Analysis and characterization of cefixime by using IR, HPLC and gas chromatography

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Abstract
To develop a new, simple, sensitive, accurate, and economical analytical methods for the estimation of related compounds in Cefixime formulations. Validate the proposed methods in accordance with USP, EUROPE and ICH guidelines for the intended analytical application. Identify whether the given sample is Cefixime by IR spectroscopy and solubility test. To determine the wavenumber in cm-1 (reciprocal to wave length region) of given sample by Infrared Spectroscopy. To determine the moisture content present in the given sample of Cefixime by Karl Fischer method. Find out the assay and related substances (impurities) of given Cefixime by using the validated method with help of High performance Liquid Chromatography. Estimate the amount of Residual solvents by Gas Chromatography. The tests were performed as per the requirements of EUROPE monograph for Cefixime.

Keywords: Cefixime, IR, HPLC and gas chromatography.

Introduction
Cefixime: Cefixime is an orally absorbed third generation cephalosporin antibiotic that was approved by the U.S. Food and Drug Administration in 1997 for the treatment of mild to moderate bacterial infections.

IUPAC Name:
(6^, 7^)-7-{[2-(2-amino-1, 3-thiazol-4-yl)-2-(carboxy ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
Mol.Formula: $C_{16}H_{15}N_5O_7S_2$
Mol.Wt: 453.452 g/mol
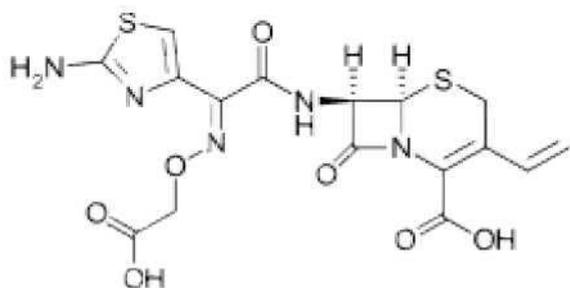
Structure of Cefixime

Therapeutic category: Antibacterial
Cefixime is a broad spectrum cephalosporin antibiotic and is commonly used to treat susceptible infections, including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections.

High Performance Liquid Chromatography (HPLC)
The modern form of column chromatography called high performance, high pressure, high resolution and high speed liquid chromatography. The equipment consists of an a high pressure, injector, eluent and reservoir, for introducing the sample, a column containing the stationary phase, a detector and recorder. The development of highly efficient micro particulate bonded phases has increased the versatility of the technique and has greatly improved the analysis of multi component mixtures.

methoxyimino)acetyl]amino}-3-

Gas Chromatography
Gas-liquid chromatography (GLC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance or separating the different components of a mixture (the relative amounts of such components can also be determined). In some conditions, GC can help in identifying a compound. In beginning chromatography, GC can be used to prepare pure compounds from a mixture. The major factor in separation is due to analytes having different affinities for the stationary phase.

Experimental Procedure
Standard test procedure Solubility: The qualitative visual test for solubility has been included confirm that incoming batches of Cefixime drug substance specifications and it is based on Europe.
Cefixime is slightly soluble in water and soluble in methanol
Reagents:
1. Water
2. Methanol

Procedure

Place 10 mg of sample in 10 ml of H2O; place 1 gm of sample in 30 ml of methanol. Shake vigorously for 30 seconds at an interval of 5 minutes. Observe the solubility behaviour for 30 minutes. It is considered to be completely soluble, if none of the particles or droplet of the solute is observed.

Identification

a) Infrared Spectroscopy: Mix the sample with KBr to finely powdered and prepare the pellets by pressing the mixture in a die and identified by infrared absorption spectroscopy. If the spectra obtain show difference, dissolve the sample and Reference substance separately in CH3OH, evaporate to dryness and record the new spectra using the residues. Suspend 0.5 gm of sample in H20 and dilute to 10 ml with same solvent.

a) pH:

OPTIMIZED METHOD

Column:	Novapak (150 mm x 3.9 mm) C 18, 4p
Flow rate:	1 ml/min
Detection:	254 nm
Column oven temp:	40 c
Injection vol:	10 pl
Run time:	20 min

Preparation of tetrabutyl ammonium hydroxide solution: Dissolve 8.2 gm of tetrabutyl ammonium hydroxide in 800 ml of water. Adjust the pH to 6.5 ± 0.05 with dilute phosphoric acid and dilute to 1000 ml with water.

