www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN( USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 114-121, Jan-Mar 2010

# A LCMS compatible Stability-Indicating HPLC Assay Method for Cefdinir

**U.C.Mashelkar, Sanjay D.Renapurkar***

[1]Principal S.S. & L.S. Patkar College of Arts and Science. S.V.Road, Goregaon West Mumbai 400062, India

[1]Department of Chemistry, S.S. & L.S. Patkar College of Arts and Science. S.V.Road, Goregaon West Mumbai 400062, India

*Corres.author: renapurkars@rediffmail.com
Telephone no 9820550036*

**Abstract:** A stability-indicating HPLC method for the quantitative determination of Cefdinir is described. Cefdinir is a semi-synthetic cephalosporin belonging to third generation antibiotic. The drug is an oral aminothiazolyl hydroxyimino cephalosporin derivative.

Separation was achieved on a Inertsil C18 HPLC column using a mobile phase which consists of a mixture of 0.02 M ammonium formate buffer pH = 4.5 (Solvent A) and organic modifier methanol (Solvent B). The method developed was LCMS compatible and can be used to identify the degradation products. The mass spectrum provides the identity of degradation products formed and proves the specificity of the method unambiguously which demonstrates the stability-indicating power of the developed method.

Degradation studies were performed on bulk samples of Cefdinir under acidic conditions (using 1.0 N hydrochloric acid), basic conditions (0.05 N sodium hydroxide), neutral conditions, oxidation (0.1 % v/v hydrogen peroxide), thermal (105 °C) and photolytic conditions (UV light 254 nm).

Degradation was observed under hydrolytic (acidic and basic), oxidative and photolytic stress conditions. Similar degradation products have been described by Yoshihiko Okamoto et al and serve as representative model for developing method. The method was further validated with respect to linearity, accuracy, precision and robustness.

**Keywords :** Cefdinir, HPLC method, LCMS compatible method and stability indicating

## Introduction

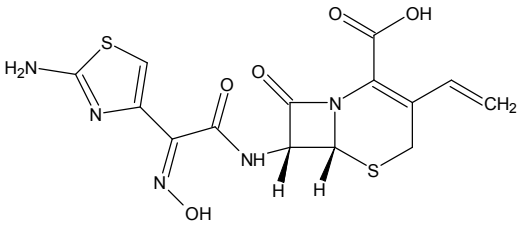
Cefdinir is a semisynthetic cephalosporin antibiotic. The drug is an oral aminothiazolyl hydroxyimino cephalosporin. Cefdinir is structurally similar to other oral (cefpodoxime proxetil, ceftibuten) or parenteral (cefepime, cefotaxime, ceftazidime, ceftriaxone) cephalosporins that contain an aminothiazolyl side chain at position 7 of the cephalosporin nucleus; however, cefdinir contains an unsubstituted oxime group instead of methoxyimino group contained in many aminothiazolyl cephalosporins. The oxime group may contribute to improved activity against gram-positive bacteria. Like cefixime, cefdinir contains a vinyl moiety at position 3 of the cephalosporin nucleus which makes the drug suitable for oral administration.

Chemically, Cefdinir is [6R-[6α, 7β (Z)]]-7-[[(2-amino-4- thiazolyl) (hydroxyimino) acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.. So

far to our present knowledge no LCMS compatible stability-indicating analytical method for Cefdinir has been published in the literature[1-7], although there are few methods for separation of Cefdinir and its related compounds have been published [5,7].

The present work deals with development of stability indicating assay method for Cefdinir and investigates the degradation of the drug under stress conditions such as acid hydrolysis, base, hydrolysis, oxidation, thermal and photolytic. The work also includes validation of HPLC method developed.

.**Structure of Cefdinir, drug substance**



## Experimental

### Reagents and Chemicals

Sample of Cefdinir was received through the kind courtesy of German Remedies limited, Mumbai, India The drug substance was used as received. HPLC (gradient) grade Methanol was purchased from Merck, Germany. Ammonium formate AR grade was procured from Qualigens India whereas sodium hydroxide, hydrochloric acid and hydrogen peroxide were procured from Qualigens India. High purity water was prepared with a Millipore Milli Q plus system.

