Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1920 - 1926
Received 16 February 2021; Accepted 08 March 2021.

Rs-Method Development of Cefuroxime Acid for Impurities by Hplc

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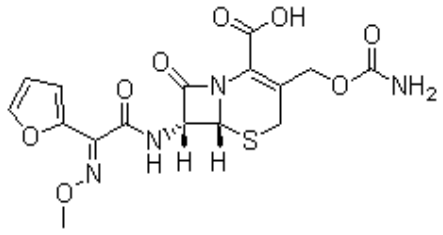
ABSTRACT: HPLC method has been developed for determination of Cefuroxime Acid with its related substances in alaboratory mixture By performing several hidden trials in the laboratory finally a selective, sensitive,robust and accurate method was developed with the following chromatographic set up Column Betasil C1(250*4.6mm,5µ) mobile phase Methanol: Buffer (50:50v/v), flow rate 1.0 ml/min Sample cooler temp 10°C±1.0°C,Column oven Temp. 30°C±1°C,Injection volume 10 µl and eluent was monitored at 278 nm.. After the method is finalised it was validated and it was found to be accurate and precise. The proposed HPLC method developed is found to be selective,sensitive,robust and accurate for the quantification of Cefuroxime acid. The method is capable of detecting lower limit of quantification to even 10ng.Hence the method is found to be suitable and selective for the Related substance method it helps to quantification all impurities. Linearity, regression value, recovey, % RSD of method precision, LOD and LOQ values were found with in the limits. In this method impurities were well seperated from the main peak. This method was found to be satisfactory. Limits for reporting threshold and total impurities were 0.1% and 2.0%, respectively, as per Q3B(R) Impurities in New drug Products.

Keywords:HPLC,Related substance,column,mobile phase.

INTRODUCTION:A related substance is a pharmaceutical industry term for contaminants which are structurally similar to the active drug (the are chemically "related"). In practice, the term is often applied to impurities and contaminants in a drug whether or not they are chemically related. The importance should be self-evident[1].An assay is a quantitative or qualitative measurement of a particular component in a sample.In contrast, purity is the analysis of impurities in the sample. It is mainly a quantitative measurement. Therefore, the main difference between assay and purity is the type of components and measurements[13].

Drug Profile: Cefuroxime is beta-lactum type antibiotic.Cefuroxime is beta-lacum type antibiotic.More specifically,it is a second-generation cephalosporin.cephalosporins work the same way as pencillins Cefuroxime is effective against the following organism:Areobic Gram–positive:Staphylococcus aureus,Streptococcus pneumonia[2].

Chemical Structure:



Chemical Formula: $C_{16}H_{16}N_4O_{8S}$
Molecular Weight: 424

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IUPAC Name: (6R,7R)-3-[[(amainocarbonyl)oxy]methyl]7-[[(2z)-2-furanyl methoxy imino)acetyl]amino]-8-oxo-5-thio-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid[3].
Categories: Areobic Gram-negative:Escherichia coli,Haemopilus influenzae.
Material and Method: Cefuroxime acid working standard was procured from Nectar life sciences Ltd.Derabassi[5].

Instrumentation:WATERS 2695system was usedfor liquid chromatography method development and validation an auto sampler (ALS) and a Betasil C1 (250*4.6 mm,5micron) column and the detector consisted of UV/VIS operated at 278 nm. Empower Softwar (version-2) was used for data processing[6].

Chemical & Reagents: A pharmaceutical grade sampleof Cefuroxime acid was obtained from Nectar Life Sciences Ltd Derabassi.HPLC grade of Mobile Phase,AR grade of Ammonium dihydrogen phoshphate & HPLC grade water use for the method development and validation[7].

