Original Article

A developed and validated stability-indicating reverse-phase high performance liquid chromatographic method for determination of cefdinir in the presence of its degradation products as per International Conference on Harmonization guidelines

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Abstract

The present article deals with the development and validation of a stability-indicating, reverse-phase high performance liquid chromatographic (RP-HPLC) method, for the determination of cefdinir on a Waters RP Spherisorb C-18 column (250 mm × 4.6 mm, 5 μm). A mobile phase consisting of water (pH adjusted to 3.0 with orthophosphoric acid) : acetonitrile : methanol 13:5:2 (v/v/v) was used. The flow rate was 1 mL min^{-1}. The separation was performed at room temperature. Detection was carried out at 286 nm, using a PDA detector. The developed method was statistically validated for the linearity, accuracy, specificity, Limit of Detection (LOD), and Limit of Quantitation (LOQ). The specificity of the method was ascertained by forced degradation studies, by acid and alkali degradation, oxidation, photolysis, and heat degradation. The degraded products were well-separated from the analyte, with significant differences in their retention time values. The Beer Law was obeyed over a concentration range of 0.05 – 15.00 μg mL^{-1} and the correlation coefficient was 0.999.

Key words: Cefdinir, degradation products, reverse-phase high performance liquid chromatographic, validation

INTRODUCTION

Cefdinir is an advanced generation, broad / extended-spectrum oral semisynthetic cephalosporin.[1] The drug is an oral aminothiazolyl hydroxyimino cephalosporin. Cefdinir a broad spectrum cephalosporin is effective against enteric gram-positive and gram-negative bacteria. Cefdinir is stable in the presence of some, but not all, β-lactamase enzymes. As a result, many organisms are resistant to penicillins and some cephalosporins are susceptible to cefdinir.[2] It has been proven to be effective for common bacterial infections of the ear, sinus, throat, and skin.[3]

Chemically, cefdinir [Figure 1] 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.[4] 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 rather than the methoxyimino group contained in many aminothiazolyl cephalosporins. The oxime group may contribute to the improved activity against gram-positive bacteria. Similar to cefixime, cefdinir contains a vinyl moiety at position 3 of the cephalosporin nucleus, which makes the drug

suitable for oral administration.[5-6] 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.

A literature survey revealed that few analytical methods are available for the estimation of cefdinir from bulk drugs / dosage forms[7-16] and from human plasma.[17] The reported method for the estimation of cefdinir includes HPLC,[7-11] the ultraviolet (UV) spectroscopic and colorimetric method,[12-13] and the spectroflourimetric method of analysis.[14] There is also a liquid chromatography-tandem mass spectrometry (LCMS-MS) method reported for the estimation of cefdinir from human plasma.[17] The earlier reported methods were either less sensitive[12-14] or involved costlier techniques like LCMS-MS.[16-17] Hence, the objective was to develop a simple and cost-effective method for the analysis of cefdinir.

The International Conference on Harmonization (ICH) guidelines require that stress testing be carried out to elucidate the inherent stability characteristics of the active substances. It suggests that degradation products that are formed under a variety of conditions should be identified and the degradation pathway established. It is stated that testing should include the effect of temperature, humidity, oxidation, photolysis, and acid and base hydrolytic conditions.[18] An ideal stability-indicating method is the one that quantifies the drug *per se* and also resolves its degradation products. Stability is considered to be one of the most important criteria in pharmaceutical quality control. Only a stable preparation would promise precise delivery of the drug to the patients. Expiration dating on any drug product is based on scientific studies at normal and stressed conditions.[19]

With this background, an attempt has been made to develop and validate a stability-indicating RP-HPLC method, for the accurate quantitation of cefdinir in bulk drugs, in the presence of its degradation products.

EXPERIMENTAL

Reagents and materials

Cefdinir bulk drug (98% pure) was supplied by Glenmark Pharmaceuticals Ltd. (Mumbai, India).

