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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR THE DETERMINATION OF RELATED IMPURITIES OF CEFUROXIME AXETIL IN BULK AND IN PHARMACEUTICAL FORMULATION

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ABSTRACT

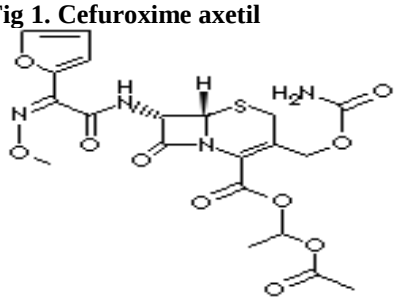
A new simple**,** precise, accurate and selective RP-HPLC method has been developed and validated for Cefuroxime axetil in oral dosage form. The method was carried out on a Waters symmetry, C8 (150mmX4.6mmX5µ i.d) column with a mobile phase consisting of milli Q water, methanol and glacial acetic acid (49:50:01) and flow rate of 1.5 mL min^{-1}. Detection was carried out at 278 nm. The retention time for Cefuroxime axetil was found to be 6.413 min. The Cefuroxime axetil % recovery was within the range between 92.5- 102.8%. The percentage RSD for precision of the method was found to be not more than 15% and correlation coefficient was 0.98.The method was validated as per ICH guidelines. The developed method was validated for precision, accuracy, sensitivity and robustness. The developed method can be used for routine analysis of titled drug in formulation.

Keywords: Cefuroxime axetil oral suspension, RP-HPLC, C8-column, Related impurities, Method development and Validation.

INTRODUCTION

Cefuroxime is chemically 1-acetoxyethyl ester; (6R-(6alpha,7beta(Z)))-3-(((aminocarbonyl)oxy)methyl)-7-((2-furanyl(methoxyimino) acetyl) -amino)-8-oxo- 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 1-(acetyloxy)ethyl ester [1]. The molecular formula of Cefuroxime Axetil is $C_{20}H_{22}N_4O_{10}S$, and it has a molecular weight of 510.48. It has the following structural formula and shown in fig (1).

Fig 1. Cefuroxime axetil



Cefuroxime axetil is in the amorphous form and white in colour. Cefuroxime, like the penicillins, is a beta-lactam antibiotic. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, it inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefuroxime interferes with an autolysin inhibitor. The axetil moiety is metabolized to acetaldehyde and acetic acid. After oral administration it is absorbed from the gastrointestinal tract. Absorption is greater when taken after food (absolute bioavailability increases from 37% to 52%). Cefuroxime is effective against the following organisms: Aerobic Gram-positive Microorganisms: Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes. Aerobic Gram-negative Microorganisms: Escherichia coli, Haemophilus influenzae (including beta-lactamase-producing strains), Haemophilus parainfluenzae, Klebsiella pneumoniae,

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Moraxella catarrhalis (including beta-lactamase-producing strains), Neisseria gonorrhoeae (including beta-lactamase-producing strains). Spirochetes: Borrelia burgdorferi. Cefuroxime axetil is the prodrug for the treatment of many different types of bacterial infections such as bronchitis, sinusitis, tonsillitis, ear infections, skin infections, gonorrhea, and urinary tract infections [2].

MATERIALS AND METHOD

Chemicals and Reagents

HPLC grade Methanol and Glacial acetic acid was obtained from Rankem laboratories. Analytical grade Hydrochloric acid, Sodium hydroxide and Hydrogen peroxide were obtained from Rankem laboratories. Working Standard (Cefuroxime axetil) obtained as a gift sample from reputed laboratory.

Oral suspension formulation (Ceftin 125mg) manufactured by Glaxo Smithkline Company containing labelled amount of 125mg $5mL^{-1}$ of Cefuroxime axetil oral suspension was purchased from the local market.

Equipments

The instrument was a Waters HPLC model 2695 equipped with 2996 photodiode array detector. The system was connected with the help of Waters empower Chromatographic software in a computer system for data collection and processing. The analytical column used is C8.