Chromatographic Conditions*:* Mobile phase A was prepared by Dissolve 2.3gm of ammonium dihydrogen phosphate in 100 ml of water[8]. filter through a 0.45um membrane filter and degas.Mobile Phase B was Methanol. analysis was carriedout on an Waters 2695 series HPLC system. analytes were conducted on an analytical Betasil C1 column(250*4.6 mm),5 µm, 250 × 4.6mm with a detection wavelength of 277 nm. operating temperature of the column was set at 30°C. injection volume was 10ul and the flow rate was maintained at 1.0 mL/min. run time was 35minutes[9].

Marker Solution Preparation: Weight accurately and transfer about 12.5mg each of Cefuroxime acid ,6.5mg Hydroxy Cefuroxime,7-ACA and Hydroxy 7-ACA and 1mg SMIA into a 50ml volumetric flask, dissolve in about 5ml of 2% Ammonium bicarbonate solution(w/v) and make up to the make with Diluent[10].

Sample Preparation:12.5mg of Cefuroxime acid sample was transferred into a 50 ml volumetric flask, dissolve and make up to the volume with Diluent[12].

Stability and System suitability: Tests Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis[13].

Result & Discussion: From the standard stock solution, 250ppm solution was scanned by PDA under spectrum mode for 200-400nm wavelength range and a sharp peak was obtained at 273nm in Diluent using as a solvent.

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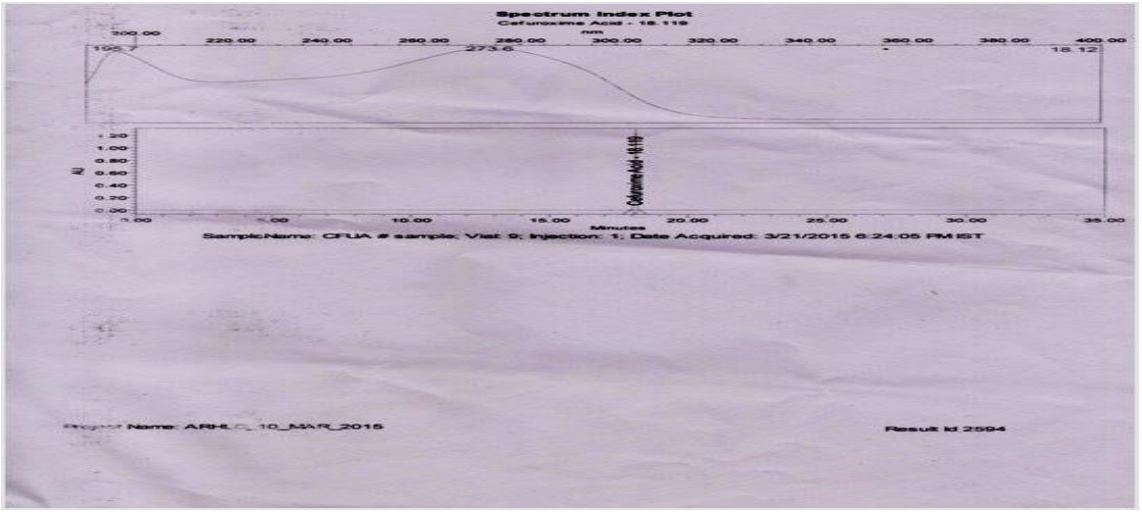

Method Optimization: Different trials were performed for method optimization.

Day	Column	Method	Resolution	Remark
Day 1	Symmetry C18	Isocratic	Sepration between Hydroxy Cefuroxime and Cefuroxime acid peak is very less	Slow elution
Day 2	Symmetry C18	Isocratic	Sepration between OH-7-A CA and 7-ACA peak is very less	Slow elution
Day 3	Betasil C1	Gradient	Sepration between Hydroxy 7-ACA and SMIA is very less	Slow elution
Day 4	Betasil C1	Gradient	Hydroxy CFU and Cefuroxime acid peaks were well sperated but OH-7-ACA and SMIA is still less	Acceptable recovery but unstable peak
Day 5	Betasil C1	Gradient	All peaks are well seprated from each other with good shapes	Good recovery but with peak enhancement