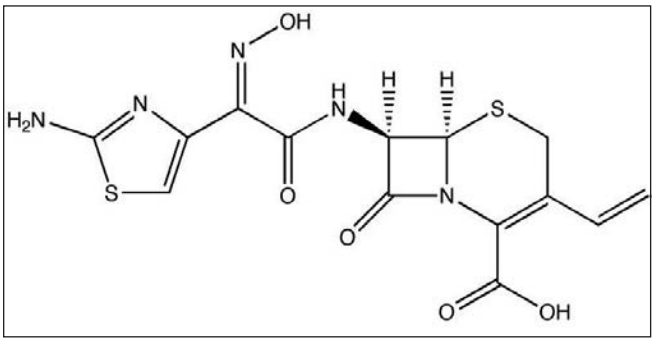

Figure 1: Chemical structure of cefdinir

Acetonitrile and methanol (HPLC grade) were procured from Merck (Darmstadt, Germany). Deionized and ultra pure water (Millipore grade) used in all experiments was obtained from Milli-Q System (USA). The 0.45-µm Nylon pump filter was obtained from Advanced Microdevices (Mumbai, India). Orthophosphoric acid, used for adjusting the pH of the buffer solution (AR grade), was procured from (S. D. Fine Chemicals). Sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) (all AR grade) were purchased from Qualigens Fine Chemicals (Mumbai, India).

Instruments

The instrument used was the Shimadzu (Japan) Liquid Chromatographic system, equipped with an LC-10AT, VP pump, and a photodiode array detector system (SPD-10A, VP). The output signal was monitored and processed using Clarity software on a Pentium computer (HCL-Mumbai).The column used was Waters RP Spherisorb C-18 (250 mm × 4.6 mm i.d. × 5 µm particle size)

Chromatographic conditions

Chromatographic separation was achieved at room temperature on a reverse phase column, using a mobile phase consisting of orthophosphoric acid in water (pH 3.0) : acetonitrile : methanol in a ratio of 13 : 5 : 2 (v/v/v). Before use it was filtered through a 0.45-µm Nylon filter and degassed in an ultrasonic bath. The flow rate was set at 1.0 mL min^{-1}. The injection volume was 20 µL and ultraviolet (UV) detection was performed at 286 nm. For analysis of samples from forced degradation, a photodiode-array detector was used in scan mode, in the range of 200 – 400 nm.

Preparation of the mobile phase

HPLC grade water of 650 mL (pH adjusted to 3.0 with orthophosphoric acid) was mixed with 250 mL

of acetonitrile and 100 mL of methanol. A final mobile phase of 1000 mL, (pH 3.0), was then filtered through a 0.45-μm Nylon filter and sonicated in an ultrasonic bath for 15 minutes.

Preparation of the standard solution

The standard stock solution (1 mg mL^{-1}) of cefdinir was prepared by dissolving the working standard in methanol : acetonitrile 4 : 1 (v/v). Standard calibration solutions (0.05 - 15.0 μg mL^{-1}) for the assessment of linearity were prepared from this stock solution, by dilution with the mobile phase. The solution was then filtered through a 0.45 μm Nylon filter. The filtered solution was then injected into the HPLC system.

Induced degradation of cefdinir

Acid- and Base-Induced Degradation

Acid-induced forced degradation was performed by adding 10 mL stock solution (1 mg mL^{-1}) of cefdinir to 10 mL each of methanol and 0.1 M HCl, and refluxing the mixture at 60°C for approximately six hours. The solution was then left to reach ambient temperature, neutralized to pH 7 by addition of 0.1 M NaOH, and then diluted to 100 mL, with a mobile phase, so as to get a concentration of 100 μg mL^{-1}. From this solution 10 mL was diluted to 100 mL to get the final concentration of 10 μg mL^{-1}.

Base-induced forced degradation was performed by adding 10 mL stock solution (1 mg mL^{-1}) of cefdinir to 10 mL each of methanol and 0.1 M NaOH and refluxing the mixture at 60°C for approximately six hours. The solution was then left to reach ambient temperature, neutralized to pH 7 by addition of 0.1 M HCl, and then diluted to 100 mL with a mobile phase, so as to get a concentration of 100 μg mL^{-1}. From this solution 10 mL was diluted to 100 mL to get the final concentration of 10 μg mL^{-1}.

