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VALIDATION OF REVERSED - PHASE HPLC METHOD FOR THE ESTIMATION OF CEFIXIME IN CEFIXIME ORAL SUSPENSION.

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ABSTRACT: A simple, fast, precise and accurate, high performance liquid chromatographic method for estimation of Cefixime in Cefixime Oral Suspension was validated using C18 [Hypersil ODS], 4.6x250mm, 5 microns with mobile phase composition of Tetrabutylammonium hydroxide solution: Acetonitrile (760:240), flow rate of 2.0 ml/min and UV detection at 254 nm. Tetrabutylammonium hydroxide solution was prepared by diluting 25ml of 0.4M Tetrabutylammonium hydroxide solution with water to obtain 1000ml of solution, and pH was adjusted to 6.5 with 1.5M Phosphoric acid. The linearity of the method was observed over concentration range of 140- 260 mcg/ml. The accuracy of the proposed method was determined by recovery studies and found to be 99.17-101.00%. The proposed method is precise, accurate selective, reproducible and rapid for the determination cefixime in cefixime oral suspension.

KEYWORDS: Cefixime, RP-HPLC, Cefixime Dry Syrup.

INTRODUCTION:

5-Thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)[(carboxymethoxy) imino] acetyl] amino]-3-ethenyl-8-oxo-, trihydrate, [6*R*-[6α, 7β (*Z*)]]-.(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid, 7^2-(*Z*)-[*O*(carboxymethyl)oxime] trihyzdrate.

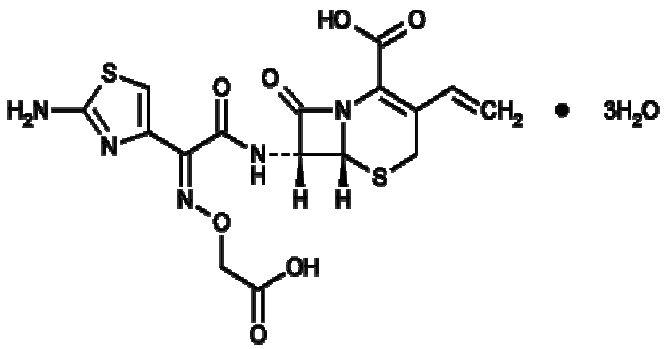

Fig 1. Structure of Cefixime Trihydrate

Cefixime is third generation oral cephalosporin, effective against a wide spectrum of sensitive gram positive, gram negative and anaerobic bacterial pathogens including β-lactamase producing strains. The high performance liquid chromatographic instrument used was Waters with PDA detector. The solvents used were of HPLC grade and other chemicals used were of AR grade. Standard cefixime and formulation were procured from Micro Labs Ltd.

EXPERIMENTAL STUDIES:

INSTRUMENTS:

High performance liquid chromatography, Waters with PDA detector.

Analytical weighing balance, pH Meter, Centrifuge Apparatus, Laboratory Accessories.

Chromatographic conditions:

Equipment	:	HPLC with PDA/UV detector
Analytical Column	:	ODS Hypersil, (250mm x 4.6mm), 5µ
Flow rate	:	2.0 ml/minute
Column oven Temperature	:	40^{o} C
Detector Wavelength	:	254 nm
Injection volume	:	10 µl
Run time	:	15 minutes

Standard Preparation:

About 56 mg of cefixime trihydrate was weighed and dissolved in 30 ml of buffer in a 50 ml volumetric flask and keep it for sonicate for about 5 minutes. Cooled room temperature and then the volume made up with the same solvent system. 10 ml of the above solution was diluted to 50 ml with buffer and mixed.

Sample Preparation:

About 2.95 gm of the suspension were weighed accurately, an equivalent to about 56 mg cefixime trihydrate was transferred to a 50 ml volumetric flask, About 30 ml of buffer was added and sonicate for about 10 minutes. Cooled room temperature and then the volume made up with the same solvent and mixed. 10 ml of the above solution was diluted to 50 ml with buffer and mixed**.**

System Suitability:

Chromatograph the standard preparation five times and record the peak responses as directed under the procedure. The test is not valid unless

a) The resolution R, between Cefixme and Cefixime E-isomer is NLT 2.0
b) The tailing factor for the analyte peak is NLT 0.9 & NMT 2.0.
c) The relative retention times are about 0.9 for cefixime (E)-isomer and 1.0 for Cefixime.
d) The column efficiencies is NLT 4000 theoretical plates for the analyte peak.

Procedure:

Separately inject 10 µL of standard preparation in five replicates and assay preparation in duplicate into the chromatograph and measure the peak responses for the major peaks.