### Chromatographic Conditions HPLC

A Waters Alliance 2695 Separations model LC system equipped with a photodiode array detector was used. The output signal was monitored and processed using Empower software (Waters) on a Pentium computer (HP). The column used was Inertsil C18 (150 X 4.6 mm, 5 micron) and the mobile phase consisted of solvent A (0.02 M of ammonium formate pH = 4.50 ± 0.05 with formic acid in HPLC grade water) and solvent B (Methanol). The flow rate was 1.0 ml/min. The HPLC gradient was T/B: 0/5, 5/5, 20/50, 25/50, 28/5, and 30/5 % v/v. (where, T is time in minutes and B is concentration of solvent B). The column temperature was maintained at 35 °C and the analysis was carried out at wavelength, = 285 nm. The injection volume was 20 µl. A mixture of ammonium formate 0.05 M in water adjusted to pH = 7.00 ± 0.05 and methanol 90:10 was used as diluent.

### HPLC-PDA-ESI-MS (LC-MS)

The analyses were conducted using a HPLC coupled with LCQ Advantage (ThermoElectron, USA) mass spectrometer operating in the positive and negative ion modes with electrospray ionisation (ESI) source. The mass spectra were obtained as an average of 50 scans.
The chromatographic conditions were maintained the same as described above, however the flow of mobile phase from HPLC was directed into the ESI source at a flow rate of 0.2 ml/min by using a flow splitter. ESI source conditions were as follows: heated capillary temperature 300 °C; sheath gas (Nitrogen) flow rate 40 units (ca. 0.60 L/min); spray voltage 4.5 kV; capillary voltage 25 V; tube lens off set voltage 25 V. For ESI-MS/MS, the precursor ions were first isolated by applying an appropriate waveform across the end cap electrodes of the ion trap to resonantly eject all trapped ions except those ions of the m/z ratio of interest. The isolated ions were then subjected to a supplementary ac signal to resonantly excite them and to cause collision-induced dissociation (CID). The relative collision energy was set to a value at which product ions were produced in measurable abundance varying from 18 to 40 %. The isolation width used in the MS/MS experiments was 3unit.

### Preparation of Standard Solutions

Working solution of 200 ppm (µg/ ml) was prepared for assay determination and carrying out degradation studies.

### Specificity

The specificity of the method for Cefdinir was carried out in the presence of its degradation products (figure I) degradant I, II, III, IV and V. Degradant I and II are product of hydrolysis of Cefdinir observed under basic conditions. Degradant III is hydrolysis product formed under acidic to neutral conditions as well under photolytic conditions. Degradation products IV and V are products of oxidation formed under oxidative stress conditions.
Degradation studies were performed on the bulk drug to provide an indication of the stability-indicating property and specificity of the proposed method. Forced degradation studies were carried under conditions of photolysis (UV light at 254 nm), thermal (105°C), acid (1.0 N HCl), base (0.05 N NaOH) and oxidation (0.1 % H2O2) to evaluate the ability of the proposed method to separate Cefdinir from its degradation products. For
thermal studies, the study period was 12 hours, whereas treatment with acidic, basic media and under oxidative stress was carried out for 6 hours The degradation under photolytic stress conditions were carried out for 5 hours. The degradation product observed under acidic and photolytic stress conditions were similar.
Peak purity assessment was carried out on the stressed samples of Cefdinir by using PDA
detector. Specificity was also demonstrated by subjecting the degraded samples to LCMS analysis using the same method. The mass detector showed excellent mass purity for each of the degradation product and the original drug substance.

## Method Validation

### Precision

Precision of the method was evaluated by carrying out six independent assays of a test sample of Cefdinir against a reference standard and calculating the percent RSD. The reproducibility of the method was also

evaluated using a different analyst and a different instrument in the same laboratory.

