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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD IN THE DETECTION OF CEFDINIR IN BULK DRUG AND CAPSULE DOSAGE FORM

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Cefdinir, Isocratic RP-HPLC, UV-Visible detector, Method validation.

ABSTRACT

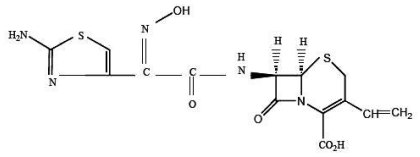
A simple, specific, accurate, rapid, inexpensive isocratic Reversed Phase – High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Cefdinir in bulk and capsule dosage forms. RP-HPLC method was developed by using Develosil C_{18} column(150×4.6mm,5µm).The mobile phase is composed of 0.05M phosphate buffer (P^H – 4.2 , adjusted with ortho phosphoric acid) and acetonitrile (30:70v/v). The flow rate was set to 0.8 mL/min. The responses were measured at 230nm using UV – Visible detector. The Retention time of Cefdinir was found to be 2.26 minutes. Linearity was established in the concentration range of 30 - 70µg/ml with correlation coefficient 0.999. The validation of the developed method was carried out for linearity, accuracy, precision, specificity and robustness. The developed method can be used for routine quality control analysis of Cefdinir in pharmaceutical capsule dosage form.

INTRODUCTION:

Cefdinir is an advanced generation, broad-spectrum Oral semi synthetic cephalosporin. Cefdinir is effective against gram positive and grand negative bacteria. It has been proven to effective for common bacterial infections of the ear, sinus, throat and skin.

Chemically, Cefdinir is 6R-[6α, 7β(Z)]-7-(2-amino-4-thiazolyl) hydroxyimino) acetyl] amino]-3-ethyl-8-oxo-5-Thia-1-azabicyclo-(4.2.0.)- Oct -2-one-2-carboxylic acid. The antibiotic has been approved for the treatment of community-acquired pneumonia, acute exacerbations of chronic bronchitis, acute maxillary sinusitis, treatment of respiratory and urinary tract infections, and for uncomplicated skin and skin structure infections. Thus, the third generation of cephalosporin is of great value in medical treatment due to its broad spectrum bactericidal effect.

Structure:



Experimental:

Chemicals and reagents: The reference sample of cefdinir standard was kindly supplied as gift sample by Aurabind Pharma, Hyderabad .Potassium dihydrogen ortho phosphate and ortho phosphoric acid were A.R. grade from SD fine chemicals limited, Mumbai. Methanol was A.R. grade from Loba chemicals, Mumbai. Acetonitrile was HPLC grade from Loba chemicals, Mumbai.

Chromatographic conditions: The chromatographic separation was carried out

using Develosil C_{18} column (150mmX4.6mm, 5µ) as stationary phase with injection volume of 20µL.The mobile phase comprised of 0.05M potassium dihydrogen ortho phosphate (P^H adjusted to 4.2 with ortho phosphoric acid) and Acetonitrile in the ratio of 30:70v/v at a flow rate of 1.0 mL/min and UV detection wavelength was 230nm.

Preparation of mobile phase:

0.05M phosphate buffer was prepared by dissolving 1.36g of potassium dihydrogen orthophosphate and 2.0mL of triethylamine(TEA) in 800mL distilled water. Adjust P^H to 4.2 with ortho phosphoric acid and add sufficient water to produce 1000mL.Filter the solution through 0.45µm membrane filter and sonicate to degas. The above prepared buffer and acetonitrile was mixed in the ratio of 30:70v/v.

Preparation of Standard solution:

Weigh accurately Cefdinir standard equivalent to 25mg of Cefdinir and transfer into 25ml volumetric flask, add 10ml of diluent, sonicate to dissolve and makeup to the volume with diluent. Pipette out 0.5ml of this solution into 10ml volumetric flask and dilute the volume up to the mark with diluent to obtain a concentration of 50 µg/ml. Filter the solution through 0.45µm nylon membrane filter.