RESULTS AND DISCUSSION:

METHIOD VALIDATION:

The described method has been validated for the assay of Cefixime Trihydrate using following parameters.

1. LINEARITY: The linearity of detector response is established by plotting a graph to concentration and area of Cefixime Trihydrate standard and determining the correlation coefficient. Linearity of Cefixime Trihydrate in Standard preparation from 70% to 130% of Test Concentration (0.2000mg/mL Cefixime Trihydrate) were prepared and injected into the HPLC system. The detector response was found to be linear from 70% to 130% of Test Concentration for Cefixime Trihydrate Standard with a correlation coefficient value is greater than is 0.9997. The results are given below.

Table 1: Linearity of Cefixime Trihydrate in Standard Preparation from 70% to 130% of Test Concentration.

S. No.	% Test conc.	Concentration (µg/mL)	Average Peak Area
1	70	139.60	1282767
2	80	159.37	1470879
3	90	179.40	1646585
4	100	199.10	1824547
5	110	218.84	2025006
6	120	238.37	2190547
7	130	258.46	2401025
Slope			**9336.26**
Intercept			**-23631.23**
Regression Co-efficient			**0.9997**

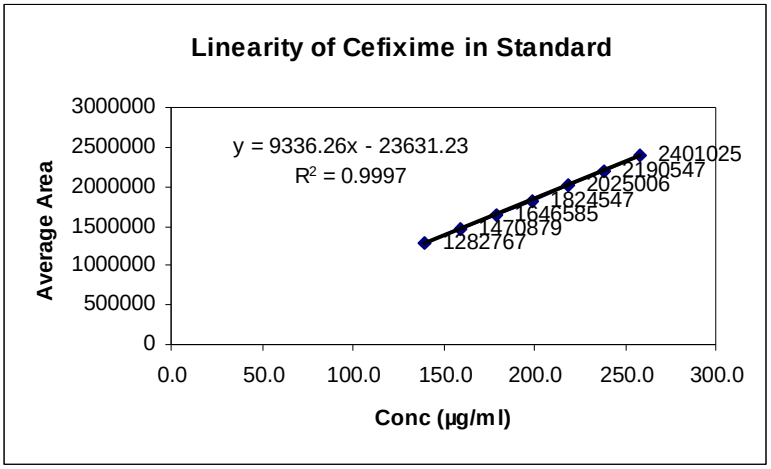

Figure: 2

2. PRECISION: The Precision and Intermediate precision of the method was established by estimating the Assay for six different sample preparations of the same batch. Two different analysts carried out the analysis of Assay of same batch of Cefixime in Cefixime oral Suspension 100mg/5ml using two different instruments two different columns on two different days.

Table 2: Statistical analysis for Precision and Intermediate precision of Cefixime oral Suspension 100mg/5ml.

Sample ID	Assay (% Labeled amount)	
	Precision [Analyst 1]	Intermediate precision [Analyst 2]
Sample-1	105.00	104.44
Sample-2	104.43	104.51
Sample-3	104.76	104.82
Sample-4	103.93	104.42
Sample-5	104.92	104.58
Sample-6	104.81	104.20
Mean	**104.64**	**104.50**
SD	**0.399**	**0.205**
%RSD	**0.4**	**0.2**

3. ACCURACY

Recovery Studies in presence of placebo:

The accuracy of the method was determined by recovery experiments. The recovery was performed by adding Cefixime Trihydrate working standard to Placebo (Cefixime oral Suspension (100mg/5ml) excipients mixture) in the range of 70% to 130% of Test concentration (70%, 80%, 90%, 100%, 110,120 & 130%) and expressed as % recovered.

The mean percentage recovery is 99.99 for Cefixime Trihydrate. The analysis precision expressed as %RSD is 0.4.

Table 3: Recovery of Cefixime Trihydrate from placebo

S. No	Level %	gm added	Wt/ml	Peak Area	gm Recovered	% Recovery	Mean	SD	% RSD
1	70%	940.02	1.1821	1319937	949.46	101.00	99.87	0.992	1.0
2		939.52		1316589	934.12	99.43			
3		938.25		1314569	930.50	99.17			
4	80%	1074.51	1.1826	1492646	1066.33	99.24	99.60	0.317	0.3
5		1073.86		1495689	1071.92	99.82			
6		1074.51		1494569	1071.85	99.75			
7	90%	1208.91	1.1829	1684794	1211072	100.23	100.25	0.074	0.1
8		1208.61		1684532	1210.87	100.19			
9		1207.21		1682356	1211.20	100.33			
10	100%	1343.24	1.1835	1872922	1349.54	100.47	100.46	0.110	0.1
11		1343.08		1873652	1351.49	100.57			
12		1343.56		1874526	1348.14	100.35			
13	110%	1477.38	1.1840	2048219	1473.65	99.75	99.67	0.065	0.1
14		1477.56		2045695	1472.02	99.62			
15		1476.59		2042356	1471.39	99.65			
10	120%	1611.80	1.1844	2235698	1604.54	99.55	99.65	0.120	0.1
11		1611.56		2234950	1606.46	99.63			
12		1612.36		2236526	1608.35	99.78			
13	130%	1745.95	1.1850	2420258	1741.97	99.77	99.84	0.104	0.1
14		1745.62		2420635	1744.94	99.96			
15		1746.21		2421365	1742.53	99.79			
	Over all statistical analysis				**Mean**		**99.92**		
					SD		**0.494**		
					% RSD		**0.5**		