Oxidative degradation

To study the effect of the oxidizing conditions, 10 mL stock solution (1 mg mL^{-1}) of cefdinir was added to 10 mL of 30% H_2O_2 solution and the mixture was refluxed at 60°C for approximately six hours. The solution was left to reach ambient temperature and then diluted to 100 mL with a mobile phase, so as to get a concentration of 100 μg mL^{-1}. From this solution 10 mL was diluted to 100 mL to get the final concentration of 10 μg mL^{-1}.

Thermal degradation

To study the effect of temperature, approximately 50 mg cefdinir was stored at 80°C for 48 hours. Then it was dissolved in 10 mL of methanol and the volume was adjusted to 50 mL, with a mobile phase. The above solution was further diluted with the mobile phase to give a solution of final concentration equivalent to 10 μg mL^{-1} of cefdinir.

Photolysis

To study the effect of UV light, approximately 50 mg of cefdinir was exposed to short and long wavelength UV light (254 nm and 366 nm, respectively) for 48 hours, and then dissolved in 10 mL of methanol and the volume made up by the mobile phase, in a 50 mL volumetric flask, and then 1 mL of stock solution was further diluted with the mobile phase, to give a solution of final concentration equivalent to 10 μg mL^{-1} of cefdinir.

Twenty microliters of the resulting solution was injected into the HPLC, and the chromatograms were recorded. The stability samples were analyzed using the PDA detector, to determine the peak purity, as the method was found to be rugged in nature. The results of the percent degradation are shown in Table 1.

Table 1: Percent degradation of cefdinir and retention time of degradation products

Condition	Retention time of drug/ degradation products (Min)	Peak area (μV. sec)	Percent degradation (%)
Untreated stock solution	7.67	983771.51	--
Acid hydrolysis	7.27	785660.54	20.14
Base hydrolysis	5.55	29732.13	48.83
	7.52	503391.21	
	8.48	59871.18	
	14.23	11896.09	
Oxidation	6.07	25428.11	31.20
	7.97	676871.38	
	9.57	1188.24	
Thermal degradation	7.58	785781.21	20.12
	9.84	87754.07	
Photolytic degradation	7.56	899626.31	8.55

RESULTS AND DISCUSSION

Method development

The chromatographic conditions were optimized to develop a stability indicating assay method for cefdinir. The column, Waters RP Spherisorb, gave good peak shape with response at an affordable retention time. Various composition of solvents were tried in order to get a maximum resolution of the peaks. Finally, the optimum separation was achieved using the mobile phase consisting of water pH adjusted to 3.0 with orthophosphoric acid : acetonitirile : methanol, in the ratio of 13 : 5 : 2 (v/v/v) at a flow rate of 1 mL min^{-1}. The detection was performed at 286 nm.

Method validation

The method was validated for linearity, limits of detection (LOD) and quantification (LOQ), system suitability, precision, accuracy, specificity, robustness, and stability in accordance with the ICH guidelines.[18] Peak purity was determined with the use of the photodiode-array detector.

Calibration curve

The linearity response for cefdinir was determined by injecting solutions with concentrations of 0.05 – 15.00 μg mL^{-1}. Each solution was injected in triplicate, keeping the injection volume constant (20 μL). The linear regression data for the calibration curve indicated that the response was linear over the concentration range studied, with the coefficient of correlation, r^2 value (0.999), and slope (137.47). Results from the regression analysis with system-suitability data are listed in Table 2.

Table 2: Results from regression analysis and system suitability of cefdinir

Description	Value
Retention time (min)	7.67
Linear range (μg/ml)	0.05 – 15.00
Limit of detection (μg/ml)	0.03
Limit of quantification (μg/ml)	0.05
Slope (*m*)	137.47
Correlation coefficient (*r*)	0.999
Tailing factor	1.32
Theoretical plates	7362.85

Detection limits

The limits of detection (LOD) and quantification (LOQ) for cefdinir were determined as the amounts for which signal-to-noise ratios were 3 : 1 and 10 : 1, respectively, by injecting a series of dilute solutions of known concentration. LOD and LOQ for cefdinir were 0.02 and 0.05 μg mL^{-1}.