Chromatographic condition

The mobile phase consists of a mixture of Milli Q water (49 volumes), methanol (50 volumes) and glacial acetic acid (01 volume) was filtered through 0.45µm nylon membrane filter and degassed for 10 minutes before use. The injection volume was 20µL with a flowrate 1.5mL min^{-1} and detection wavelength 278nm having gradient condition and run time 80

Standard preparation

Stock solutions were prepared by accurately weighing 125 mg of Cefuroxime axetil working standard and transferred into 50mL volumetric flask, containing 15mL of methanol. The flasks were sonicated for 10 mins to dissolve the solids. Volumes were made up to the mark with mobile phase to give 2500 µg mL^{-1}. Aliquots from the stock solutions were appropriately diluted with mobile phase to obtain working standards of 7.5 µg mL^{-1} of drug. Filtered about 2mL through 0.45 µm nylon membrane filter. Typical standard chromatogram of Cefuroxime axetil is shown in Fig (2) [3-9].

Preparation of Resolution solution

Heated 20mL of standard stock solution for 60 min at 60°C on waterbath and cooled to room temperature. Filtered about 2 ml through 0.45 µm nylon membrane filter (Fig. 3).

RESULTS AND DISCUSSION

Estimation of Cefuroxime axetil in Oral Suspension

The HPLC procedure was optimised with a view to develop accuracy, precise and stable assay method. Cefuroxime axetil was run in different mobile phase composition and different pH ranges of mobile phase with different C8, columns at gradient temperature. The flow rate was also varied from 0.5 to 1.5mL min^{-1}. The mobile phase consists of a mixture of milli Q water (49 volumes), methanol (50 volumes) and glacial acetic acid (1 volume) was filtered

Through 0.45 µm nylon membrane filter before use. The column used is water symmetry, C8, 5 µm column having 150 X 4.6 mm i.d.

Weighed the content of one bottle of Cefuroxime axetil for Oral suspension and recorded the weight. Reconstituted as per the labels. Weighed and transferred accurately equivalent to 100 mg of Cefuroxime into a 50 ml volumetric flak, added 15 ml of methanol, sonicated for 20 minutes to dissolve the material, diluted to volume with mobile phase and mixed. Filtered about 2 ml through 0.45 µm nylon membrane filter. The sample solution was chromatographed similar to standard solution and concentrations of Cefuroxime Axetil in oral suspension were calculated.

Method Validation

The described method has been validated for the assay of Cefuroxime axetil using following parameters.

System precision

The standard solution were prepared as the same way as the above. Ten replicated injections were made into the HPLC system. The percentage RSD were calculated from ten replicate injections and presented in Table (1).

System suitability

Heated 20mL of standard stock solution for 60mins at 60°C on waterbath and cooled to room temperature. Filtered about 2mL of this through 0.45µm HNN filter. Injected about 20 µL portion of resolution solution into the chromatograph recorded the chromatogram and measured the response for major peaks. The resolution between Cefuroxime Axetil diasteroisomers B and A should be not less than 2.5. The resolution between Cefuroxime Axetil diasteroisomers A and Impurity B should be not less than 2. Results are shown in Table (2).

Specificity

Specificity of the method was carried out by injecting the diluted placebo, drug substance and drug product at different stress condition like hydrolytic degradation, oxidative degradation, water degradation studies, photolytic degradation studies, heat stress and

humidity stress. There is no interference from impurities of degradation products at the RT of main peak. Hence the developed analytical method was specific for cefuroxime axetil in oral suspension. Results are shown in Table (3).

Precision

The repeatability of the method was established by six replicate injection of the drug product with 1mL of impurity stock solution were spiked. The percentage RSD were calculated and presented in Table (4). From the data obtained, the developed RP-HPLC method was found to be precise.

LOD and LOQ

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Linagliptin was found to be 0.9 µg mL^{-1}.The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 2.95µg mL^{-1} for Linagliptin respectively.

Linearity and Range

Linearity was studied by preparing standard solution at seven different concentration levels. The linearity range was found to be 0-75 µg mL^{-1}. 20µL of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting peak areas (Y axis) against the amount of drug in µg mL^{-1} (X axis). Peak area of linearity range and the parameters were calculated.