**Linearity**
Linearity test solutions were prepared from stock solution at seven concentration levels of
analyte (50, 100, 150, 200, 250, 300 and 400 ppm). The peak area versus concentration data was performed by least-squares linear regression analysis. The calibration curve was drawn by plotting Cefdinir average area for triplicate injections and the concentration expressed as a percentage.

**Accuracy**
The accuracy of the method was evaluated in triplicate at three concentration levels i.e. 100, 150, 200, 250 and 400 ppm in bulk drug sample. The % recoveries were calculated.

**Robustness**
To determine the robustness of the method, experimental conditions were purposely altered. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, it was changed from 0.9 to 1.1 ml/min while the other mobile phase components were maintained constant. The effect of column temperature on resolution was studied at 30 and 40°C instead of 35°C while the other mobile phase components were held constant.

## Results and Discussion

**Optimisation of Chromatographic Conditions**
The main objective was to separate Cefdinir from its degradation products. Different types of column chemistry like C-18. C8, phenyl were considered. Also different lengths of column (100, 150 and 250 mm) and brands were compared. However a Inertsil C18 column (150 X 4.6 mm, 5 micron) was found suitable. A mobile phase consisting of a mixture of solvent A and B is given above. The flow rate of the mobile phase was 1.0 ml/min. The HPLC gradient was kept as T/B: T/B: 0/5, 5/5, 20/50, 25/50, 28/5, and 30/5 % v/v. The column was maintained at 35°C and the wavelength was monitored at 285 nm. Under the optimised conditions, Cefdinir and its degradation products were well separated and the method was found to be specific for Cefdinir and its degradation products.

The mass spectrometer conditions were optimised for obtaining a good signal and high sensitivity for Cefdinir and its degradation products. The conditions like capillary voltage
and spray voltage along with tube lens voltage were varied to maximise the response for Cefdinir and its degradation products, even at a very low concentration. The above mentioned conditions were found suitable for the purpose.

**Results of Degradation Studies**
The degradation of Cefdinir and its degradation products have been studied by K.V Rao et al and Y. Okamoto etal [6,8,9]. The knowledge of these products forms foundation for developing a new stability indicating assay method for Cefdinir. The degradation products formed can be quantified as well as can be identified using LCMS. Also any new product formed can be characterised by LCMS using MS/MS i.e. fragmentation studies.Cefdinir drug substance degrades under basic conditions at ambient temperature forming two degradation products (degradant I and II) [8,9] and under acidic conditions at ambient temperature forming a single major degradation product (degradant III) [8,9].
Cefdinir degrades under oxidative stress conditions and forms two degradation products (degradant IV and V). Degradation product V is a major degradation product which is. S-oxide formed by oxidation of Cefdinir. Degradation product IV is formed in minor amount and the concentration does not increase significantly with respect to time, whereas the degradation product V shows significant increase with time. Hence only degradation product V was considered for identification. These products formed are unstable and hence could not be isolated for further characterisation. However the mass spectrum observed during the LCMS analysis suggests the formation of these degradation products.[6]

Peak purity test results and the mass purity [figure II] confirm that the Cefdinir peak was homogeneous and pure in all the analysed stress samples. The mass balance of stressed samples was greater than 98.0 (Table 1). The assay of Cefdinir is unaffected in the presence of degradation products confirming the stability-indicating power of the method.

**Precision**
The percent RSD of assay of Cefdinir during assay method precision studies was well within 1% thus confirming good precision of the method.

**Linearity**
A linear calibration plot for the method was obtained over the calibration ranges 50–600 ppm with a correlation coefficient greater than 0.999. Linearity was checked over the same concentration range for three consecutive days. The results showed that an excellent
correlation exists between the peak area and concentration of the analyte.

**Accuracy**
The percentage recovery of Cefdinir in bulk drug samples was in the range of 98.0 to 102.0 % w/w.

**Robustness**

In all the deliberately varied chromatographic conditions (flow rate and column temperature) no significant change in assay value was observed, which confirms the robustness of the method.

**System suitability**

The system suitability was established by evaluating parameters like asymmetry factor and plate count. The results were established by six replicates of Cefdinr solution (200 ppm), which prove the method suitability for intended purpose.