Precision

Precision was measured in terms of repeatability of application and measurement data. Repeatability of the standard sample was carried out using six replicates of the same injection (0.15, 5.00, 12.00 μg mL^{-1}). It showed very low relative standard deviation (RSD) of the peak area for the cefdinir standard. These studies were also repeated on different days to determine inter-day precision. Table 3 presents the precision data obtained for the method.

Accuracy

The accuracy of the method was determined by spiking the working standard of cefdinir into placebo at different concentration levels (0.15, 5.00, 12.00 μg mL^{-1}). The resulting solutions were injected in triplicate and the results obtained were compared with the expected results and the recovery of the added drug was expressed as a percentage. Table 3 presents the accuracy data obtained for the method.

Robustness

Robustness of the method was investigated by varying the chromatographic conditions such as change of flow rate (± 10%), organic content in the mobile phase (± 2%), wavelength of detection (± 5%), and pH of buffer in the mobile phase (± 0.2%). Robustness of the developed method was indicated by the overall % RSD between the data at each variable condition. The observed RSD was 1.47% for the flow rate change, 0.96% for the organic content change, 0.37% for the detection change, and 0.74% for the pH of mobile phase change. In all the conditions the RSD was < 2%, indicating a robust method.

Table 3: Precision and recovery data

Actual concentration (μg/ml)	Precision		% Recovery	
	Measured Concentration (μg/ml) ± S.D.; % R.S.D.			
	Intra-day	Inter-day	Intra-day	Inter-day
0.150	0.15 ± 0.004; 2.67	0.15 ± 0.008; 2.33	100.09	100.27
5.000	5.09 ± 0.115; 2.26	5.10 ± 0.111; 2.18	101.80	102.01
12.000	12.10 ± 0.182; 1.50	12.05 ± 0.311; 2.58	100.83	100.42