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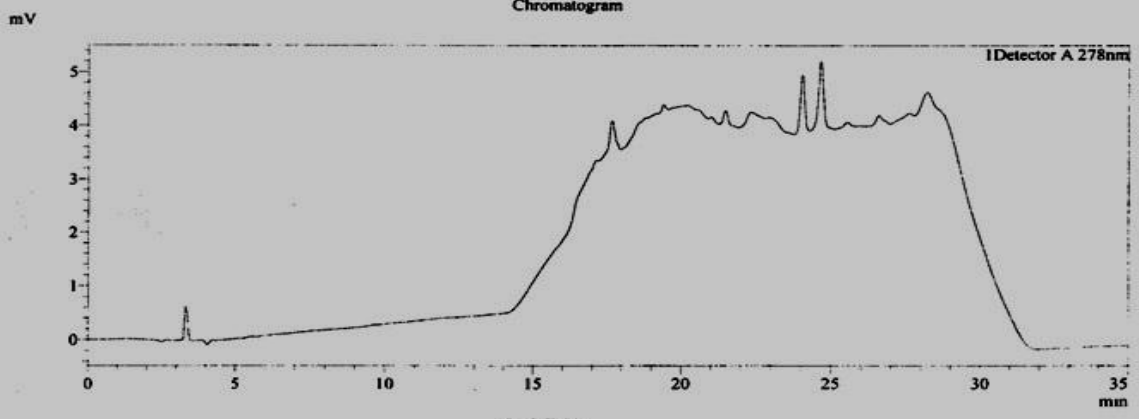
NECTAR LIFESCIENCES LTD. UNIT-II

Sample Information D:\ARHLC-05\DATA\DATA-2015\MAY-2015\12.05.2015\002.lcd

Sample Name	: CFUA # RS
Sample ID	: CFUA # Blank
Tray#	: 1
Vail#	: 2
Injection Volume	: 10 uL
Data Filename	: 002.lcd
Method Filename	: Cefuroxime acid # project.lcm
Date Acquired	: 3/8/2014 1:37:01 AM
Data Processed	: 5/13/2015 10:58:42 AM

Chromatogram



Peak Table

Detector A 278nm

Peak#	Name	Ret. Time	Area	Area%	RRT
Total					

Analyzed by :
Date :

Checked by :
Date :

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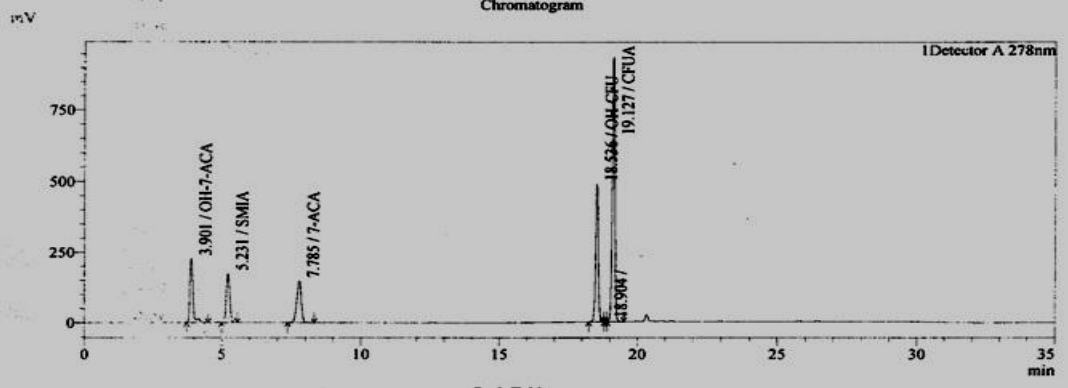
ANALYTICAL RESEARCH & DEVELOPMENT
NECTAR LIFESCIENCES LTD. UNIT-II

