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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT, VALIDATION AND DETERMINATION OF RESIDUAL COMPONENTS OF CEFIXIME IN PHARAMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple rapid and precise Rp-hplc method for cefixime in a pharmaceutical solid dosage form has been developed and validated. Chromatography was performed on a 150mm x 3.9mm i.d 4µparticle C18 Column with Tetra butyl ammonium hydroxide and Aceto nitrile 77:23(v\v) PH-7 adjusted with phosphate buffer as mobile phase at a flow rate of 1ml\min. UV detection was performed at 254nm.Run time 20 min; cefixime retension time is 9.010.The method was validated for accuracy, precision, linearity, LOD, LOQ, Robustness, Ruggedness, specificity, and sensitivity in accordance with ICH guide lines. Validation revealed the method is specific, rapid, accurate, precise, reliable and reproducible. Calibration plot was linear over the concentration ranges 20µg/ ml for cefixime. Limit of detection is 0.14µg/ml and limit of quantification is 2 µg/ml for cefixime. The high recovery and low coefficients of variation confirm the suitability of the method for cefixime drug in tablets. The validated method was successfully used for quantitative analysis of cefixime tablets. Find out the impurities of given cifixime by using the validated method with help of HPLC. Estimate the amount of residual solvents by Gas chromatography.

KEYWORDS: RP-HPLC, GC, Validation, Residual Solvents, Impurities, Cefixime.

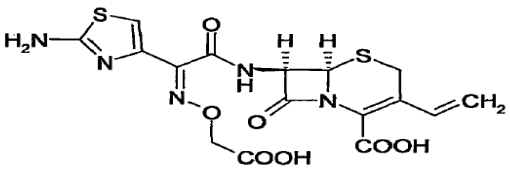
INTRODUCTION

Cefixime chemically(6R,7R,)-7-[2-(2-amino-1,3-thiazole-4-yl)-(carboxymethoxyimino) acetyl)amino]-3-ethelinyl8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid. It is used as antibiotic useful to treat a number of bacterial infection. It is used as bactericidal action inhibition of cell wall synthesis it binds on the pencillin binding proteins which inhibits the final trans peptidation step of the peptide glycon synthesis in the bacterial cell wall, Thus inhibiting biosynthesis & arresting cell wall assembly resulting in bacterial cell death. Cefixime also includes Otitis media, Strepthroat, Pneumonia, Urinary tract infection, Gonorrhea & Lyme disease for gonorrhoea typically only one dose is required. In the united states it is a secod line treatment to cefitriaxone for gonorrhoea. It is taken by mouth.

Different assay techniques ha ve been used for quantitative estimation of cefixime. The UV spectroscopy determination, H PLC Determination, Gas chromatography d etermination.

Mol. Formula: C16H15N5O7

Mol.Wt: 453.452 g/mol



Chemical structure of Cefixime

Experiment

MATERIALS AND METHODS

Cefixime was supplie d by Apollo Pharmacy in Guntur, India as gift sample & used as such Methanol used was Spectro grade from IOCL in chennai LTD, India. Water used was HPLC Grade, Tetra butyl am monium hydroxide -AR grade, Phosphate buffer – AR grade, Tetra hydro furon , ethyl acet ate, Acetonitrile –HPLC grade.

Instrumentation

Quantitative HPLC was performed on low pressure gradient LC-2010CHT SHIMADZU High liquid chr

omatographic instrument foerr the analysis. GC was perform on an low pressure gradient GC -2010CHT SHIMADZU gas chromatographic instrument for the analysis. Instrument is provided with solvent delivery system with PDA Detector & gas chromatography Instrument is provided with solvent delivery module with FID Detector SHIMADZU Phenomix ODS Reverse phase column (150mmx3.9mm) an auto injector & window based CLASS VP software was used for its automatic op eration, recording &analysis. A METTLER TO LEDO Analytical balance was used for weigh ing the materials.

Standard test procedure

Solubility

The qualitative visual test for solubility has been included into the specification to confirm that incoming batches of Cefixime drug substance comply with solubility 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 H_2O; 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

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 PH: suspend 0.5 gm of sample in H20 and dilute to 10 ml with same solvent.

Optimized Method

Column: Novapak (150 mm x 3.9 mm) C_{18}, 4µ
Flow rate: 1 ml/min
Detection: 254 nm
Column oven temp: 40^0 C
Injection vol: 10 µl
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 P^H 6.5 ± 0.05 with dilute phosphoric acid and dilute to 1000 ml with water.

Preparation of Mobile phase

Mix 770 ml of tetrabutyl ammonium hydroxide solution with 230 ml of acetonitrile and homogenize. Filter the solution through 0.45 µ 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 P^H 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 µg/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 P^H 6.5 is selected. Mixture of tetrabutyl ammonium hydroxide and acetonitrile 77:23V/V was selected due to low retention time and high plate count.

