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STABILITY INDICATING ASSAY PROCEDURE FOR METHOD DEVELOPMENT AND VALIDATION OF CEFDINIR IN ITS TABLET DOASGE FORM BY USING RP-HPLC

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ABSTRACT

A rapid and precise, linear, specific and suitable Reverse Phase High Performance Liquid Chromatographic method has been developed for the estimation of Cefdinir in its tablet dosage form. Chromatography was carried out on a Develosil C18 (4.6 × 150mm, 5 µm) column using a mixture of ACN and phosphate buffer (pH 3, adjusted with ortho phosphoric acid) in the ratio of 40:60 as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 254 nm. The retention time of Cefdinir was 6.31 min respectively. The method produced linear responses in the concentration range of 0-25 µg/ml. The method of precision for the determination of assay was below 2.0% RSD. The method is useful in the quality control of bulk and other pharmaceutical formulations.

KEYWORDS: Cefdinir, RP-HPLC, Stability indicating Studies, Validation, Tablet dosage form.

INTRODUCTION

The term 'chromatography' covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase.[1]

Cefdinir is chemically (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(N-hydroxyimino)acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid belongs to the class of organic compounds known as cephalosporins. Cefdinir is a semi-synthetic, broad-spectrum antibiotic in the third generation of the cephalosporin class, proven effective for common bacterial infections of the ear, sinus, throat and skin.

It used for the treatment of the respiratory, skin, soft tissue and ENT infections caused by *H. influenzae* (including b-lactamase producing strains), *H. parainfluenzae* (including b-lactamase producing strains), *S. pneumoniae* (penicillin-susceptible strains), *S. pyogenes*, *S. aureus* (including b-lactamase producing strains) and *M. catarrhalis*. The chemical formula cefdinir is $C_{14}H_{13}N_5O_5S_2$. It is sparingly soluble in in Methanol, freely Soluble in Ethanol and Acetonitrile. The chemical structure of Cefdinir is following on Figure – 1and literature survey revealed that numerous methods have been reported for estimation of Cefdinir in pharmaceutical formulations has been reported. The main objective of the work was to develop simple, fast, inexpensive, sensitive and accurate methods which could be applied to analyse Cefdinir in pure form and in other pharmaceutical dosage form.[2]

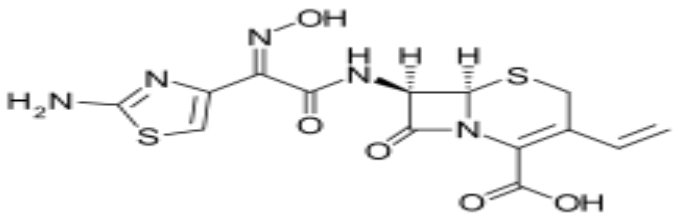

Fig.01: Chemical Structure of Cefdinir.

MATERIAL AND METHODS

Reagents and Materials

Acetonitrile (ACN), Phosphate Buffer. The HPLC system employed was HITACHI L2130 with D Elite 2000 software with Isocratic with UV-Visible Detector (L-2400).

Preparation of Standard solution

Working concentration should be around 10 µg/ml.

Accurately weighed around 25 mg of cefdinir working standard, taken in to a 25 ml volumetric flask, then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000mcg/ml.

Further dilutions has been made to get the final concentration of 10 µg/ml.

Preparation of Test Solution

Diluted quantitatively an accurately measured volume of label claim solution with diluents to obtain a solution containing about a linear range.

Chromatographic Condition

Prepare a mixture of 600ml (60%) of Buffer and 400ml of Acetonitrile (40%) sonicated to degasse and the solvents were pumped from the solvent reservoir in the ratio of Buffer, Acetonitrile pH 3 (60:40 v/v) in to the column. The flow rate of mobile phase was maintained at 1.0 ml/minute and the detection wavelength was set at 254 nm with a run time of 10 minutes. The volume of injection loop was 20 µl prior to injection of the drug solution. The column was equilibrated for at least 25min with the mobile phase flowing through the system. The column and whole system kept in ambient temperature.

Table 1: Optimized Chromatographic Conditions.