The mean results for six replicates are given below,

**Parameters Observed / Preferred**

| **Parameters** | **Observed** | **Preferred** |
|---|---|---|
| % RSD (for area) | 0.81 | less than 2.0 |
| % RSD (Retention time) | 0.20 | less than 1.0 |
| Peak tailing factor(as per USP) | 1.05 | less than 1.3 |
| Plate count (N) (as per USP) | 52000 | more than 25000 |

## Conclusions

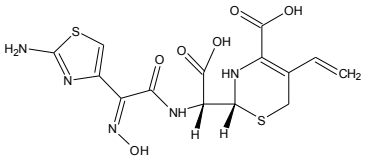
The gradient RP-HPLC method developed for quantitative determination of Cefdinir is precise, accurate and selective. The method was completely validated by obtaining satisfactory data for all the method validation parameters tested. The studies suggest that the method can be used for assessing the stability of bulk samples of Cefdinir.

**Table I : Degradation studies and mass balance for Cefdinir drug substance**

| **Degradation Studies** | **Time** | **Assay (by % area)** | **Assay (% w/w)** | **Remarks** |
|---|---|---|---|---|
| Control | | 100 % | 98.2% | |
| Thermal at 105°C | 12 days | 100 % | 98.0 % | No significant degradation observed |
| Base hydrolysis (at RT) | 6 h | 86.8 % | 86.2 % | Degradation product I and II observed |
| Acid hydrolysis (at RT) | 6 h | 88.1 % | 87.5 % | Degradation product III observed |
| Oxidation by $H_2O_2$ | 6 h | 88.7 % | 88.8 % | Degradation products IV and V observed |
| Photolytic Condition | 5 h | 53.9 % | 52.7 % | Degradation product III observed |

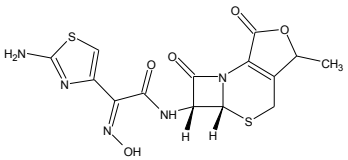
**Figure I: Degradation products of Cefdinir and their structures.**

**Degradation product I**



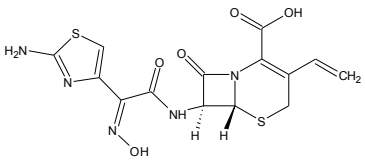
β-Lactam ring opened product of Cefdinir
Molecular weight = 413, $(M+H)^+$ = 414

**Degradation product III**



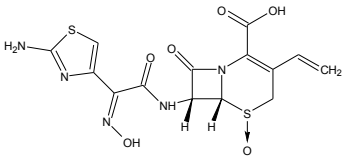
Cefdinir –lactone product
Molecular weight = 395, $(M+H)^+$ = 396

**Degradation product II**



Cefdinir 7-epimer
Molecular weight = 395, $(M+H)^+$ = 396

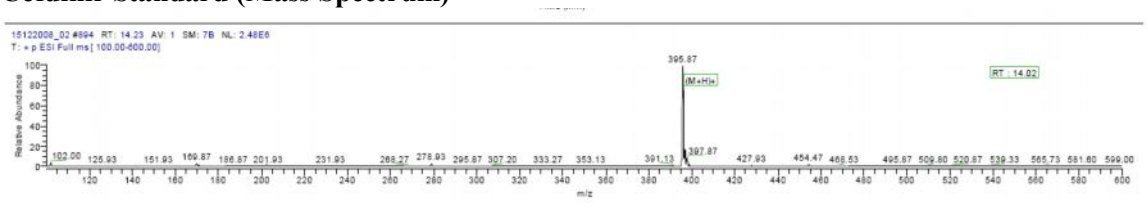
**Degradation product IV**



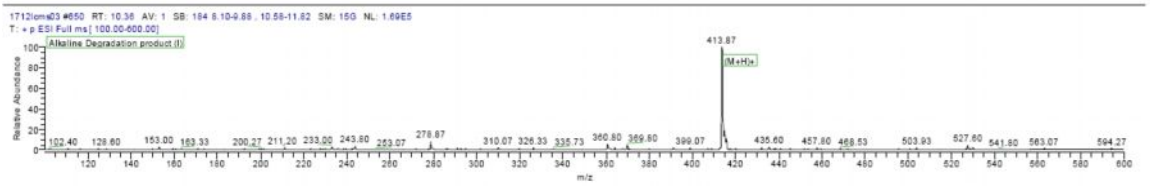
Cefdinir Sulfoxide
Molecular weight = 411, $(M+H)^+$ = 412