Selection of column

Novapak (150 mm x 3.9 mm) C_{18}, 4µ column is selected due to high stability at Environmental p^H 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.10µl injection volume was fixed due to good peak shape of cefixime without distortion.

Assay

System suitability sample was injected before analysis. System suitability result 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 0.45µ 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 0.45µ membrane filter.

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 µ membrane filter.

Procedure: Seperately inject 10 µl 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 anhydrous basis) = AT\AS X WS\50 X 50\WT X P X 100\100-Z

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 sample peak.

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

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)

System suitability: Inject 10 µl of resolution solution into the chromatogram, using the givenChromatographic 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 10min, respectively.
3. The RSD of the areas of cefixime determined from Six replicate injections of Standard solution is NMT 1.0%.

Method Validation

Specificity: Standard solution and sample solution were injected into the chromatographic system.

Retention times obtained from standard and sample were compared for identification of analyte.

Accuracy: The accuracy was performed by spiking standard into 80%, 100% and 120% working concentration samples of Cefixime.

Standard stock 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 0.45µ membrane filter.

Sample stock 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 µ membrane filter.

Preparation of standard spiked 80% sample

0.5 ml of standard stock solution was spiked into 4 ml of sample stock solution present in a 50 ml volumetric flask and volume was made up to the mark with diluent.

Preparation of standard spiked 100% sample

0.5 ml of standard stock solution was spiked into 5 ml of sample stock Solution present in a 50 ml volumetric flask and volume was made up to the mark with diluent.

Preparation of standard spiked 120% sample

0.5 ml of standard stock solution was spiked into 6 mL of sample stock solution present in a 50 ml volumetric flask and volume was made up to the mark with diluents.

Precision

System Precision: The system precision was established by injecting six replicate injections of standard solution into the chromatographic system by maintaining the optimized chromatographic conditions.

Method Precision: Six replicate samples of drug product at 100 µg/ml of concentration were prepared and injected into the chromatographic system.

Linearity: A series of solutions of drug substance standard were prepared in the concentration range from 10 % to 110 % of test concentration to demonstrate linearity for assay by using single plot and injected in to the chromatographic system. A calibration graph is plotted between amount of drug (µg/ml) and chromatographic peak area.

Preparation of standard stock solution

Accurately 50 mg of cefixime working standard was weighed and transferred into a 50 ml clean dry volumetric flask. 35 ml of diluent was added to cefixime and sonicated for 10 minutes with intermittent shaking. Finally the volume was made up to mark with diluent.(1000 µg/ml).

Table. 1.

S. No	Pippeted from stock (ml)	Volume of flask (ml)	Concentration in PPM	%Linearity level
1	0.1	10	10	10
2	0.3	10	30	30
3	0.5	10	50	50
4	0.7	10	70	70
5	0.9	10	90	90
6	1.1	10	110	110

Robustness: As part of evaluation of robustness, deliberate change in the flow rate and temperature composition was made to evaluate the impact on the method.

Effect of variation of flow rate: Sample solution was prepared and analysed by injecting into the chromatographic system maintaining flow rates i.e. less flow (0.8 ml/min), more flow (1.2 ml/min) and actual flow (1.0 ml/min).

Effect of variation of temperature of HPLC column
Sample solution was prepared and injected into the chromatographic system by changing the temperature of HPLC column. The injections were injected at high temperature (45 °C) and at low temperature (35 °C).

LOD and LOQ: According to USP "LOD is the lowest concentration of analyte that can be detected, but necessarily not quantititated, under the stated experimental conditions."According to USP "LOQ is the lowest concentration in a sample that may be measured with an acceptable level of accuracy and precision, under stated experimental conditions."

Forced Degradation Studies
Acid hydrolysis: The solution for acid hydrolysis were prepared in methanol and 0.1N HCl in the ratio of 50:50 (v/v) and refluxed at 60^{o} c about 6 hours.

Alkali hydrolysis: The solution for alkali hydrolysis were prepared in methanol and 0.1N HCl in the ratio of 50:50 (v/v) and refluxed at 60^{o} c about 6 hours.

Neutral hydrolysis: The solution for neutral hydrolysis were prepared in methanol and H_2O in the ratio of 50:50 (v/v) and refluxed at 60^{0} c about 6 hours.

Oxidation: The solution for acid hydrolysis were prepared in methanol and 3% H_2O_2 in the ratio of 50:50 (v/v) and refluxed at 60^{o} c about 6 hours.

Photo Degradation: Photo degradation was induced by exposing samples prepared in methanol to UV light (100-280nm) for 6 hrs. For this samples were place into an irradiation chamber equipped with a 30 W UV – lamp.

Thermal Degradation: The samples were exposed to dry heat in oven at 70^{0}c about 48 hrs and the sample were prepared in methanol and injected to chromatographic system.

Preparation of Dilute phosphoric acid solution: Dilute 115 gm ortho phosphoric acid to 1000 ml with water.