Preparation of sample solution:

Weigh and finely powder 20 capsules (**Adcef**). Weigh capsule powder equivalent to 25mg of Cefdinir formulation and transfer into 25ml volumetric flask, add 10ml of diluent, sonicate for 15min and dilute to the volume up to the mark with diluent. Pipette out 0.5ml of this solution into 10ml volumetric flask and dilute the volume up to the mark with diluent to obtaining a concentration of 50 µg/ml. Filter the solution through 0.45µm nylon membrane filter.

Selection of detection wavelength:

The UV spectra of various diluted solutions of Cefdinir mobile phase were recorded using UV spectrophotometer. The peak of maximum absorbance was observed at 230nm. This wavelength was used for detection of Cefdinir.

VALIDATION OF THE PROPOSED METHOD:

The developed method of analysis was validated as per the ICH for the parameters like system suitability, linearity, precision, accuracy and robustness.

System suitability:

System suitability was used to verify reproducibility of the chromatographic system. It is checked by repetitive injection of the drug solution at the concentration level 50µg/ml for Cefdinir to check the reproducibility of the system. At first the HPLC system was stabilized for 40 min. One blank followed by six replicates of a single calibration standard solution of Cefdinir was injected to check the system suitability. The parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken and results were presented in table 2.

Linearity:

The linearity graphs were obtained over the concentration range of 30-70µg/ml of Cefdinir. The representative chromatograms indicating the Cefdinir were shown in Fig. 5to 9. A calibration curve was plotted between concentration and area response and statistical analysis of the calibration curve is shown in fig.9.

Precision:

Intra-day and inter-day precision study of Cefdinir was carried out by estimating corresponding responses 3 times on the same day and on 3 different days for the concentration of 50µg. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2.0. The results for intra-day and inter-day precision were presented in Table 6 and Table 7 respectively.

Accuracy:

The accuracy of the method was determined by calculating recovery of Cefdinir by the method of addition. Known amount of Cefdinir at 80%, 100% and 120% was added to a pre quantified sample solution.

Fig 1: Detection of wavelength of Cefdinir

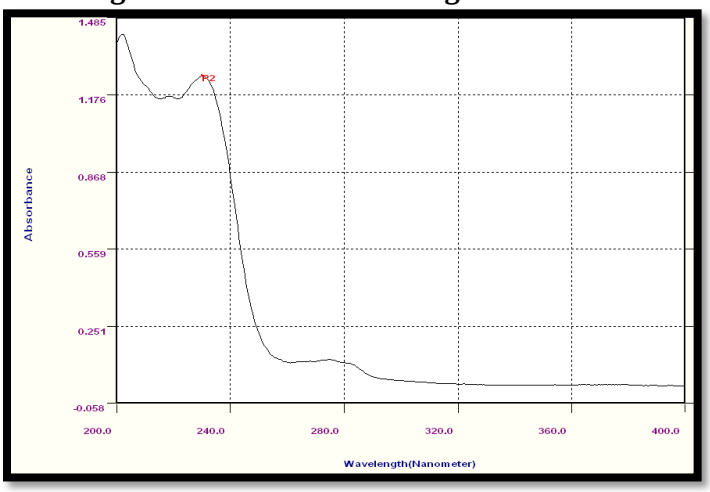
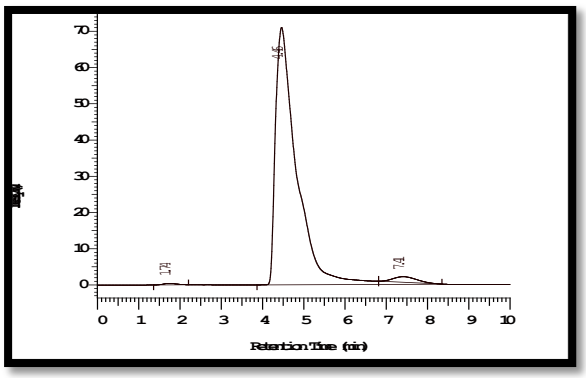