Preparation of Mobile phase: Mix 770ml of tetra butyl ammonium hydroxide solution with 230 ml of acetonitrile and homogenize. Filter the solution through 0.45 p membrane filter.

Preparation of Diluent:

Use mobile phase as diluent.
METHOD DEVELOPMENT FOR ASSAY Selection of diluent
Cefixime is freely soluble in water and methanol. Mobile phase tetrabutyl ammonium hydroxide pH 6.5: acetonitrile 77:23 V/V was selected because of its more extraction efficiency with less base line disturbances.

Selection of detector wave length

Based on the spectrum obtained by 10 pg/ml sample of Cefixime in water. The absorption maxima 254 nm were selected as detector wavelength.

Selection of mobile phase composition

After screening of experiments by using different compositions of buffer and organic phases pH 6.5 is selected. Mixture of tetrabutyl ammonium hydroxide and acetonitrile 77:23 V/V was selected due to low

retention time and high plate count.

Selection of column

Novapak (150 mm x 3.9 mm) C 18, 4p column is selected due to high stability at environmental pH and less retention time for Cefixime peak with good peak shape and high plate count.

Fixing of flow rate and injection volume

1.0 ml/min was selected due to optimum retention time for cefixime with high plate count.
10 pl injection volume was fixed due to good peak shape of cefixime without distortion. Assay System suitability sample was injected before analysis. System suitability results indicate the suitability of chromatographic system for assay analysis.

Preparation of Resolution solution

Weigh and transfer about 10mg of cefixime working standard into a 10 ml volumetric flask. Dilute to volume with water. Heat this solution on a water bath for 45 min and cool, filter the solution through 0.45p membrane filter.
Note: Use this solution promptly.

Preparation of Standard solution

Accurately weigh and transfer 50 mg of cefixime working standard into a 50 ml volumetric flask. Add 35 ml of diluent and sonicate to dissolve. Dissolve in and dilute to volume with diluent, filter the solution through 0.45p membrane filter.

System suitability

Inject 10 pl of resolution solution into the chromatogram, using the given chromatographic parameters and record the peak responses.
1. The resolution between Cefixime E- isomer and cefixime peaks is NLT 2.0
2. The Retention time of cefixime E-isomer and cefixime peaks are around 9 and 10 min, respectively.
3. The RSD of the areas of cefixime determined from Six replicate injections of Standard solution is NMT 1.0%

Preparation of Sample solution

Accurately weigh and transfer about 50 mg of sample to be analyzed into a 50 ml volumetric flask. Add 35 ml of diluents and sonicate to dissolve. Dilute to volume with diluent. Filter the solution through 0.45 p membrane filter.

Procedure

Seperately inject 10 pl of sample solution in duplicate into the chromatograph record the chromatograms and measure the peak responses of cefixime.

Calculation

Assay of Cefixime (%w/w on an anhydrousbasis) where

AT = Average of the area of the cefixime peak in the chromatogram obtained from the sample peak.

AS = Average of the area of the cefixime peak in the chromatogram obtained from the standard peak.

WS = Weight of the standard in mg.

WT = Weight of the sample in mg.

P = Purity of the cefixime working standard (% W/W on as is basis)
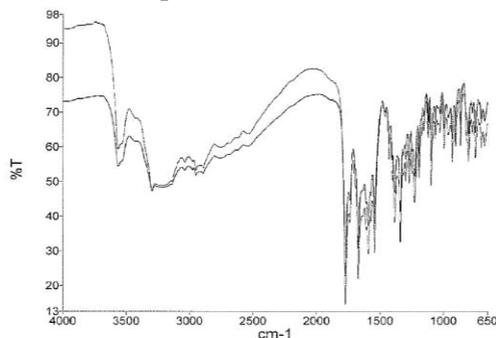
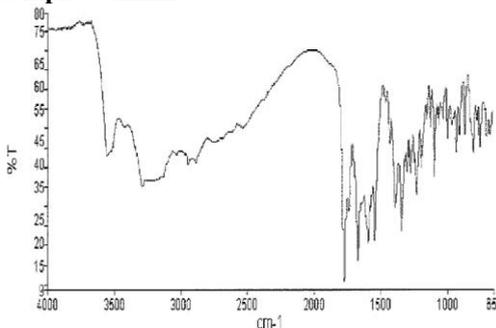Z = Water content of the sample (% W/W by KF)