Preparation of P^{H} 6.5 Buffer solution: Mix 6.5 ml of tetra butyl ammonium hydroxide (equivalent to 8.5g) in H_2O and dilute to 800 ml in H_2O. Adjust the P^{H} to 6.5 ± 0.05 with dilute phosphoric acid and dilute to 1000 ml with water.

Preparation of mobile phase
Mix P^{H} 6.5 buffer solution and Acetonitrile in the ratio 77 : 23 and homogenize. Filter and adegas the solution through 0.45µ membrane filter.

Preparation of phosphate buffer P^{H} 7.0 solution
Weigh and dissolve about 28.4 gm of anhydrous disodium hydrogen orthophosphate in 800ml of H_2O.Adjust P^{H} to 7.0 using 345 g/L solution (19.7 ml in 100 ml) of phosphoric acid and dilute to 1000 ml with water.

Preparation of Resolution solution
Accurately weigh and dissolve about 10 mg of Cefixime working standard in 10 ml of water. Heat on a water bath at about 90˚C for 45 minutes. Cool the solution to room temperature, filter the solution through 0.45µ membrane filter.

Preparation of Standard solution A
Accurately weigh and transfer about 25 mg of Cefixime working standard into a 25 ml of volumetric flask. Dissolve in about 2 ml of p^{H} 7.0 phosphate buffer solution and dilute to volume with Mobile phase. Filter the solution through 0.45µ membrane filter.

Preparation of standard solution B
Pipette out 1.0 ml of standard solution A into a 100 ml volumetric flask and diluted to 100 ml with mobile phase. Filter the solution though 0.45 µ membrane filter.

System suitability: Chromatograph the resolution solution using the given chromatographic parameters.The resolution between cefixime E-isomer and cefixime peaks is NLT 2.0 .The retention time of cefixime E-isomer and cefixime peaks are about 16.0 and 18.0 min respectively.The relative standard deviation of the area of cefexime peak determined from 6 replicates injection of standard solution A is NMT 1.0%

Preparation of sample solution
Accurately weigh and transfer about 25 mg of sample solution into 25 ml of volumetric flask Dissolve in about 2 ml of P^{H} 7.0 phosphate buffer solution and dilute to volume with mobile phase. Filter the solution through 0.45µ membrane filter.

Note: Use sample solution with in 4 hrs of its preparation.

Preparation of Blank solution
Transfer 2 ml of P^{H} 7.0 phosphate buffer solution into a 25 ml volumetric flask and dilute to volume with mobile phase. filter the solution through 0.45µ membrane filter.

Procedure: Inject 10 µl each of blank solution, standard solution B (in duplicate) and sample solution into the

chromatograph, using the given chromatographic parameters and record thechromatogram .Examine the blank chromatogram for any extraneous peaks disregard the corresponding peak observed in chromatogram of the sample solution. Also disregard the unknown peaks in the sample chromatogram whose area is less than 0.03 times that of principal peak in chromatogram obtained with standard solution B.The relative resolution time of the known impurity with respect to cefixime are given below.

Table. 2.

S. No	Name of the impurities	~RRT
1	CAVA	0.165
2	CEMO	0.428
3	Impurity B.Ph. Eur (diastereoisomer-1)	0.510
4	Impurity B.Ph. Eur (diastereoisomer-2)	0.540
5	Impurity-Eph. Eur	0.693
6	Impurity-DPh. Eur	0.894
7	Cefixime	1.000
8	Impurity APh.Eur	1.230
9	Impurity Cph. Eur	1.595
10	In house impurity-G	1.898

Determine the peak responses of all eluting peaks from the chromatographic report and calculate the % of Cefixime present in the sample.

Calculation

Any Individual known Impurity =At/As X Ws/25 X 1/100 X25/Wt X P. Highest unknown Impurity =AT1/AS X WS/25 X 1/100 X 25/WT X P Where,

AT = Area of any individual known impurity peak in the chromatogram of sample solution.

AT1 = Area of highest unknown impurity peak in the chromatogram of sample solution.

AS = Average of area of Cefixime peak in the chromatograms obtained from standard solution B.

WS = Weight of standard taken in mg.

WT = Weight of sample taken in mg.

P = potency of Cefixime standard (%W/W, on as is basis) as Cefixime.

Residual Solvents by GC

Chemicals and Reagents

N, N Dimethyl formamide, Acetone, Ethyl acetate, Tetrahydrofuran were purchased from Rankem.

Instrumentation

Gas chromatograph equipped with a flame ionization detector , a split injector and ahead space sampler was used 0.32 mm internal diameter coated with poly stationary phase of 1.0µm film thickness (DB – 1301 Column).

Preparation of Blank Solution

Pipette out 2.0 ml of diluent into a head space vial and seal with crimp cap.