Parameters	Conditions
Column	C_{18} Develosil ODS HG-5 RP 150 mm × 4.6mm 5 µm Particle Size
Mobile Phase	Buffer : Acetonitrile (60:40)
Flow Rate	1.0 ml / minute
Wave Length	254 nm
Injection Volume	20 µl
Run time	10 min
Column Temperature	Ambient
Sampler Cooler	Ambient

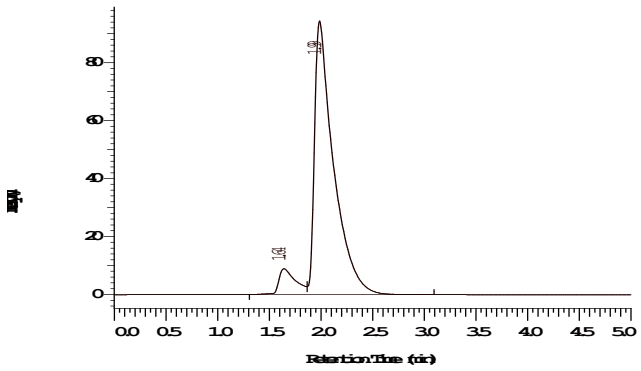

Fig 2: Optimized Chromatogram.

Method of validation[3]

The method was validated for accuracy, Precision, linearity, Specificity, limit of detection, limit of quantization and robustness by following procedures.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of pure drug of Cefdinir were taken and added to the pre-analyzed formulation of concentration 10 µg/ml. From that percentage recovery values were established. The results were shown in table no.2.

Table 2: Accuracy readings of cefdinir.

Sample ID	Concentration (µg/ml)		% Recovery of Pure Drug	Statistical Anlysis
	Pure Drug	Formulation		
S_1 : 80 %	8	10	99.18	Mean = 98.97 %
S_2 : 80 %	8	10	98.78	S.D. = 0.200083
S_3 : 80 %	8	10	98.97	%RSD = 0.202152
S_4 : 100 %	10	10	99.87	Mean = 99.54 %
S_5 : 100 %	10	10	99.54	S.D. = 0.33
S_6 : 100 %	10	10	99.21	%RSD = 0.3315
S_7 : 120 %	12	10	99.32	Mean = 99.567%
S_8 : 120 %	12	10	99.65	S.D. = 0.33%
S_9 : 120 %	12	10	99.98	RSD = 0.331159

Precision

Repeatibiliy

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Cefdinir (API). The percent relative standard deviation were calculated for cefdinir are presented in the table no 3.

Table 3: Repeatability.

HPLC Injection Replication of Cefdinir	Area
Replicate -1	1664475
Replicate -1	1702511
Replicate -1	1673681
Replicate -1	1700887
Replicate -1	1693875
Average	1687086
Standard Deviation	17069.27
% RSD	1.011761

Linearity & Range

Caliberation Curve

The calibration Curve showed good linearity in the range of 0-25 µg/ml, for Cefdinir (API) with correlation coefficient (r^2) of 0.994 (Fig 3). Atypical calibration curve has the regression equation of Y = 14694 + 67023 for cefdinir.

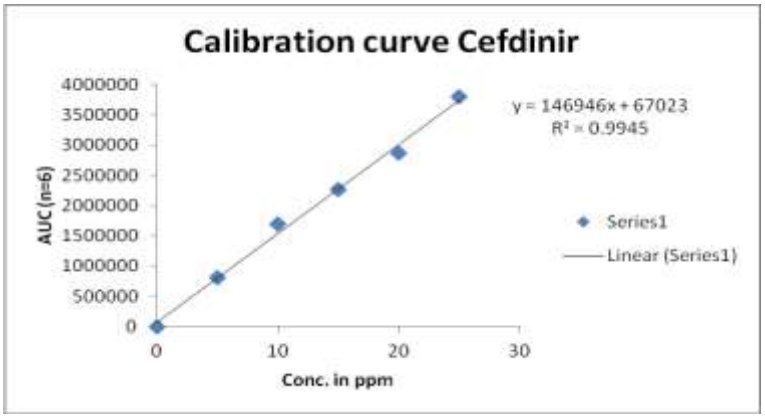

Fig 3: Calibration curve for cefdinir.