Sample Information D:\ARHLC-05\DATA\DATA-2015\MAY-2015\12.05.2015\005.lcd

Sample Name	: CFUA # RS
Sample ID	: CFUA # Marker
Tray#	: 1
Vail#	: 1
Injection Volume	: 10 uL
Data Filename	: 005.lcd
Method Filename	: Cefuroxime acid # project.lcm
Date Acquired	: 3/8/2014 1:37:01 AM
Data Processed	: 5/13/2015 11:06:37 AM

Chromatogram



Peak Table

Detector A 278nm

Peak#	Name	Ret. Time	Area	Area%	RRT
1	OH-7-ACA	3.90	1750609	12.32	0.20
2	SMIA	5.23	1498643	10.55	0.27
3	7-ACA	7.78	1640217	11.55	0.41
4	OH-CFU	18.54	3243000	22.83	0.97
5		18.90	4657	0.03	0.99
6	CFUA	19.13	6069902	42.72	1.00
Total			14207029	100.00	

Analyzed by :
Date :

Checked by :
Date :

Discussion: Optimization of the mobile composition was carried to get proper resolution of thedrug in between 11.00 which is considered as a good chromatography. Varioussolvent systems were tried on silica gel HPLC plates but after lot of trials it wasfound that this combination can be separated on reverse phase HPLC plates. Withmost of the solvent systems, either tailing of the drug spots or proper resolution ofthe drug peaks was not taking place.

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Optimization of the mobile phase

Mobile phase	Composition/v	Phase
Ammonium di-hydrogen phosphate(isocratic)	100	Reverse
Ammonium di-hydrogen phosphate(isocratic)	100	Reverse
Ammonium di-hydrogen phosphate(isocratic)	100	Reverse
Ammonium di-hydrogen phosphate (gradient)	100	Reverse
Ammonium di-hydrogen phosphate (gradient)	100	Reverse

Several mobile phase compositions were tried to get proper resolution of the drug peaks. A satisfactory separation and good RF values were obtained by using the mobile phase composition ammonium di-hydrogen phosphate (v/v gradient).Quantification was achieved with UV detection at 278 nm based on peak area.

System Suitability: Result table of system suitability

S.No.	Analyte		ISTD		Area Ratio
	Peak Area	RT	Peak Area	RT	Analyte Peak Area/Istd Peak Area
1	61	2.206	72	2.21	N/AP
2	2857352	2.231	469785	2.22	6.0823
3	2792886	2.227	500373	2.22	5.5816
4	2777860	2.234	468246	2.23	5.9325
5	2772347	2.231	487866	2.22	5.6826
6	2756152	2.233	472497	2.22	5.8332
7	2852677	2.237	494981	2.23	5.7632
8	45	2.221	36	2.25	N/AP
Mean	2801545.7	2.2322	482291.3	2.22	5.8126
S.D		0.00337		0.00363	0.17900
%C.V		0.15		0.16	3.08

Result:

Mean=17099.8(blank matrix) Mean=1783746.2(at LOQ level of analyte& IS)

S.D.(±)=402.57(blank matrix) S.D.(±)=32523.60(at LOQ level of analyte& IS)

%C.V.=2.35(blank matrix) %C.V.=1.82(at LOQ level of analyte& IS)

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Discussion: According to result table blank matrix 5 and blank matrix 6 is selected for the further exercise.%C.V also falling in the acceptance criteria i.e. %C.V≤20% at the LOQ level.Hence the exercise is passed and fulfilling the acceptance criteria.

Conclusion: By performing several hidden trials in the laboratory finally a selective, sensitive,robust and accurate method was developed with the following chromatographic set up Column Betasil C1(250*4.6mm,5µ) mobile phase Methanol: Buffer (50:50v/v), flow rate 1.0 ml/min Sample cooler temp 10°C±1.0°C,Column oven Temp. 30°C±1°C,Injection volume 10 µl. After the method is finalised. The proposed HPLC method developed is found to be selective,sensitive,robust and accurate for the quantification of Cefuroxime acid. The method is capable of detecting lower limit of quantification to even 10ng.Hence the method is found to be suitable and selective for the Related substance method it helps to quantification all impurities.

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