Preparation of Standard Solution

Weigh accurately each about 35 mg of Acetone, Tetra hydro furan and Ethyl acetate into a 25 ml volumetric flask containing about 10 ml of diluents. Dilute to volume with Diluents and mix. Dilute 5.0 ml of the above solution to 25 ml with diluents Accurately transfer 2.0 ml of this solution into six headspace vials, crimp cap and seal And keep for injection.

System Suitability

Inject the blank and the standard solution into the chromatograph using the above given chromatographic and headspace parameters.Record the chromatograms and measure the peak responses.Examine the blank chromatogram for any extraneous peaks. There should be no Interference from the blank at the retention time of analyte peaks those were obtained From standard solution. The resolution between ethylacetate and tetrahydrofuran peaks is not less than 2.0.

Preparation of Sample solution

Accurately weigh and transfer about 500 mg of sample into two headspace vial Add 2.0 ml of diluent to each of the vial Seal the vials immediately with crimp cap.

Procedure

Place the sealed headspace vials of sample solution and perform the headspace analysis. As per the given chromatographic and headspace parameters. Record the peak responses.The retention time of the solutions are given below.

Table : 3

S. No	Name of the solvent	Retention time (min)
1	Acetone	14.86
2	Ethyl acetate	23.02
3	Tetra hydrofuran	23.43

Calculation: Acetone (in PPm) =AT/AS X WS/25 X 5/25 X 2/WT X 10

Where,

AT = Area of Acetone peak in the chromatogram of the sample solution.

AS =Average of the area of Acetone peak in the chromatogram of the standard solution.

WS = Weight of standard acetone in mg.

WT = Weight of Sample in mg.

Similarly calculate the content of other solvent using the area of the respective peak in the chromatogram of the sample and the standard solution.

Report the average of the results obtained from the analysis of sample preparation in duplicate as the content of each individual residual solvent.

RESULTS AND DISCUSSION

Description

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

Solubility

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

Identification

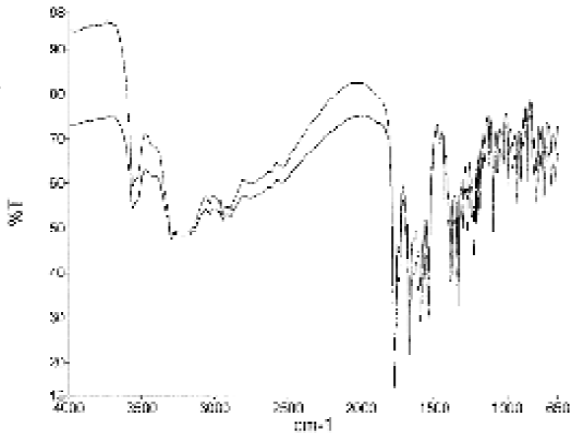


Fig. No. 1: IR Spectrum of Both Standard and Sample Cefixime.

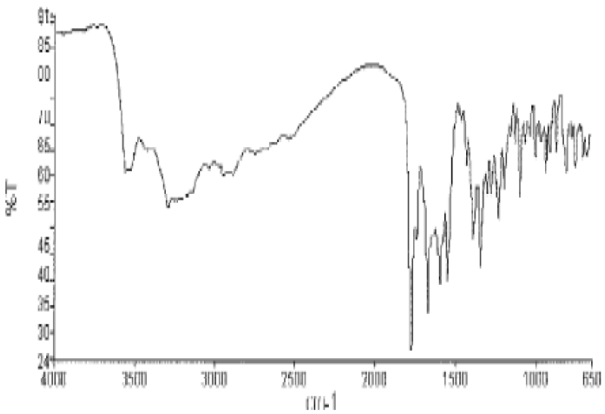


Fig. No. 2: IR spectrum of Standard Cefixime.

Table. 4.

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	1591.52	39.24
4	1669.97	33.41
3	1737.26	47.87
2	1771.89	26.08
1	3295.75	54.03