Stability

In order to determine the stability of both standard and sample solutions during analysis both the solutions were analyzed over a period of 8 hours at room temperature.

Robustness

Influence of all changes in chromatographic conditions such as change in flow rate (± 0.1 ml/min), Temperature (± 2^{o}C), wave length of detection (±2nm) & acetonitrile content in mobile phase (± 2%) studied to determine the robustness of the method are also in favor to develop RP- HPLC method for the analysis of Cefdinir (API).

Table 4: Robustness test.

Change in Parameter	% RSD
Flow (1.1 ml/min)	0.02
Flow (0.9 ml /min)	0.08
Temperature (27^{0}C)	0.04
Temperature (23^{0}C)	0.16
Wavelength of detection (206 nm)	0.05
Wavelength of detection (204 nm)	0.07

RESULTS AND DISCUSSION

The mixture of Acetonitrile and phosphate buffer was selected as mobile phase and the effect of composition of mobile phase on the retention time of cefdinir was investigated. A short run

time and the stability of peak asymmetry were observed in the ratio of Develosil C 18 (4.6 × 150mm, 5 µm) with mobile phase comprising of buffer (pH 3, adjusted with ortho phosphoric acid) in the ratio of 40:60 v/v, at the flow rate 1 ml/min. The detection was carried out at 254 nm. The retention time of cefdinir was found to be 6.31 min which indicates a good base line. The caliberation of cefdinir was obtained by plotting the peak area ratio versus the concentration of cefdinir over the range of 0-25 µg/ml and it was found to be linear with r^2 = 0.994. The regression equation of cefdinir concentration over its peak area ratio was found to be Y = 14694X + 67023 (100%), where X is the concentration of Cefdinir and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in figure no 3. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters were given in table 3. The high percentage of recovery of cefdinir was found to 99.98% indicates that the proposed method is highly accurate. The absence of additional peaks indicates no interference of the recipients used in the tablets.

FORCED DEGRADATION STUDIES

ACID HYDROLYSIS

An accurately weighed 10mg of pure drug was transferred to a clean and dry 10ml volumetric flask. To which 0.1N HCL was added and make up to the mark and kept for 24 hrs, from that 0.1 ml was taken in to a 10ml volumetric flask and mark with mobile phase, then injected in to the HPLC system against a blank of HCL (After all optimized parameters).

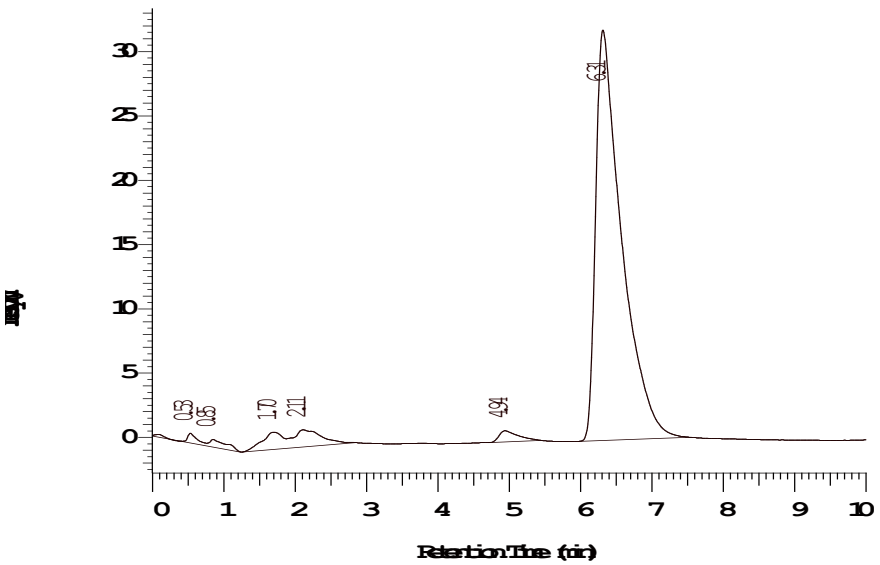

Fig: 4: Acid degradation of Cefdinir.