4. STABILITY OF ANALYTICAL SOLUTIONS: The stability of analytical solutions was established by injecting the standard solution and Sample solution at periodic intervals up to 24 hours (0, 3, 6,9,12,15,18,21 and 24 Hours) by keeping the Auto sampler temperature at RT (25°C). The responses of standard solution and Sample solution were measured and the % differences of peak Area were calculated. The values are presented in the Table.

Table 4: Stability of Standard and Sample solution for Cefixime.

Time Interval	Standard		Sample	
	Standard Peak Area	% Difference	Sample Peak Area	% Difference
0 Hour	1821253	-	1963315	-
3 Hour	1825630	0.2	1961795	0.1
6 Hour	1832644	0.6	1954237	0.5
9 Hour	1818082	0.2	1964676	0.1
12 Hour	1813278	0.4	1961420	0.2
15 Hour	1825853	0.3	1959873	0.1
18 Hour	1827949	0.4	1964826	0.5
21 Hour	1824245	0.2	1954532	0.2
24 Hour	1827953	0.4	1959056	0.3

The % difference of peak Area of Standard solution and Sample solution that were injected at periodic intervals were found to be within the specified limit. The solutions of standard and sample preparations prepared as per the method prescribed for the Assay of Cefixime in Cefixime oral Suspension 100mg/5ml are stable up to 24 hours at room temperature (25°C).

5. ROBUSTNESS: Robustness of the method was established by determining the %Assay of Cefixime in Cefixime oral Suspension 100mg/5ml by deliberately modifying the chromatographic conditions specified under the method like flow rate, % Organic modifier in Mobile phase, pH of the buffer in Mobile phase, column oven temperature and wavelength to lower and higher sides of the actual values. The values obtained are given in the table.

Table 5: Robustness results for Cefixime in Cefixime oral Suspension 100mg/5ml.

S. No	Parameter	Condition	Average Std Peak Area	Average Sample Peak Area	Assay (mg/ 5ml)	Assay (% Labeled amount)
1	Flow Rate	2.0 ml /min	1829829	1936871	104.80	104.80
				1939788	104.88	104.88
		1.8 ml / min	2044406	2150852	104.97	104.97
				2152444	104.96	104.96
		2.2 ml / min	1685294	1763240	103.59	103.59
				1755802	103.07	103.07
2	Wavelength	254nm	1829829	1936871	104.80	104.80
				1939788	104.88	104.88
		249nm	1842558	1950097	104.79	104.79
				1952625	104.85	104.85
		259nm	1899774	2009189	104.71	104.71
				2012429	104.80	104.80
3	pH of buffer in mobile phase	pH 6.5	1870149	1996000	104.56	104.56
				1995963	104.25	104.25
		pH 6.3	1879809	1998825	104.17	104.17
				1999147	103.88	103.88
		pH 6.7	1875025	2000658	104.54	104.54
				1998547	104.12	104.12
4	% Organic in Mobile phase	765:235	1848210	1938525	104.86	104.86
				1943617	104.79	104.79
		760:240	1870728	1936606	103.50	103.50
				1936367	103.00	103.00
		755;245	1870049	1938564	10364	10364
				1933719	103.04	103.04
5	Column oven temperature	38^{o}C	1834559	1976967	104.73	104.73
				1974858	104.24	104.24
		40^{o}C	1852801	1975914	103.64	103.64
				1975935	103.27	103.27
		42^{o}C	1865086	1980069	103.18	103.18
				1973728	102.47	102.47

6. SPECIFICITY: The specificity was established by injecting blank (Diluent), Placebo of Cefixime oral Suspension 100mg/5ml (mixture of excipients other than active Ingredient) and Cefixime Trihydrate standard preparations into the chromatograph. It was observed that there was no

interference from the peaks obtained for the chromatograms of blank, placebo and Cefixime peak obtained for the chromatogram of standard preparation. Hence, the chromatographic method used for the estimation of Cefixime in Cefixime oral Suspension 100mg/5ml is selective and specific.