**Figure II : Mass spectrum Cefdinir and its degradation products obtained by LCMS Analysis**

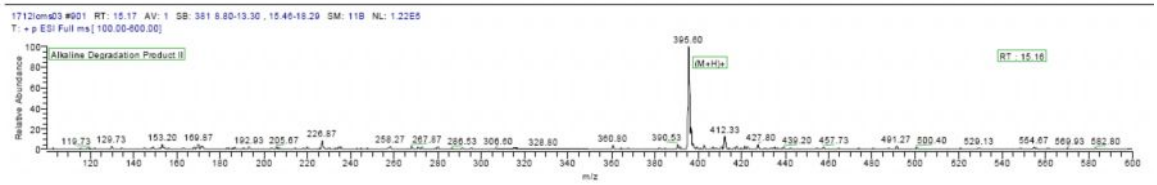
**Cefdinir Standard (Mass Spectrum)**



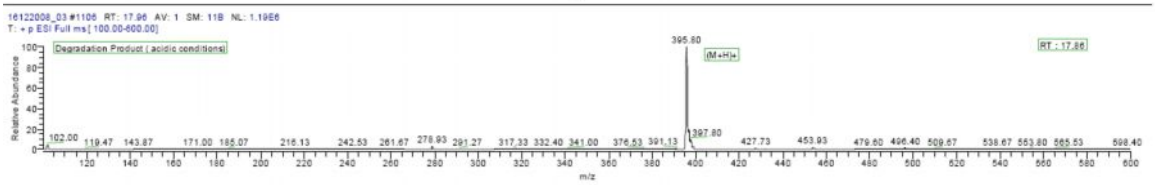

**Mass Spectrum of Degradation Product (I) β-Lactam ring opened product of Cefdinir**




**Mass Spectrum of Degradation Product (II) - Cefdinir 7-epimer**



**Mass Spectrum of Degradation Product (III) - Cefdinir –lactone product**




**Mass Spectrum of Degradation Product (IV) - Cefdinir Sulfoxide**

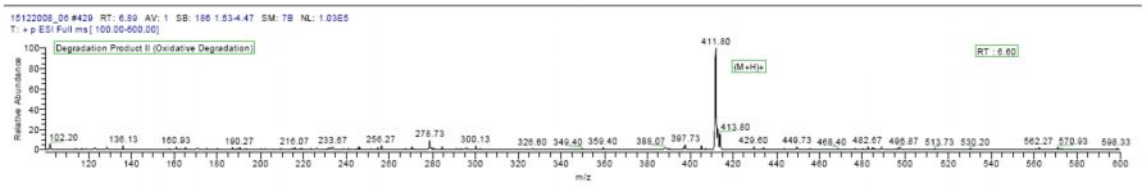
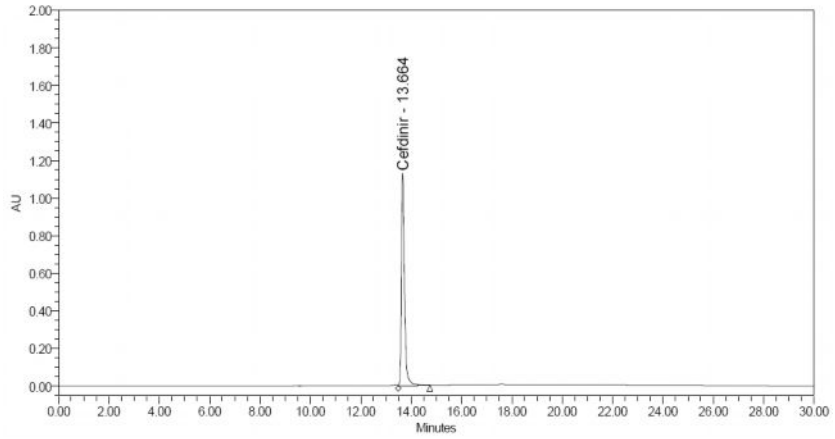
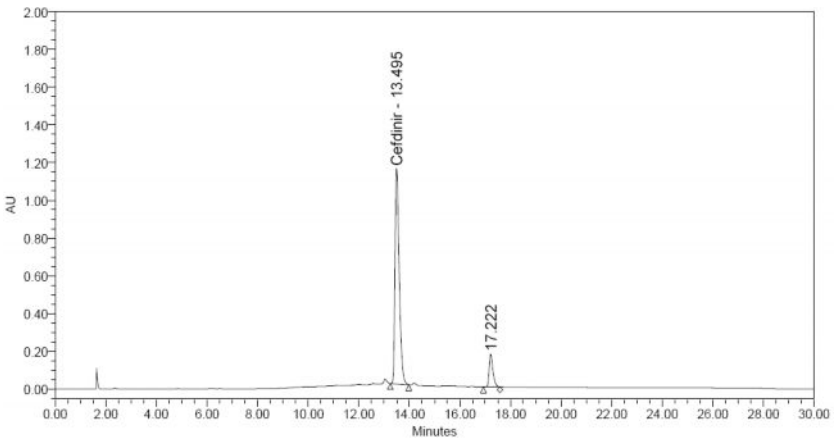



