14
Temperature variation ±5°C	+5°C	3429	1.1	0.12
	-5°C	3116	1.1	0.52
Mobile phase Variation± 10%	10	3594	1.1	0.17
	-10	3947	1.1	0.23

❖ Method is robust for changes like column oven temperature, flow rate and organic phase of mobile phase.

4.7 *Solution stability of analytical solutions*

Cefuroxime standard and sample solutions were kept for about 48 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The response of these was compared with respect Initial standard solution

and sample solution.

Table 1.8: Results for solution stability of standard at room temperature.

Time Interval	similarity factor
Initial	-
24hrs	1.0
48hrs	1.0

Table: 1.9 Results for solution stability of standard in Refrigerator.

Time Interval	similarity factor
Initial	-
24hrs	1.0
48hrs	1.0

Table: 2.0 Results for solution stability of sample at room temperature.

Time Interval	%Assay	% of Assay difference
Initial	100.41	NA
12hrs	99.85	0.56
24hrs	91.65	8.76

Table: 2.1 Results for solution stability of sample in Refrigerator.

Time Interval	%Assay	% of Assay difference
Initial	100.41	NA
12hrs	100.10	0.31
24hrs	99.25	1.16

- Standard solutions are stable for 24 hours when stored at room temperature and 2-8°C in refrigerator.
- Sample solutions are stable for 12 hours when stored at room temperature (RT) and 24 hours 2-8°C in refrigerator.

5.0 CONCLUSION

An RP-HPLC method for estimation of Cefuroxime was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Cefuroxime in bulk drugs and formulations. The optimized method consists of mobile phase pH 3.4 acetate buffer and acetonitrile in the ratio of (10:1 v/v) with Zodiac C8 (150 x 4.6mm, 5µm)column. The retention time of Cefuroxime was found to be 3.08 minutes. The developed method was validated as per ICH Q2A (R1) guidelines. The proposed HPLC method was linear over the range of 26-156 ppm, the correlation coefficient was found to be 0.9999. The percentage recoveries (accuracy) with found in the range of 100.7 to 101.8 for Cefuroxime. Relative standard deviation (%RSD) for method precision and intermediate precision was found to be 0.30 and 0.25. Solution stability of the Standard stable for 24 hours when stored at room temperature and 2-8°C in refrigerator. Sample solutions are stable for 12 hours when stored at room temperature (RT) and 24 hours 2-8°C in refrigerator.

We have developed a fast, simple and reliable analytical method for determination of Cefuroxime in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Cefuroxime. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Cefuroxime in its different pharmaceutical dosage forms.

ACKNOWLEDGMENT

The authors are grateful to Department of Chemistry, Vignan Degree College, Guntur, Andhra Pradesh, India, for providing facilities to carry this research work.

Conflict of interests

The authors claim that there is no conflict of interest.

6.0 *REFERENCES*

1. B.P. British Pharmacopoeia. Published on the Recommendation of the Commission, 2010; 3: 2476, 3026.
2. Powell DA, James NC, Ossi MJ, et al. Pharmacokinetics of Cefuroxime Axetil Suuspension in Infants and Children. Antimicrob Agents Chemother, 1991; 35(10): 2042–2045.
3. Game, MD, Sakarkar DM, Gabhane KB, et al. International Journal of Chem Tech Research, 2010; 2(2): 1259–1262.
4. Amir SB, Hossain MA, Mazid MA. Journal of Scientific Research, 2014; 6(1): 133–141.
5. Chaudhari SV, Ashwini K, Anuradha A, et al. Indian J Pharm Sci, 2006; 68(1): 59–63.
6. Ranjane PN, Gandhi SV, Kadukar SS, et al. J Chromatogr Sci, 2010; 48(1): 26–28.
7. Shah NJ, SK Shah, VF Patel, et al. Indian Journal of Pharmaceutical sciences, 2007; 69(1): 140–142.
8. Sireesha KR, Deepali VM, Kadam SS, et al. Indian J Pharm, 2004; 66: 278–282.
9. Bulitta JB, Landersdorfer CB, Kinzig M, et al. Antimicrob Agents Chemother, 2009; 53(8): 3462–3471.
10. Ingule P, Dalvi SD, Deepali D, et al. IJPPS, 2013; 5(4): 179–181.
11. Sengar Mahima R, Santosh V Gandhi, Upasana P Patil, et al. International Journal of Chem Tech Research, 2009; 1(4): 1105–1108.
12. Ivana I, Lijljana Z, Mira Z. J Chromatogr A, 2006; 1119(1–2): 209–215.
13. Santosh Kumar P. Research Journal of pharmaceutical biological and chemical sciences, 2012; 3(3): 223–228.
14. Garbacki P, Teżyk A, Zalewski P, et al. Chromatographia, 2014; 77(21–22): 1489–1495.

15. Raj KA, Divya Y, Deepthi Y, et al. International Journal of chemTech Research, 2010; 2(1): 334–336.
16. Pavankumar K, Jagadeeswaran M, Caroline G, et al. IAJPR, 2013; 3(7): 5062–5070.
17. Yuqian Du, Yinglei Z, Zhonggui He, et al. Asian J Pharma Sci, 2013; 8(5): 287–294.
18. Sonia K. International journal of medicinal chemistry & analysis, 2013; 3(2): 70–74.
19. FDA, ICH-Q1A (R2): Stability Testing of New Drug Substances and Products, vol. 68, US Food and Drug Administration, Washington, DC, USA, 2nd edition, 2003.
20. FDA, ICH-Q1B: Photo-Stability Testing of New Drug Sub-Stances and Products, vol. 62, US Food and Drug Administration, Washington, DC, USA, 1997.
21. FDA, ICH-Q2 (R1): Validation of Analytical Procedures: Text and Methodology, vol. 60, US Food and Drug Administration, Washington, DC, USA, 1995.
22. United State Pharmacopoeia, The U.S. Pharmacopeia Convention, United State Pharmacopoeia, Rockville, Md, USA, 30th edition, 2007.