BASIC HYDROLYSIS

An accurately weighed 10 mg of pure drug was transferred to a clean and dry 10 ml volumetric flask. To which 0.1 N NaOH was added and make up to the mark and kept for 24 hrs from that 0.1 ml was taken in to a 10ml volumetric flask and make up to the mark with mobile phase, then injected in to HPLC system against a blank of NaOH (after all optimized conditions).

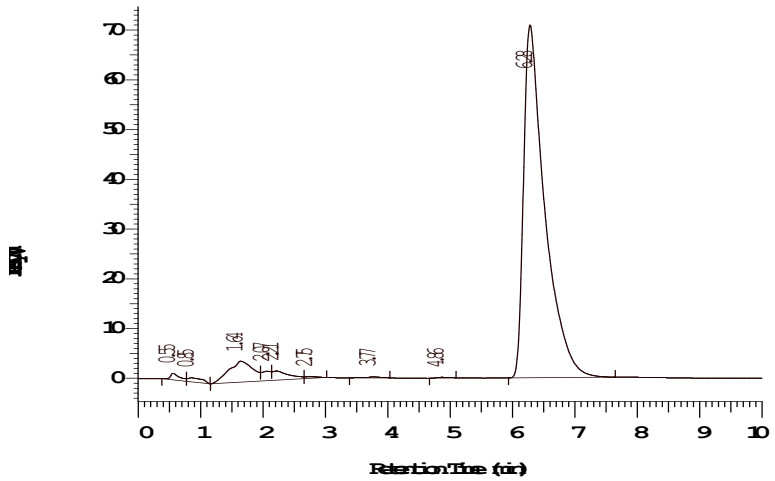

Fig 5: Basic hydrolysis.

OXIDATION WITH (3%) H_2O_2

An accurately weighed 25 mg of pure drug was transferred to a clean and dry 25ml volumetric flask. To which 3% H_2O_2 was added and make up to the mark and kept for 24 hrs from that 0.2ml was taken in to a 10 ml volumetric flask and make up to the mark with diluents then injected for HPLC analysis.

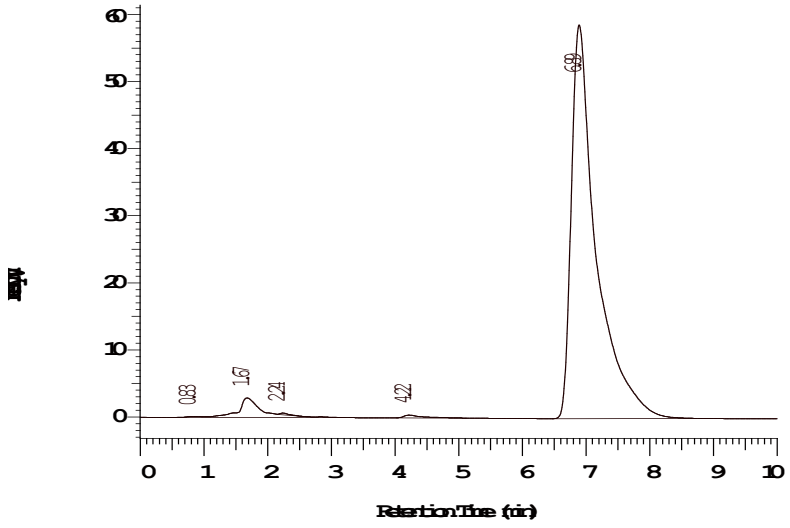

Fig 6: Oxidation with (3%) H_2O_2.

Table 5: Results of forced degradation Studies.

Stress Condition	Time	Assay of active substance	Assay of degraded products	Mass balance
Acid Hydrolysis (0.1 M HCL)	**24 Hrs**	**78.36**	**20.23**	**98.86**
Basic Hydrolysis (0.1 M NaOH)	**24 Hrs**	**98.32**	**----------**	**98.32**
3 % Hydrogen Peroxide	**24 Hrs**	**93.31**	**----------**	**93.31**

CONCLUSION

A sensitive and selective RP- HPLC method has been developed and validated for the analysis of Cefdinir API.

The solvent system used in this method was economical. The results expressed in tables for RP-HPLC method was promising, The RP-HPLC method is more sensitive, accurate and precise compared to Spectrophotometric methods.

Further the proposed RP-HPLC method has excellent sensitivity, Precision and reproducibility**.**

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