**Figure III: Cefdinir and its degradation products obtained by HPLC Analysis**

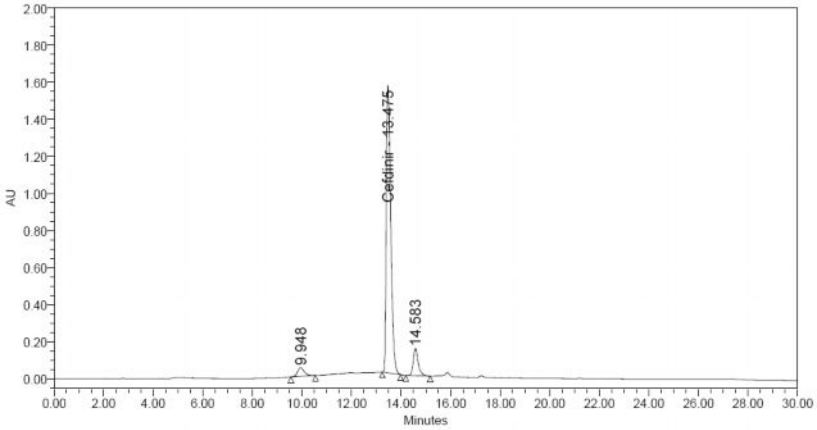
**Cefdinir Standard**



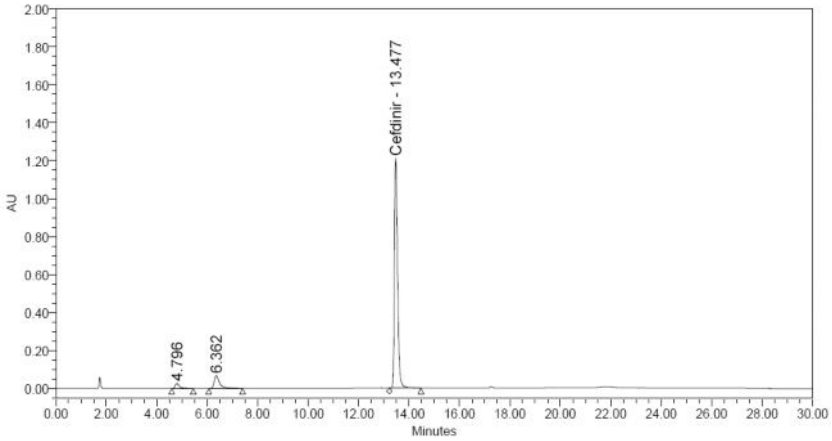

**Degradation under acidic degradation**



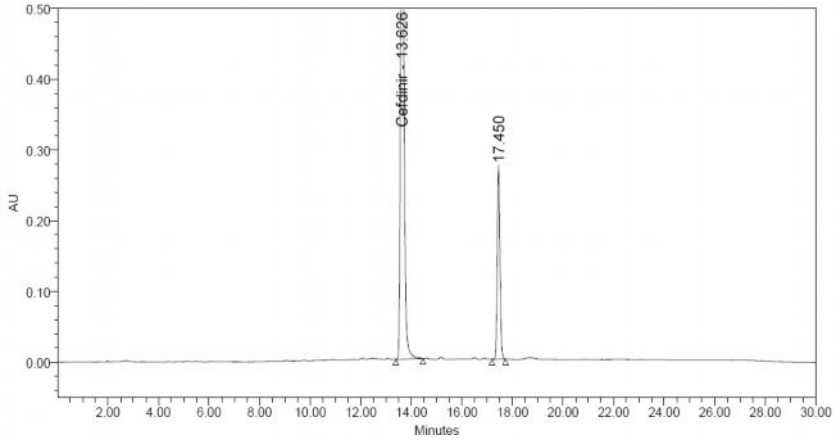
**Degradation under alkaline degradation**




**Oxidative degradation**




**Photolytic degradation**



## References


1. PB Shah, K Pundarikakshudu, Difference spectroscopic and reverse phase HPLC methods for the estimation of cefdinir in pharmaceutical dosage forms,Year: 2006, Volume 68, Issue 1, Page 90-93.

2. Zhang-jing ChenJing Zhang, Ji-cheng Yu, Guo-ying Cao, Xiao-jie Wu and Yao-guo Shi. Selective method for the determination of cefdinir in human plasma using liquid chromatography electrospray ionization tandam mass spectrometry , Journal of Chromatography B Volume 834, Issues 1-2, 13 April 2006, Pages 163-169

3. Rajeev Jain, Keisham Radhapyari, and Nimisha Jadon Electrochemical Evaluation and Determination of Cefdinir in Dosage Form and Biological Fluid at Mercury Electrode J. Electrochem. Soc., Volume 154, Issue 11, pp. F199-F204 (2007).