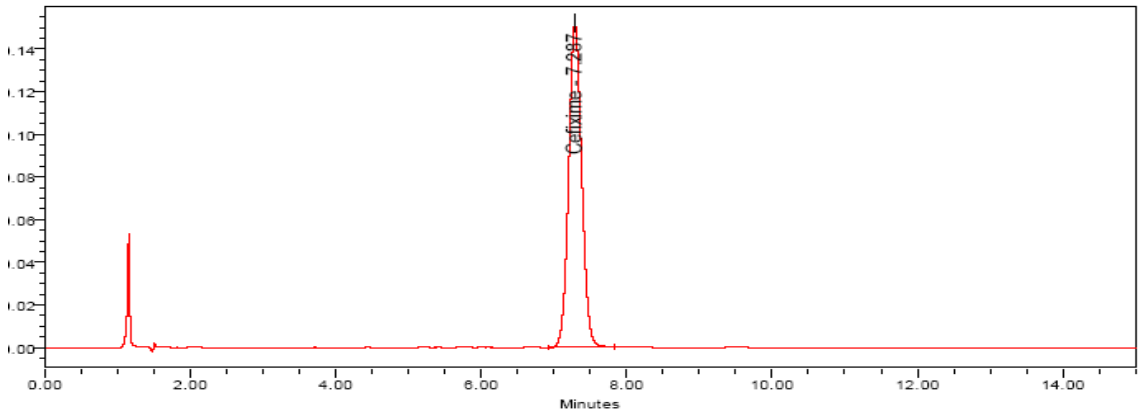

Fig.3 Standard Chromatogram:

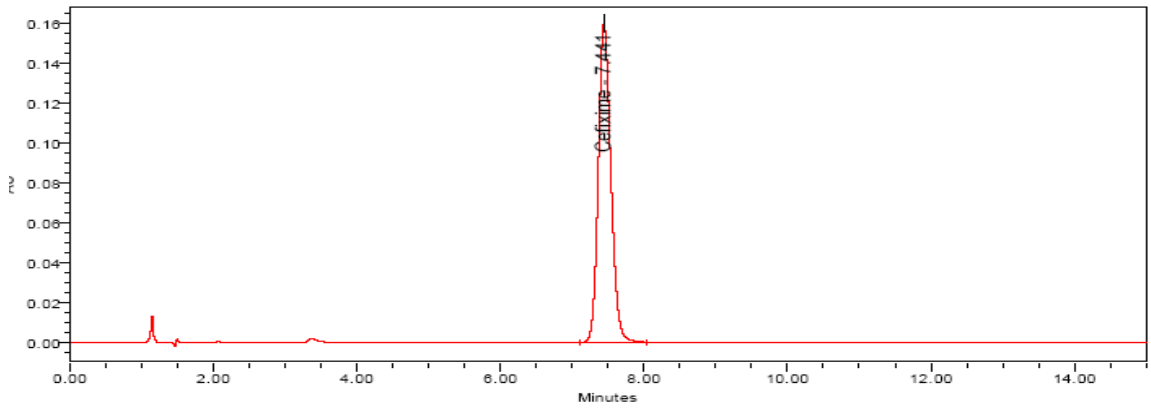

Fig. 4 Sample chromatogram:

CONCLUSION

Cefixime is available in a powder for oral suspension which when reconstituted provides 100 mg/5 ml. The powder for oral suspension is strawberry flavored and contains sodium benzoate, sucrose, and xanthan gum. Cefixime is a broad-spectrum semi synthetic Cephalosporin antibiotic for oral administration. The bactericidal action of Cefixime results from inhibition of cell-wall synthesis.

A HPLC method is validated for the estimation of Cefixime in Cefixime Dry syrup 100mg /5ml using instrument High performance liquid chromatograph.

WATERS HPLC system is being used, having PDA detector fitted with injector and column C18 [ODS Hypersil], 4.6 x 250mm, and 5 microns. Injection volume of 20 µl is injected and eluted with the mobile phase composition of Tetrabutylammonium hydroxide solution: Acetonitrile (760:240), flow rate of 2.0 ml/min and UV detection at 254 nm. Tetrabutylammonium hydroxide solution was prepared by diluting 25ml of 0.4M Tetrabutylammonium hydroxide solution with water to obtain 1000ml of solution, and pH was adjusted to 6.5 with 1.5M Phosphoric acid.

The peak of Cefixime was eluted at about 6.5 minutes. The method is validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, ruggedness and robustness. The results obtained are within the acceptance criteria. The proposed method was found to be satisfactory.

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