Table 1: Optimized Method for Cefdinir

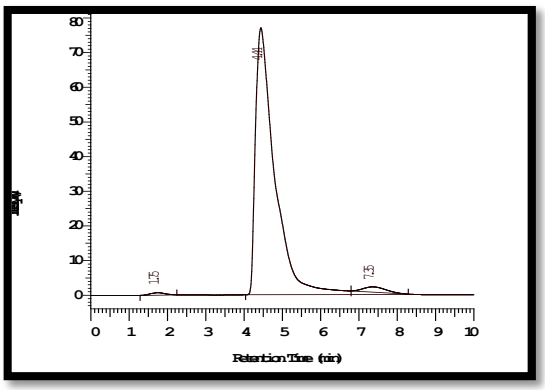
S. no	Parameter	Trail – 1	Trail - 2	Trail – 3	Trail – 4	Optimized method
1	HPLC	Hitachi L 2130 with D Elite 2000	Hitachi L 2130 with D Elite 2000	Hitachi L 2130 with D Elite 2000	Hitachi L 2130 with D Elite 2000	Hitachi L 2130 with D Elite 2000
2	Spectrophotometer	ELICO SL-120	ELICO SL-120	ELICO SL-120	ELICO SL-120	ELICO SL-120
3	Column	Hypersil C18 250mm	Hypersil C18 250mm	DEVELOSIL C18 150mm	DEVELOSIL C18 150mm	DEVELOSIL C18 150mm
4	Column temp	Ambient	Ambient	Ambient	Ambient	Ambient
5	Mobile Phase	Phosphate buffer (P^H 3.5) : acetonitrile (70:30 v/v)	phosphate buffer :acetonitrile 70:30 P^H: 4	water: Acetonitrile 50:50	phosphate buffer : acetonitrile 60:40 P^H: 4.2	0.05M Phosphate buffer(P^H-4.2): Acetonitrile(30:70v/v)
6	Flow rate	1ml/min	1 ml/ min	1.3 ml/ Mn	1.3 ml/ Min	0.8 mL/min
7	Detection wavelength	256 nm	256 nm	256 nm	230nm	225 nm
8	Inj Volume	20µL	20µL	20µL	20µL	20µL
9	Run time	10 Min	10 Min	10 Min	8 Min	6 min
10	Retention time	4.46 Min	4.44 Min	3.41 Min	2.87 Min	2.26min
11	Theoretical plates	1236	1436	2034	2300	6341
12	Tailing factor	2.46	2.32	2.01	2.38	1.78

Method Development:

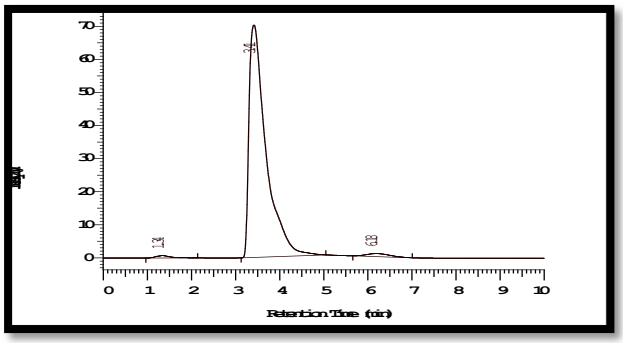
Trail – 1



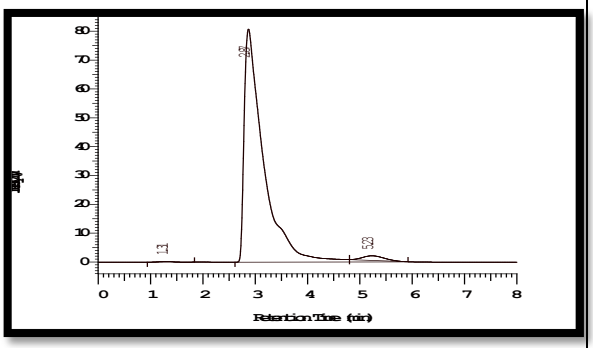
Trail – 2



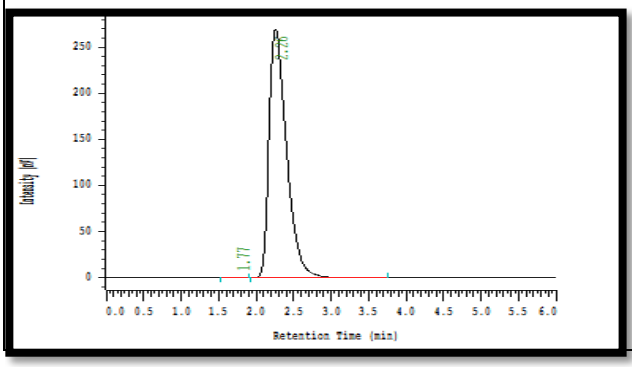
Trail – 3



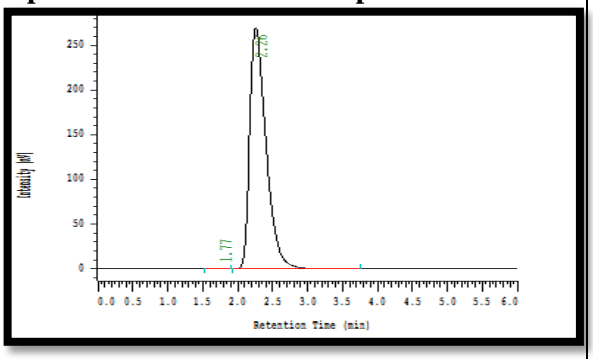
Trail – 4



Optimized method for Standard solution



Optimized method for sample solution



Results and Discussion:

Table 2: System suitability:

S. No	Concentration	Retention time	Peak area	Theoretical plates	Tailing factor
1	50	2.26	4371369	6320	1.01
2	50	2.26	4327048	6389	0.98
3	50	2.26	4372696	6366	0.99
4	50	2.26	4283857	6312	0.99
5	50	2.26	4340455	6353	0.96
6	50	2.26	4329302	6308	0.97
Avg		2.26	4339085	6341.333	0.98333
SD		0	36636.2	32.99495	0.01751
%RSD		0	0.84433	0.520316	1.78087

Table *3:* Linearity data of the method

S.no	Concentration (µg /mL)	Peak area
1	30	2789320
2	40	3508004
3	50	4371369
4	60	5304243
5	70	6090165

Figure -2

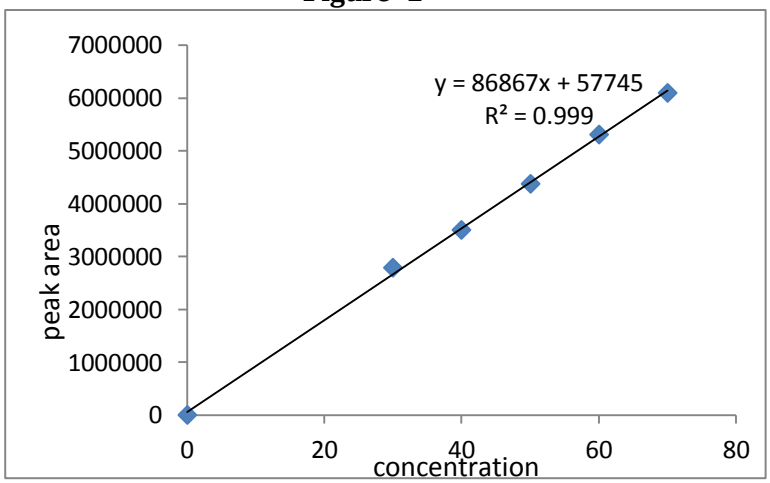


Table 4: Results of precision study (Intra-day)

Sample	Concentration	Injection No	Peak Area	% RSD(acceptance criteria<2)
Cefdinir	50µg / mL	1	4389192	1.107%
		2	4389101	
		3	4429920	
		4	4492901	

Table 5: Results of precision study (Inter-day)

Sample	Concentration	Injection No	Peak Area	% RSD(acceptance criteria<2)
Cefdinir	50µg/mL	1	4371369	0.84433
		2	4327048	
		3	4372696	
		4	4339085	

Table 6*:* Results of Accuracy

Level	Rt of Cefdinir	Peak area of Cefdinir	Recovery
80% (50µg+40µg)	2.21	8007244	99.33925
	2.22	8056564	100.7428
	2.22	8072938	100.2087
100% (50µg+50µg)	2.25	8807444	98.48498
	2.24	8890650	100.3947
	2.25	8824707	98.88119
120% (50µg+60µg)	2.24	9994211	100.5212
	2.23	9951108	99.73021
	2.24	9992432	100.4885