Table 1. System Precision

Injection number	Total peak area of Cefuroxime Axetil diasteroisomers (A&B)
1	218945
2	218332
3	217856
4	218114
5	216848
6	217949
7	217898
8	217490
9	217804
10	217333
Average	217859
% RSD	0.3

Table 2. System Suitability

System Suitability Parameter	Observed Value	Acceptance criteria
The USP resolution between Cefuroxime Axetil diasteroisomers B and A.	2.6	NLT - 2.5
The USP resolution between Cefuroxime Axetil diasteroisomers A and Impurity A	2.2	NLT - 2.0

Table 3. Specificity studies

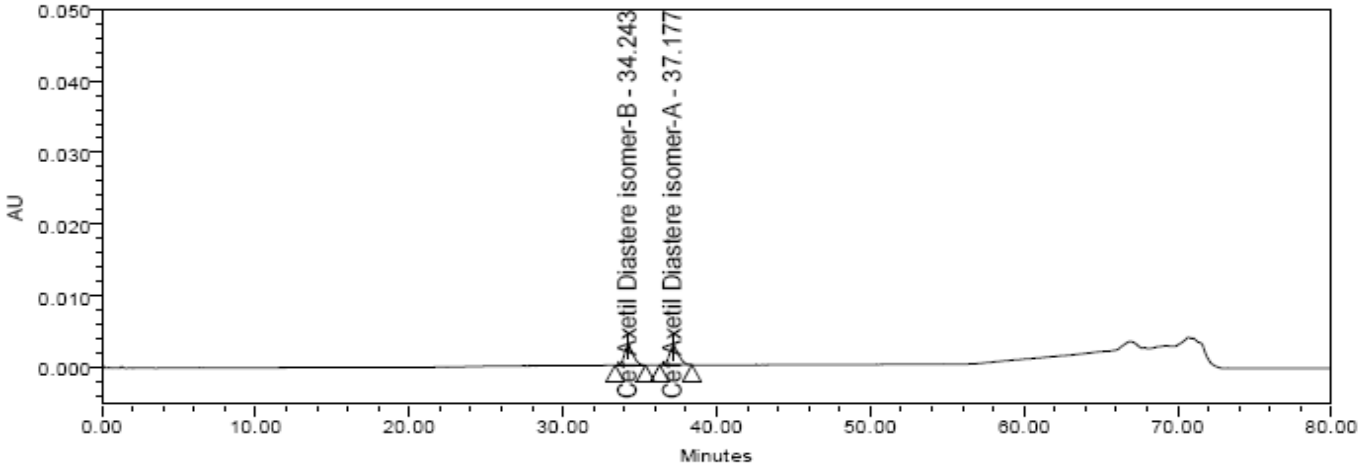
Mode of Degradation & Condition	Parameters	Drug Substance		Drug Product	
		B-Isomer	A-Isomer	B-Isomer	A-Isomer
Acid-30mL of 0.1N HCl heated on a Waterbath at 70º C for 45 min.	Purity Angle	0.109	0.108	0.147	0.092
	Purity threshold	0.257	0.228	0.255	0.248
	% degradation	15		17	
Alkali-10mL of 0.01N NaOH kept on bench top for 10 min.	Purity Angle	0.031	0.025	0.036	0.033
	Purity threshold	0.264	0.250	0.268	0.265
	% degradation	15		1	
Peroxide stress 30mL of 1% H_2O_2 and heated on Waterbath at 70º C for 30 min	Purity Angle	0.029	0.035	0.039	0.038
	Purity threshold	0.261	0.264	0.256	0.257
	% degradation	15		26	
Waterdegradation 30mL of water on a	Purity Angle	0.032	0.031	0.031	0.034

Waterbath at 70º C for 3 hours	Purity threshold	0.263	0.264	0.261	0.265
	% degradation	8		17	
Photolytic degra- dation exposed to UV radiation for 54hrs	Purity Angle	0.040	0.037	0.030	0.028
	Purity threshold	0.278	0.273	0.264	0.262
	% degradation	Nil		2	
Photolytic degra -dation exposed to Visible radiation for 288hrs	Purity Angle	0.036	0.034	0.033	0.032
	Purity threshold	0.279	0.274	1.271	1.270
	% degradation	Nil		1	
Heat stress exposed to dry heat at 105º C for 10 hours in hot air oven.	Purity Angle	0.036	0.044	0.180	0.174
	Purity threshold	0.264	0.262	0.405	0.409
	% degradation	16		28	
Humidity stress exposed at 25ºC/90%RH for 288 hours.	Purity Angle	0.037	0.031	0.037	0.032
	Purity threshold	0.280	0.274	0.269	0.266
	% degradation	Nil		Nil	