4. Akira Hishida, , Kazuhisa Ohishi,Satoru Nagashima, Mitsutaka Kanamaru, Masao Obara, and Ayako Kitada, Pharmacokinetic Study of an Oral Cephalosporin, Cefdinir, in Hemodialysis Patients Antimicrobial Agents and Chemotherapy, July 1998, p. 1718-1721, Vol. 42, No. 7.

5. Tushar N Mehta, Gunta Subbaiah, Kilambi Pundarikakshudu; Determination of Cefdinir by a stability-indicating liquid chromatographic method. J AOAC Int; 88 (6):1661-5.

6. Rao KV, Rani A, Reddy AV, Bharathi CH, Dandala R, Naidu A., Isolation, structural elucidation and characterization of impurities in Cefdinir. J Pharm Biomed Anal. 2006 Dec 14.

7. Okamoto Y, Itoh K, Namiki Y, Matsushita J, Fujioka M, Yasuda T., Method development for the determination of cefdinir and its related substances by high-performance liquid chromatography. J Pharm Biomed Anal. 1996 Apr;14(6):739-48.

8. Okamoto Y, Kiriyama K, Namiki Y, Matsushita J, Fujioka M, Yasuda T. Degradation kinetics and isomerization of cefdinir, a new oral cephalosporin, in aqueous solution. 2. Hydrolytic degradation pathway and mechanism for beta-lactam ring opened lactones. J Pharm Sci. 1996 Sep;85(9):984-9.

9. Okamoto Y, Kiriyama K, Namiki Y, Matsushita J, Fujioka M, Yasuda T. Degradation kinetics and isomerization of cefdinir, a new oral cephalosporin, in aqueous solution. 1. J Pharm Sci. 1996 Sep;85(9):976-83.


*****