Peak and intensity of Standard Cefixime

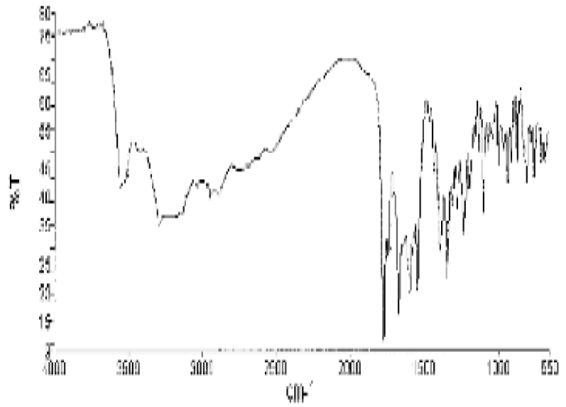


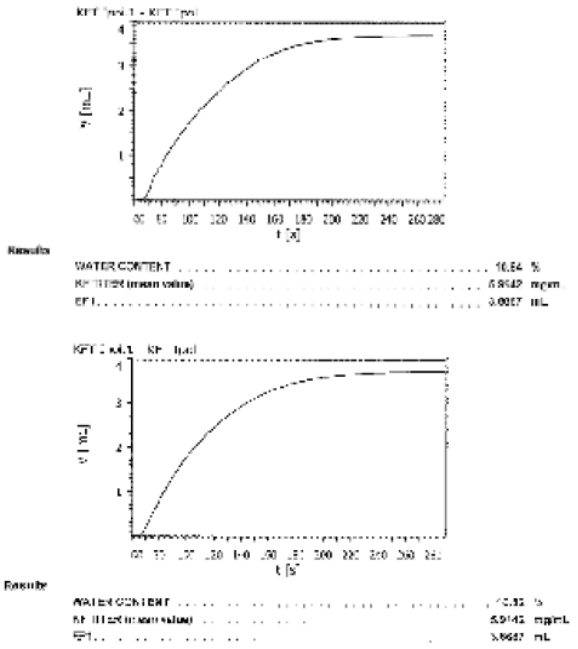
Fig. No.3: IR spectrum of pooled sample Cefixime.

Table. 5: Peak and intensity of Cefixime Sample.

Peak name	X	Y
10	1225.83	33.11
9	1337.56	23.71
8	1384.17	29.82
7	1542.03	21.33
6	1569.88	29.14
5	1591.53	20.73
4	1669.97	15.84
3	1737.26	28.89
2	1771.89	10.46
1	3295.75	53.83

Water content By KF Titrator

The water content value of given Cefixime sample is given with Trial 1 and Trial 2 graphs shown below



Average % of water content is 10.83%

The determination of water content is the important factor in drug analysis because the amount of water content value will be used for further analysis.

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.

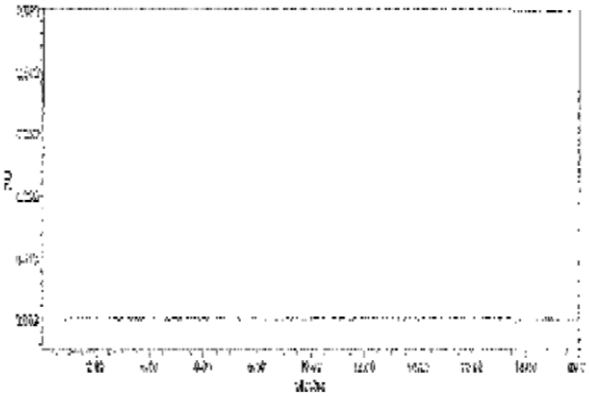


Fig. No.4 :Blank Solution of Cefixime.

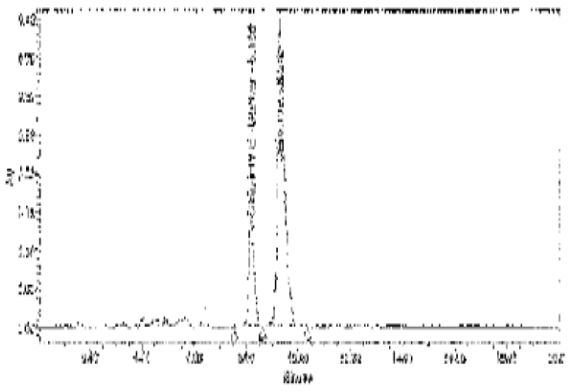


Fig. No.5: Resolution chromatogram of Cefixime.

Table. 6: Results- for System Suitability from Resolution of Cefixime Chromatogram.

S. No	Name	RT	Area (µ* sec)	%Area	Usp resolution	Int type
1	Cefixime (E) - Isomer	8.15	1947218	20.35		BB
2	Cefixime	9.255	7620714	79.65	2.4	BB

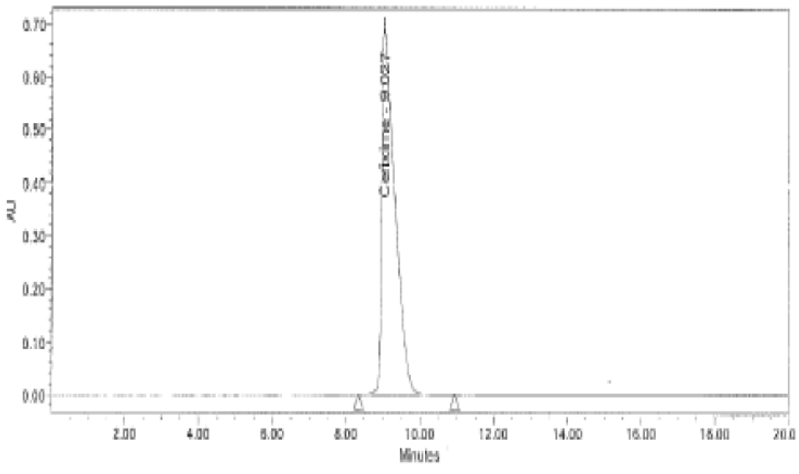


Fig. No. 6: Chromatogram for Standard solution of Cefixime.