Results and Discussion

Description

The given sample Cefixime was observed as Almost White owder and slightly hygroscopic.

Solubility: The given sample cefixime was Slightly soluble in water, soluble in methanol.

Graph 1: Spectrum of Both Standard and Sample Cefixime

Graph 2: IR spectrum of pooled sample Ceflxime

Identification

Table 1: Peak and intensity of Standard Ceflxime

Peak Name	X	Y
10	1225.83	51.99
9	1337.56	42.67
8	1384.17	48.1
7	1542.03	39.91
6	1569.88	47.72
5	1591.53	39.24
4	1669.97	33.41
3	1737.26	47.87
2	1771.89	26.08
1	3295.75	54.03

Graph 3: IR spectrum of pooled sample Ceflxime
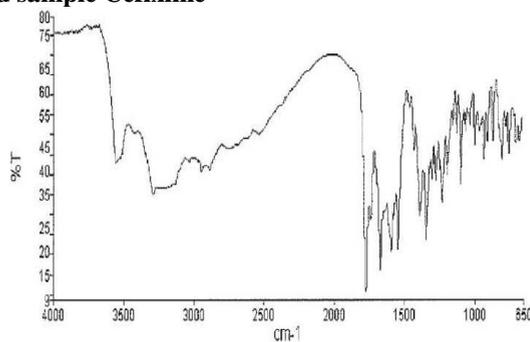
Table 2: Peak and intensity of Cefixime Sample

Peak Name	X	Y
10	1225.72	33.11
9	1337.46	23.71
8	1383.86	29.82
7	1541.92	21.33
6	1569.96	29.14
5	1591.54	20.73
4	1670.32	15.84
3	1737.26	28.89
2	1771.9	10.46
1	3296.11	53.83

Identification of Cefixime by Assay Technique

Assay is the potency of the drug, this test reveals that % of assay of given Cefixime sample using Cefixime standard