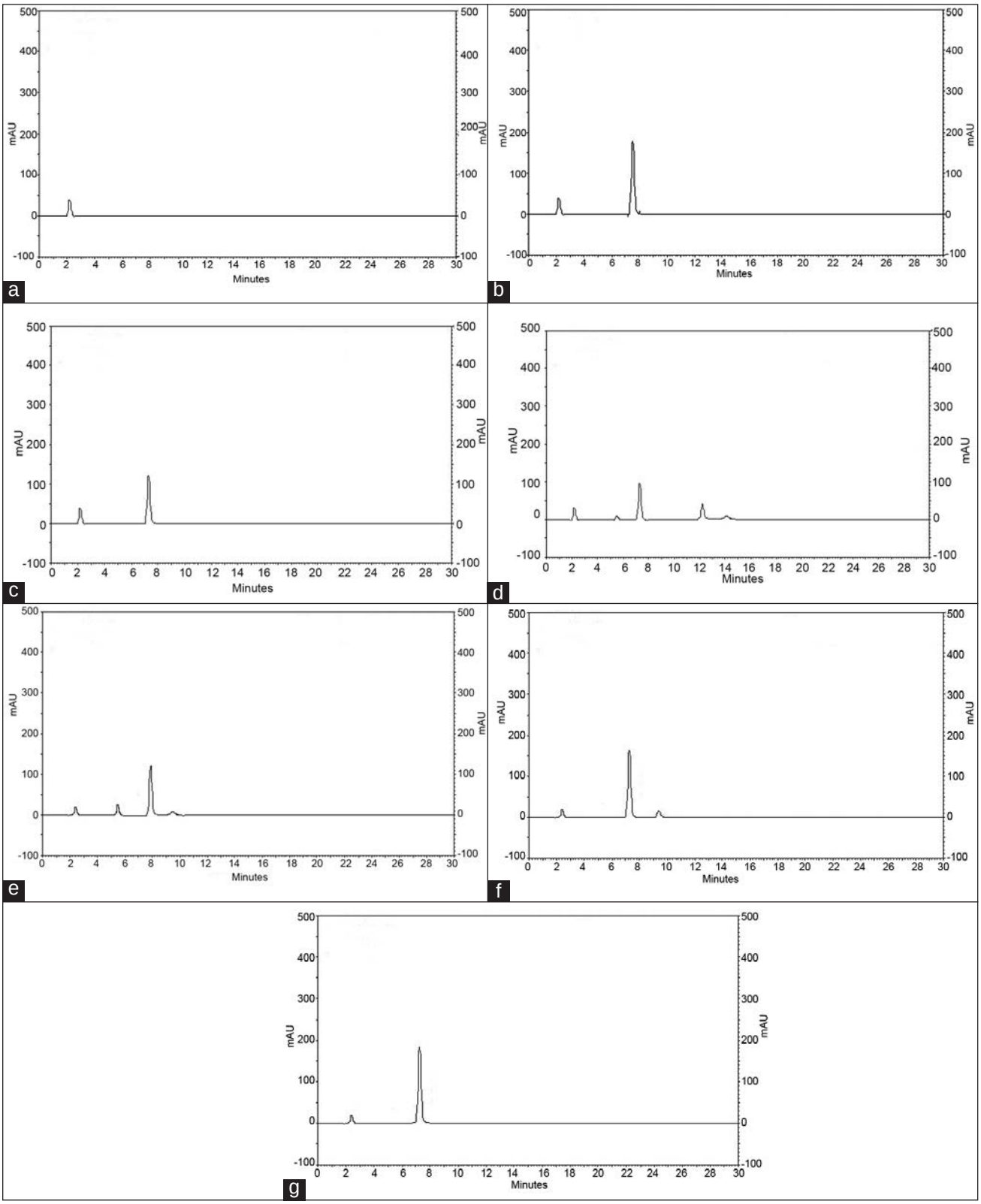

Figure 2: Representative chromatograms of cefdinir for the stability method. (a) Blank, (b) Untreated stock solution, (c) Acid hydrolysis, (d) Base hydrolysis, (e) Oxidation, (f) Thermal degradation

Stability

The solution stability of cefdinir was carried out by leaving the test solutions of the sample and reference standard in tightly capped volumetric flasks for 24, 48, and 72 hours. The results were compared with those obtained from freshly prepared standard

solutions, and calculation of RSD. The RSD values of the assay of cefdinir during solution stability experiments were within 2%. The RSD observed was 0.86, 1.27 and 1.64% at 24, 48, and 72 hours respectively.

Degradation behavior

HPLC studies on cefdinir, under different stress conditions, suggested the following degradation behavior [Table 1]. The drug gradually decreased with time, on heating at 60°C, in 0.1 M HCl [Figure 2c]. The rate of hydrolysis in acid was slower as compared to that of alkali or oxidation [Figure 2d]; 20.14% of the drug degradation was observed with acid. The drug was found to be highly labile to alkaline hydrolysis. The reaction in 0.1 N NaOH at 60°C was so fast that 48.83% of the drug was degraded in 60 minutes [Figure 2d]. Cefdinir proved labile to hydrogen peroxide (3%) at 60°C. After refluxing at 60°C for 60 minutes, 31.20% of the drug was degraded [Figure 2e]. The drug was comparatively stable to thermal degradation. Only 20.12% of the drug was degraded after refluxing at 60°C for 60 minutes, [Figure 2f]. No major degradation product was observed after exposure of the drug solution to UV light for 24 hours. Only minor degradation (8.55%) was observed. [Figure 2g].

CONCLUSION

Thus the developed HPLC method for determination of cefdinir is simple, precise, accurate, and stability indicating. Statistical analysis proves that the method is reproducible and selective for the analysis of cefdinir in bulk drugs. As the method efficiently estimates cefdinir in the presence of its degradation products, it can be employed as a stability indicating method and can also be successfully applied for the assay of cefdinir in the bulk drug and in pharmaceutical dosage forms in the pharmaceutical industry.

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