Table. 7: Results for system suitability from Standard cefixime.

S. No	Name	RT	Area (µ*sec)	%Area	Int type
1	**Cefixime**	**9.027**	**17312296**	**100.00**	**BB**

System Suitability

The resolution between Cefixime E- isomer and cefixime peaks is 1.2.

The Retention time of standard cefixime peak is 9.
The assay of given Cefixime sample was calculate
AT/AS X WS/50 X 50/WT X P X 100/100-Z
The potency of the given sample is = 99.79 %

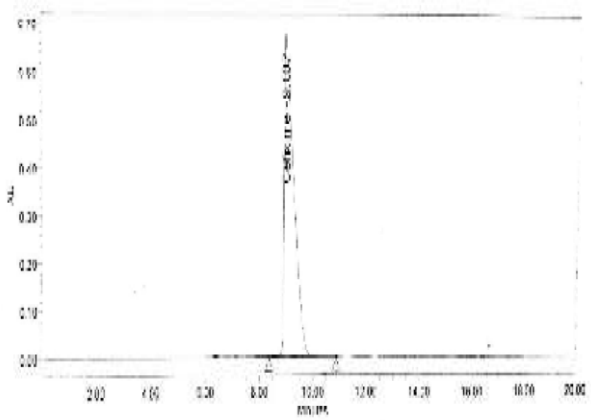


Fig. No. 8. Chromatogram for Sample.

Method Validation

Chromatogram of Specificity

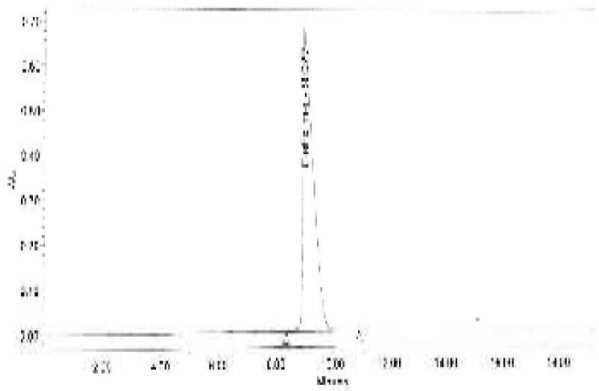


Fig.no.7. Chromatogram for Standard.

Table. no: 8. Data of specificity.

	Name	Retention time (RT)	Area	USP plate count	USP Tailing
Standard	Cefixime	9.027	17312296	4198	1.76
Sample	Cefixime	9.007	17142521	4518	1.47

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

Accuracy

Table 9: Data of accuracy.

Accuracy (%)	Amount Added (µg)	% Recovery	Mean recovery
80	80	98.95	Mean = 99.93
80	80	99.90	S.D = 0.81
80	80	100.95	% RSD = 0.81
100	100	99.20	Mean = 99.14
100	100	98.89	S.D = 0.18
100	100	99.34	% RSD = 0.18
120	120	99.23	Mean = 99.28
120	120	99.21	S.D = 0.08
120	120	99.40	% RSD = 0.08

Chromatogram of System Precision
Data of chromatograms of system precision
Table. 10.

Injection (100µg/ml)	RT	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
Avg		17288525.5
Standard Deviation		17899.88
% RSD		0.10

Acceptance Criteria
The % RSD for the areas of sample injections results should not be more than 2.0 %.

Inference: System precision was performed and % RSD is calculated. % RSD of system precision was found to be 0.10 which is within the acceptable limit.

Chromatogram of Method Precision
Data of method precision
Table: 11.

Injection (100µg/ml)	Retention time (RT)	Area
Injection -1	9.007	17142521
Injection -2	9.002	17148319
Injection -3	9.009	17269092
Injection -4	9.011	17304495
Injection -5	9.024	17271267
Injection -6	9.026	17272472
AVG		17234694.33
Standard deviation		64260.75
% RSD		0.3

Acceptance criteria: The % RSD for the areas of sample injections should not be more than 2.0%

Chromatogram of robustness
Change in flow rate
Data of effect of flow rate

Table: 12.

S. No	Flow rate (ml/min)	System Suitability Results		RT (min)	Peak area	% RSD
		USP Plate count	USP Tailing			
1	0.8	4641	1.47	9.026	17272472	0.16
2	0.8	4698	1.48	9.027	17312296	0.16
3	1.2	4197	1.43	9.007	17142521	0.02
4	1.2	4329	1.43	9.002	17148319	0.02

Acceptance criteria: The % RSD for the areas of sample injections should not be more than 2.0 %.

Change in temperature
Data of effect of chromatogram on temperature
Table. 13.