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0.050-] ----------------------------------------------------------------------------------------------------------------------
0.040
0.030
0.020
0.010-
o.ooo-j ---------------------------------------------------------------------------------------------- * ----------
2.00                    4 00      6.00      8.00      10.00     12100    14.00     16.00     18 00      20.C
```
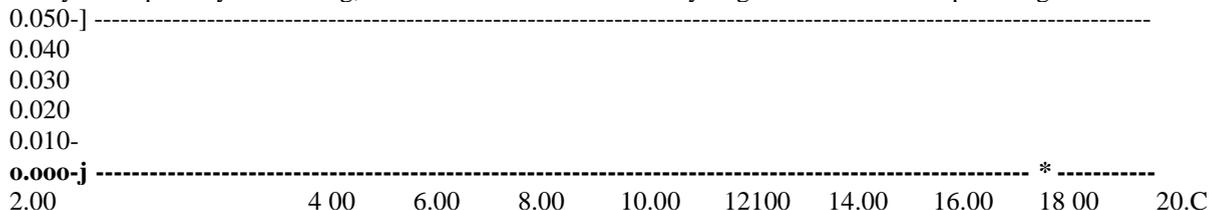
Blank Solution of Cefixime

Graph 4: Resolution chromatogram of Cefixime
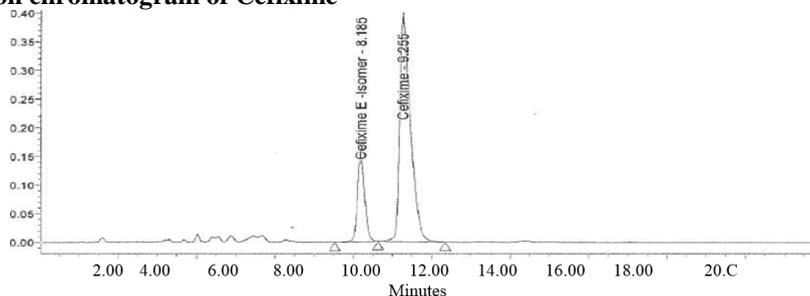
System Suitability

1.   The resolution between Cefixime E- isomer and cefixime peaks is 1.2.
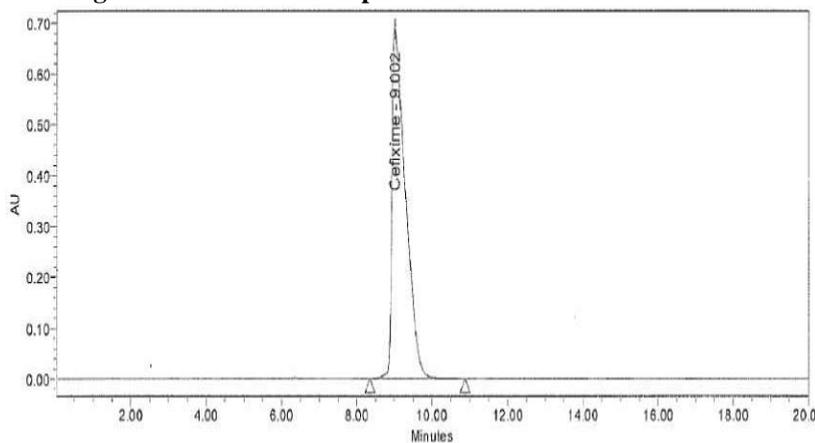
The Retention time of standard cefixime peak is 9.

Graph 5: Trail 1 Chromatogram for Ceflxime Sample

Table 3: Results for Trial 1 Ceflxime sample

	Name	RT	Area (^V*sec)	%Area	IntType
1	Cefixime	9.007	17142521	100.00	BB

Graph 6: Trail 2 Chromatogram for Cefixime Sample

Table 4

	Name	RT	Area (^V*sec)	%Area	IntType
1	Cefixime	9.002	17148319	100.00	BB

The assay of given Cefixime sample was calculated

$$\frac{AT}{AS} \times \frac{WS}{"50"} \times \frac{50}{WT} \times P \times \frac{100}{100-Z}$$

The potency of the given sample is = 99.79 %