Table 7: Robustness Results of Cefdinir by HPLC Method

Changed parameter	Peak area	R_t (min)	Theoretical plates	Tailing factor
Flow rate (±0.1ml/min)				
0.9 ml/min	4284939	2.24	6320	1.04
1.1 ml/min	4388232	2.26	6353	1.06
Column temp(±5^{0}C)				
35^{0}C	4283030	2.23	6366	1.03
45^{0}C	4371891	2.24	6389	1.08
Mobile Phase (± 2%)				
28:72	4214683	2.23	6308	1.12
32:68	4368121	2.26	6312	1.09
pH Variation (±0.2 units)				
2.8	4261438	2.24	6353	1.04
3.2	4326148	2.26	6372	1.18

Parameters	**Values of Cefdinir**	**Acceptance Criteria**	**Results**
Assay	99.931	90-110	passes
System suitability	0.844	NMT 2	Passes
Linearity	30 -70 µg/ml		
Slope(M)	86867		
Intercept(C)	57745		
Co-relation (r^2)	0.999	0.99 – 1.00	Passes
Repeatability	0.844	NMT 2	Passes
Precision			
Intermediate precision	1.336	NMT 2	Passes
Intraday precision	1.182	NMT 2	Passes
Accuracy	99.33% - 101.20%	90.0%-110.0%	Passes
LOD	1.39	NMT 3	Passes
LOQ	4.17	NMT 10	Passes
Robustness & Ruggedness			
Flow rate	0.22 - 0.39	NMT 2	Passes
Temperature	1.15	NMT 2	Passes
Mobile phase concentration	1.28	NMT 2	Passes
Flow rate	0.39	NMT 2	Passes
Wavelength	0.12 – 1.18	NMT 2	Passes
Analyst	0.022 – 0.36	NMT 2	Passes
STABILITY STUDIES			
Acid	1.76	NMT 10%	Passes
Base	4.87	NMT 10%	Passes
Peroxide	2.87	NMT 10%	Passes
Thermal	1.48	NMT 10%	Passes
Photo	2.87	NMT 10%	Passes

The recovery studies were carried out in the capsule in triplicate each in the presence of placebo. The mean percentage recovery of Cefdinir at each level was not less than 99% and not more than 101%. The results were presented in Table 8.

Robustness:

The Robustness was evaluated by the analysis of Cefdinir under different experimental conditions such as making small changes in flow rate (± 0.1 ml/min), P^H (± 0.2 units) and Mobile phase

composition (±2%), column temperature (± 5^0 C). The results were presented in Table 9.

Limit of detection:

Limit of detection is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit (generally 1%) . the limit of detection estimated from the mean of blank, the standard deviation of blank and some confidence factor. whose concentration is not greater than 3.

Limit of Quantification:

Limit of Quantification is the lowest concentration at which analyte can not only be detected but at which some predefined goals for bias and imprecision are met. LoQ may be equivalent to LOD or it may be higher concentration. Whose value is not greater than 10

RESULTS AND DISCUSSION

The mobile phase consisting of 0.05M phosphate buffer (pH-4.2): acetonitrile (30:70% v/v at 0.8 mL/min flow rate was optimized which gave sharp peak, minimum tailing factor with short runtime for Cefdinir. The retention time for Cefdinir was 2.26min. UV spectra of Cefdinir showed that the drug absorbed maximum at 230 nm, so this wavelength was selected as the detection wavelength. All validation parameters for optimized chromatographic conditions are satisfied the ICH Guidelines.

CONCLUSION

A New validated RP-HPLC method has been developed for the quantitative determination of Cefdinir in bulk and pharmaceutical capsule dosage forms. Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. The method was completely validated shows satisfactory results for all the method validation parameters tested and method was free from interference of the other active ingredients and additives used in the formulation. In fact, results of the study indicate that the developed method was found to be simple, reliable, accurate, linear, sensitive, economical, and reproducible and have short run time which makes the method rapid. Hence it can be concluded that this method may be employed for the routine quality control analysis of Cefdinir in active pharmaceutical ingredient (API) and pharmaceutical capsule preparations.

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