S. No	Temperature (^{0}C)	System suitability results		Rt (In min)	Peak area	%RSD
		Usp plate count	Usp tailing			
1	35	4395	1.45	9.027	17312296	0.02
2	35	4337	1.46	9.011	17304495	0.02
3	45	4883	1.46	0.009	17269092	0.35
4	45	4523	1.46	9.002	17148319	0.35

Acceptance criteria
The % RSD for the areas of sample injections should not be more than 2.0 %.

Inference: From the study it was found a change in flow rate ± 0.2 ml/min, and change in temperature ± 5 °C doesn't affected the proposed analytical method. Hence, the proposed method was robust for the estimation of Cefixime.

Data of Forced degradation studies
Table. 14.

S. No	Stress conditions	%drug present	
		0 hrs	6hrs
1	Acid hydrolysis :0.1NNaOH,refluxed at 60^{0}C	98.04	97.5
2	Alkali hydrolysis:0.1N NaOH refluxed at 60^{0}C	98.10	93.3
3	Neutral hydrolysis :refluxed at 60^{0}C	98.91	99.99
4	Oxidation :3%H_2O_2,refluxed at 60^{0}C	81.74	91.96
5	Photo degradation :uv light	99.18	99.85
6	Thermal degradation at 70^{0}C about 48 hr	99.37	99.10

Upon conducting the force degradation studies with cefixime sample the results were found to be within the limits.

Limit of Detection and Quantification: Detection and quantification limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, using the formula

Limit of Detection = σ × 3.3/S
Limit of Quantitation = σ × 10/S
Where σ = the standard deviation of the response.
S = the slope of the calibration curve (of the analyte).

LOD and LOQ
Table:15

Sample	LOD(µg/ml)	LOQ(µg/ml)
Cefixime	0.14	2

Identification of Related Substances by HPLC
Related substances are the substances which are related to the drug Cefixime .This test reveals that the substances which are present in the given Cefixime sample.

System suitability
The resolution between cefixime E-isomer and cefixime peaks is 3.0 .The retention time for Standard cefixime peak is 19.49 minutes.

Inference
Method precision was performed and % RSD is calculated. % RSD of Method precision was found to be 0.3 which is within the acceptable limit.

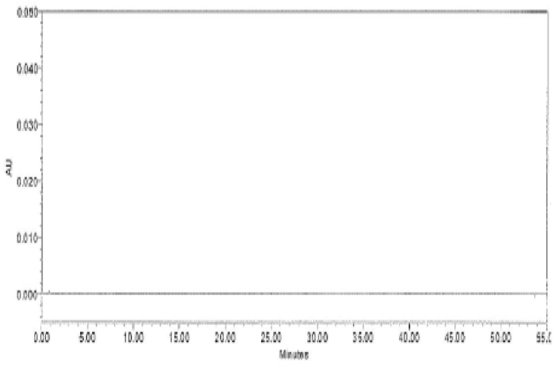


Fig. No. 9. Blank chromatogram of Cefixime.

Table. 16.

	Name	Rt	Area(µv*sec)	%Area	Int type
1	Cefixime	18.995			Missing

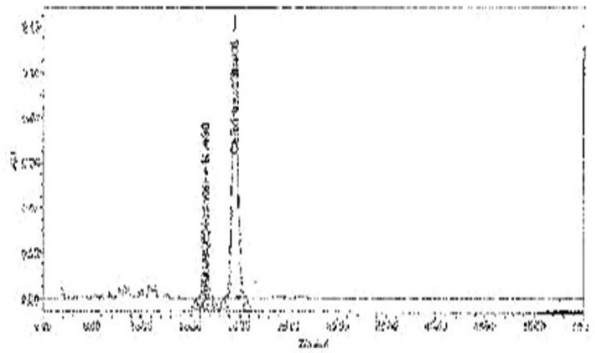


Fig. No. 10. Resolution chromatogram of Standard cefixime.

Table. 17. System suitability value for Standard Cefixime substance.

	Name	Rt	Area (µv*sec)	%Area	Usp resolution	Int type
1	Cefixime(E)- isomer	16.490	1671046	27.99		BB
2	Cefixime	19.466	4299242	72.81	3.6	BB
Sum			5970288			

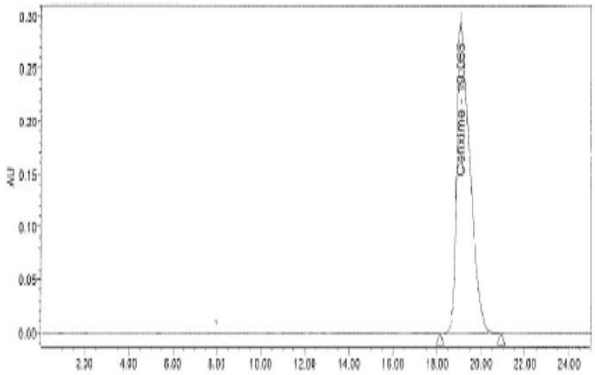


Fig. No. 11. System suitability value for standard Cefixime substance (sol-A).