Method Validation

Chromatogram of Specificity:
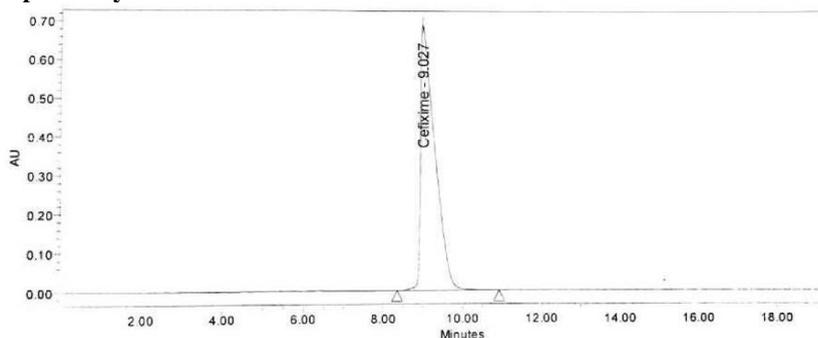
Fig. 1: Chromatogram for Standard

Table 5: Data of specificity

S.No	Name	Retention time	Area	USP plate count	USP tailing
Standard	Cefixime	9.027	17312296	4198	1.46
Sample	Cefixime	9.007	17142521	4518	1.47

Inference

The R_t of standard and test sample was found to be almost equal. Hence the method is specific for estimation of Cefixime.
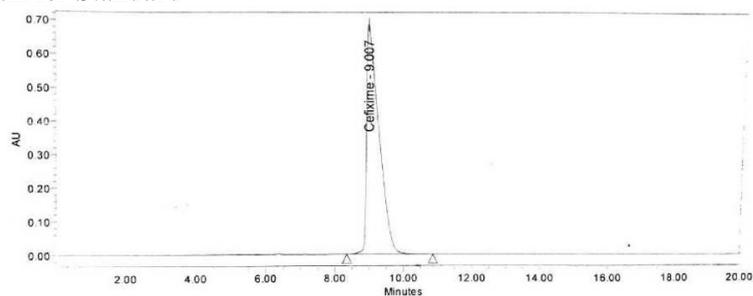
Accuracy

Table 6: Data of accuracy

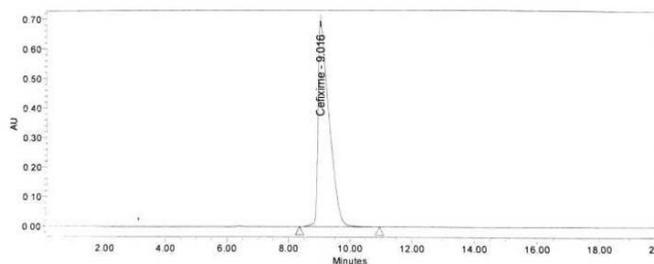|	Accuracy	Amount added (pg)	% recovery	Mean recovery
1.	80%	80	98.95	MEAN=99.93
2.	80%	80	99.90	S.D = 0.81
3.	80%	80	100.95	%RSD = 0.81
4.	100%	100	99.20	MEAN=99.14
5.	100%	100	98.89	S.D = 0.18
6.	100%	100	99.34	%RSD = 0.18
7.	120%	120	99.23	MEAN=99.28
8.	120%	120	99.21	S.D = 0.08
9.	120%	120	99.40	%RSD = 0.08

6 Chromatogram of Specificity:
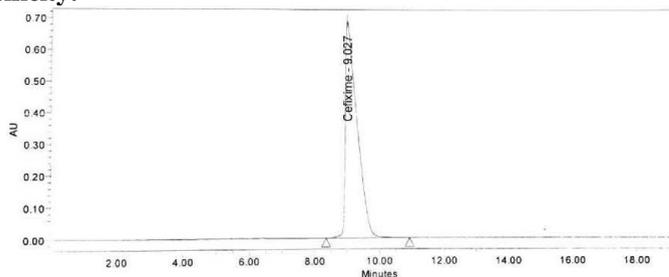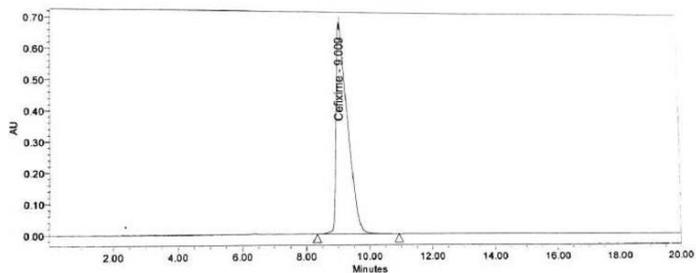
Fig. 2: Chromatogram for Standard
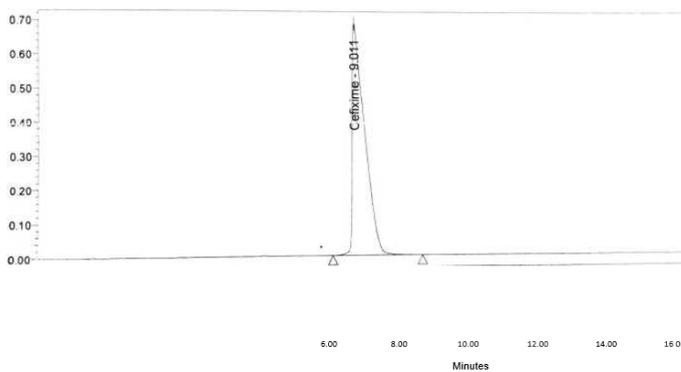

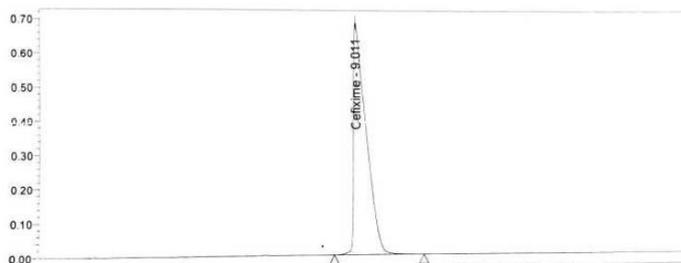**Chromatogram for sample**

Chromatogram: 1
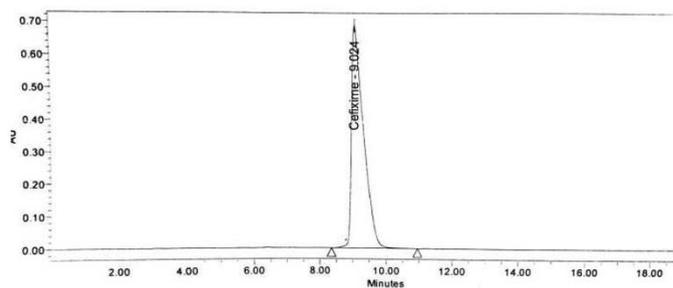

Chromatogram: 2
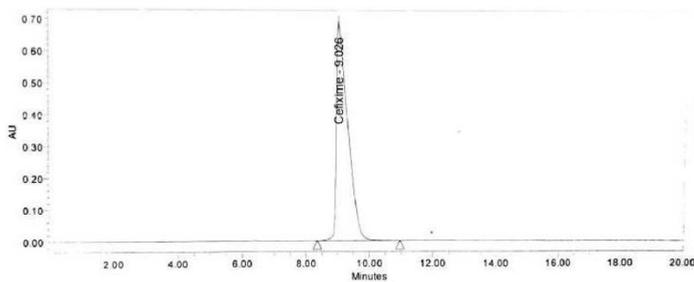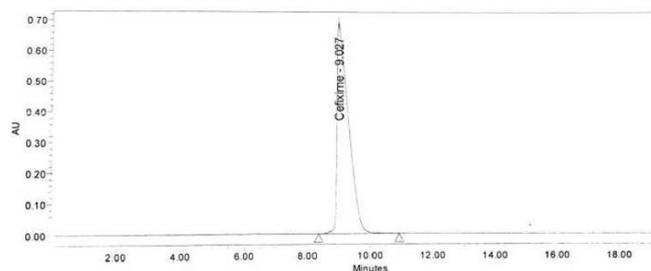