Table 4. Method Precision (Repeatability)

Imp. name	% Impurity						Avg.	SD	% RSD	RRF
	Spl- 1	Spl- 2	Spl- 3	Spl- 4	Spl- 5	Spl- 6				
Imp- 1Area	0.4679	0.4669	0.4663	0.4654	0.4660	0.4661	0.4664	0.0008	0.2	1.00
	34955	34935	34852	34797	34838	34885				
Imp- 2Area	0.6323	0.6454	0.6547	0.6637	0.6815	0.6906	0.6514	0.0244	3.7	0.75
	35428	36215	36699	37217	38212	38772				
Imp- 3Area	0.7888	0.8074	0.7912	0.8060	0.8044	0.8053	0.8005	0.0082	1.0	0.57
	33587	34436	33705	34346	34282	34359				
Imp-4 Area	0.7045	0.6986	0.6984	0.6973	0.6929	0.6929	0.6983	0.0041	0.6	0.71
	37366	37110	37058	37015	36780	36823				
Imp-5Area	0.6148	0.6141	0.6171	0.6172	0.6205	0.6185	0.6170	0.0024	0.4	0.72
	33067	33081	33207	33222	33403	33334				

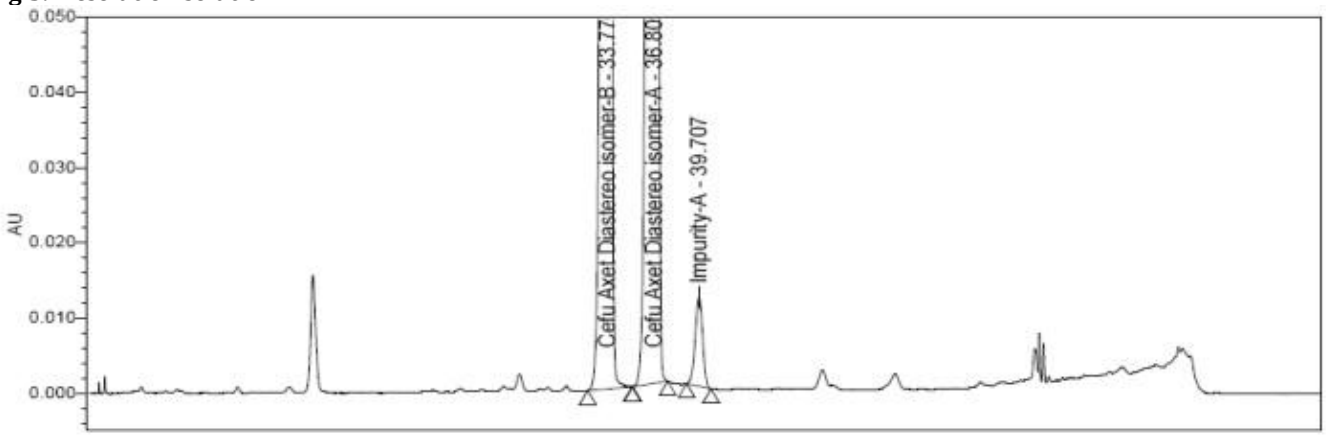
Figure 2. Standard solution



Peak Results

	Name	RT	Area	% Area	Int Type
1	Cef Axetil Diastere isomer-B	34.243	105987	49.15	BB
2	Cef Axetil Diastere isomer-A	37.177	109671	50.85	BB
Sum			215658	100.0	

Fig 3. Resolution solution



Peak Results

	Name	RT	Area	% Area	Int Type	USP Plate Count	USP Tailing
1	Cefu Axet Diastereo isomer-B	33.770	35454467	48.80	BB	39402	0.8
2	Cefu Axet Diastereo isomer-A	36.809	36796538	50.65	BB	38234	0.8
3	Impurity-A	39.707	395917	0.54	BB	31680	1.0

CONCLUSION

The proposed RP-HPLC method for the estimation of Cefuroxime axetil for the oral dosage form is simple, accurate, specific, precise, linear, sensitive and also system suitability. Hence the present RP-HPLC method is suitable for the quality control of the raw material and have suitable application in routine laboratory analysis and with a high degree of accuracy and precision. It was concluded that the method is stability indicating and could be used throughout shelf life of the drug.

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