Table. 18. Peak Results for standard solution A.

	Name	Rt	Area (µv*sec)	% Area	Int type
1	Cefixime	19.055	11890293	100.00	BB

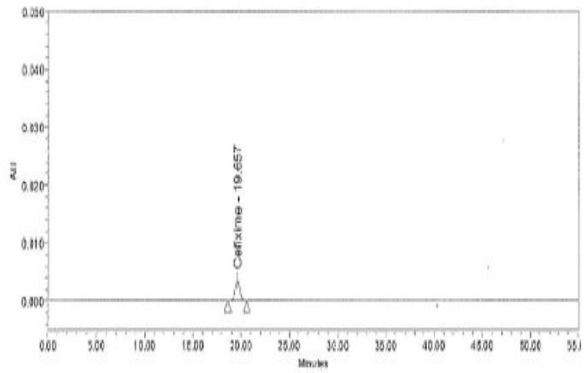


Fig.no.12. Standard Solution B.

Table. 19. Peak Results of Standard solution B.

	Name	RT	Area (µV*sec)	% Area	Int Type
1	Cefixime	19.657	119413	100.000	BB
Sum			119413		

Highest unknown impurity = 0.04 %
Total impurities = 0.4 %

Analysis of HPLC reveals the presence of some impurities which are normally the related or by products which are formed during the production of Cefixime. These impurities are also present with in the EUROPE limit. A part from these some unknown impurities were also present which will be analysed later.

Identification of Residual Solvents by GC: Residual solvents are the solvents which may present in the drug; this test reveals that whether there is any solvents are present and if the present solvents are within the specified limits .System Suitability Resolution between Acetone and Ethylacetate peak is determined.

The results are shown in the chromatogram below

Table. 20. Peak Results of standard solvents.

Peak	RT (min)	Component	BC	Area	% Area	Height
1	10883	Acetone	BB	200331	35.27	18706.6
2	21.107	Ethyl acetate	BB	125071	22.02	18246.9
3	21.610	THF	BB	242602	42.71	39236.5
Sum				568004	100.00	76190.0

From the above chromatograms, it is clear that two solvents are present in the given sample, the amount of solvents was calculated by the formula

Solvent content (in PPm) =AT/AS X WS/25 X 5/25 X 2/WT X 10’6 Where,
AT = Area of Acetone peak in the chromatogram of the sample solution.

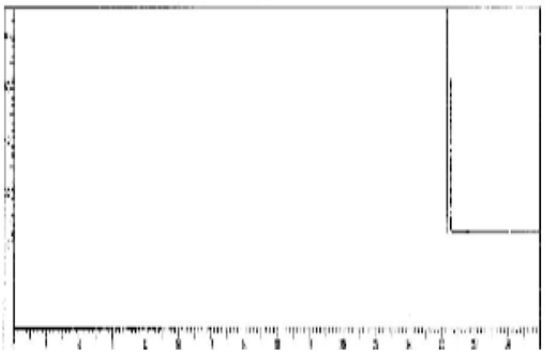


Fig.no.13. Chromatogram of Blank Solution by GC.

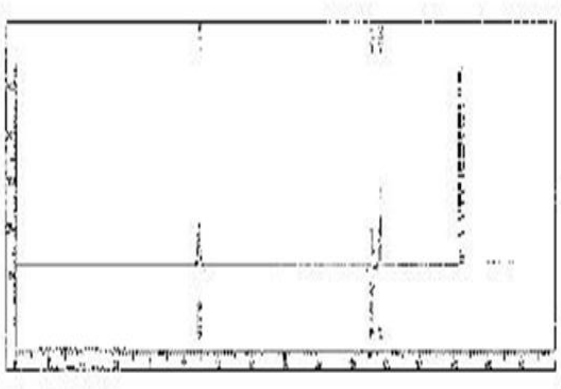


Fig. No. 14. Chromatogram of Standard Solution.

AS =Average of the area of Acetone peak in the chromatogram of the standard solution.
WS = Weight of standard acetone in mg.
WT = Weight of Sample in mg.

The above results reveals that Acetone and Ethyl acetate were not exceed the specified limits as per Europe.

Table. 21.

Residual solvents	Specified limit(ppm)	Observed value(ppm)
Acetone	NMT 5000	4225.99
Ethyl acetate	NMT 5000	5.758

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 with out 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 × 3.9mm, 4µm 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.

Residual solvent test was performed by GC. Tailing factor and plate count results are reveals that the system and the column efficiency were in good condition during analysis. From analytical and statistical data obtained the methods could be successfully applied to determination of Cefixime .The analytical method is suitable to estimate the content and purity of Cefixime. This indicate the results that the antibiotic complies with the specification.Hence, the Validated methods are in accordance with USP, EUROPE and ICH guidelines for the intended analytical application.

"From the study, we concluded that a simple, precise, accurate and economic method was developed for the routine analysis of cefixime in pharmaceutical dosage form".

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