Chromatogram: 3

Chromatogram: 4

Chromatogram: 5

Chromatogram: 6

Chromatogram: 7


Table 7: Data of chromatograms of system precision

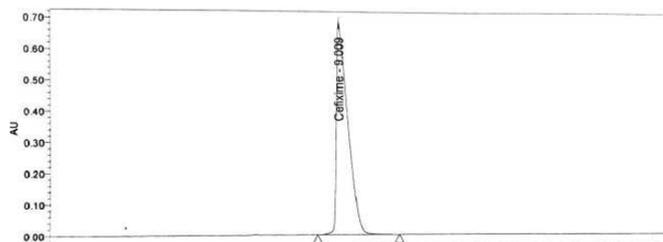| Injection (100 pg/ml)	Retention time	Area
Injection-1	9.016	17301531
Injection-2	9.009	17269092
Injection-3	9.011	17304495
Injection-4	9.024	17271267
Injection-5	9.026	17272472
Injection 6	9.027	17312296
Average		17288525.5
Standard deviation		17899.88
% RSD		0.10

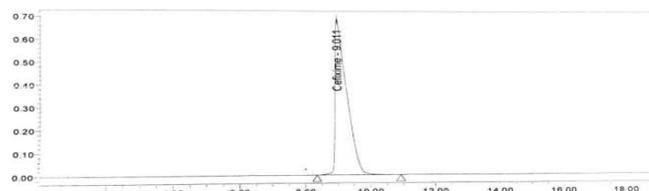**Chromatogram of method precision**

Chromatogram: 1
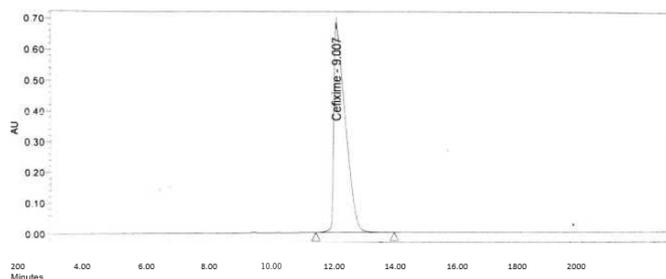
Chromatogram: 2
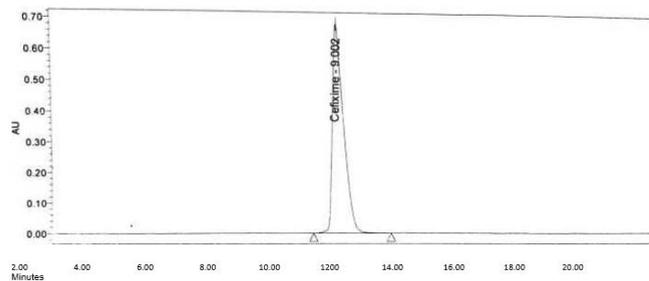
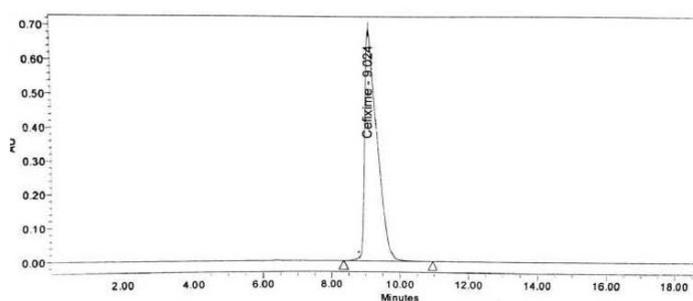

Chromatogram: 3

Chromatogram: 4

Chromatogram: 5

Chromatogram: 6
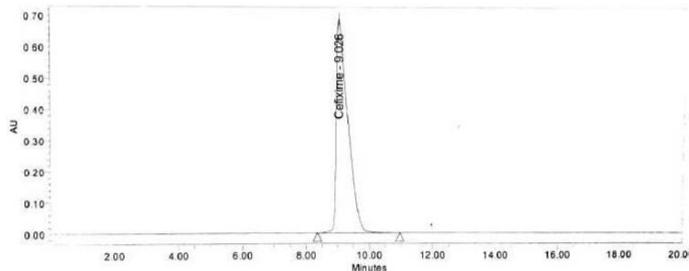
Summary and Conclusion

The antibiotic of Cefixime was subjected to both physical and chemical tests. The various tests like Description, Solubility, Identification, Water content, Assay, Related substances, Residual solvents has been performed for Cefixime.

Identification test was performed by using Infrared Spectroscopy and High performance Liquid Chromatography (HPLC) techniques.

For routine analytical purpose it is desirable to

establish methods capable of analyzing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step.

HPLC & GC methods generate large amount of quality data which serve as highly powerful and convenient analytical tool.

Assay and Related substances were performed by using HPLC technique.

Based on literature review, a HPLC method was developed on Novapak C_{18} (150 mm x 3.9 mm, 4pm particle size) column with tetrabutyl ammonium hydroxide and acetonitrile (77:23, V/V) as mobile phase at a flow rate of 1.0 ml/min with UV detection at 254 nm for estimation of Cefixime. The run time of the HPLC procedure is only 20 minutes. The Proposed RP-HPLC method was suitable technique for estimation of milnacipran hydrochloride in pharmaceutical dosage form without any interference from other excipients.

All the parameters for drug had met the criteria of ICH guidelines for method validation. The developed method may be recommended for routine and QC analysis of investigational drugs to provide simple, accurate and reproducible quantitative analysis. The % RSD of proposed method was found to be less than 2 % shows its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The low values of % RSD indicate the method is precise and accurate.

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