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Stability indicating HPLC method for the determination of Cefdinir in presence of its acid induced degradation products

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ABSTRACT

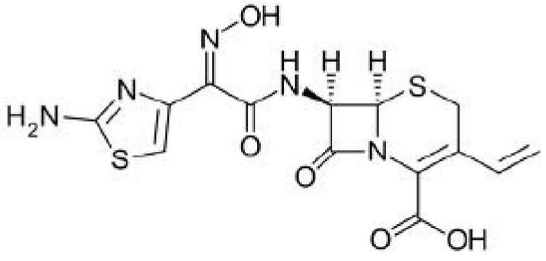
High performance liquid chromatographic technique was proposed for the determination of cefdinir (CEF) in presence of its degradation products. The method was based on HPLC separation of CEF from its acidic degradation products using YMC-Pack ODS-A column at ambient temperature with mobile phase consisting of 10mM sodium dihydrogen phosphate : acetonitrile : methanol (80: 10: 10, by volume, 0.5% triethylamine, pH was adjusted to 4.5 using O-phosphoric acid). Quantification was achieved with UV detection at 285 nm based on relative peak area. The drug was subjected to acid hydrolysis. Complete separation was achieved for the parent compound and all degradation products in an overall analytical run time of approximately 10 minutes with the parent compound CEF eluting at approximately 6.5 min. The method was linear over the concentration range of 2–25 µg/ml (r^2 = 0.9998) with a limit of detection and quantitation 0.0598 and 0.1813 µg/ml, respectively. The method has the requisite accuracy, selectivity, sensitivity and precision to assay CEF in capsules and suspension.

KEYWORDS

Cefdinir;
High Performance Liquid Chromatography
Determination;
Acidic Degradation;
Stability Indicating Study.

INTRODUCTION

Cefdinir (CEF) is chemically designated as (6R,7R)-7-[[(2Z)-(2-Amino-4-thiazolyl)(hydroxyimino) acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid[1]. The drug is not an official one . It is a semisynthetic oral cephalosporin antibiotic, structurally similar to other cephalosporins that contain an aminothiazolyl side chain at position 7 of the cephalosporin nucleus, however CEF 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[2]. It has the following structural formula:



Few methods have been reported for determination of CEF including High performance liquid chromatography[2-8], and spectrophotometry[7].

EXPERIMENTAL

Chemicals and reagents

Pure standard Cefdinir was obtained from Adwia, Egypt and its purity was certified to be 100.08% according to HPLC method[5]. All chemicals were of analytical grade and solvents were of an HPLC grade, Methanol (HPLC grade, SIGMA, Germany), Acetonitrile (HPLC grade, SIGMA, Germany), Triethylamine, hydrochloric acid (analytical grade, SGMA, Germany), Phosphoric acid and sodium hydroxide used were analytical grade (ADWIC, El-Nasr Pharmaceuticals Chemicals Co. Egypt).

Cefdin® Capsules (Batch No. 003) are labeled to contain 300mg Cefdinir per capsule, manufactured by Bristol-Mayers Squibb Egypyt for Novartis Pharma Egypt. Dinar® Suspension (Batch No. 100236/0348) is labeled to contain 125 mg cefdinir per 5ml suspension, manufactured by Kahira Pharmaceutical Company for Adwia. Co. S.A.E 10th of Ramadan city-Egypt. The two dosage forms were obtained from the local market.

HPLC instrumentation and conditions

Samples were loaded into Rheodyne 7725i injection valve, equipped with a 20-µL sample loop (Rheodyne, Berkeley, CA, USA). HPLC separation and quantitation were made on YMC-Pack ODS-A (250 × 4.6 mm i.d., 5 µm particle size, analytical column from YMC Co., Ltd. Japan) with a mobile phase consisting of 10mM sodium dihydrogen phosphate : acetonitrile : methanol (80:10:10, by volume, 0.5% triethylamine, pH was adjusted to 4.5 using O-phosphoric acid). An isocratic pump was used to deliver the mobile phase at a flow rate of 1 mL/min (Agilent 1100 Series Iso pump G1310A, Agilent Technologies, USA). The samples were filtered using 0.45 um membrane filters (Millipore, Milford, MA, USA). The UV-VIS detector (Agilent 1100 Series VWD G1314A) was set at 285 nm. Data acquisition was performed on Agilent LC ChemStation software. All determinations were performed at ambient temperature.

Preparation of mobile phase

The mobile phase was prepared by mixing 10mM sodium dihydrogen phosphate, acetonitrile, methanol and triethylamine (80:10:10:0.5 by volume) and the pH was adjusted to 4.5 using O-phosphoric acid. the prepared mobile phase was filtered through 0.45µm membrane filter and degassed before application by means of ultra-sonication for 15 min.

Solutions

CEF stock standard solution

CEF standard solution (100 µg/ml) in methanol was prepared by accurately weighing 10mg of CEF into 100 mL measuring flask and completing the volume with methanol.

Acidic degradation products (CEF Deg) stock solution

An accurately weighed amount of intact CEF (10 mg) was refluxed with 20 ml 1N HCl for 1 hour. The solution was then neutralized by adjusting the pH using NaOH and transferred quantitatively to 100 mL volumetric flask. The volume was then completed using methanol to produce concentration equivalent to 100 µg/mL. Degradation was checked every 30 minutes and complete degradation was confirmed by HPLC using 10mM sodium dihydrogen phosphate : acetonitrile : methanol (80:10:10, by volume, and the PH was adjusted to 4.5 using O-phosphoric acid.

Preparation of laboratory prepared mixture for stability indicating characterization of the method

Aliquots (0.9 – 0.2 mL) of CEF standard solution (100 µg/mL) equivalent to 9 – 2 µg/mL were accurately transferred into a series of 10 mL volumetric flasks to which aliquots (0.1 – 0.8 mL) of CEF Deg stock solution (100 µg/ml) equivalent to 1 – 8 µg/mL were accurately added. The volumes were then completed with the mobile phase to prepare mixtures containing from 10 – 80 % of CEF Deg.

Standard solutions and calibration

Different aliquots (0.2 – 2.5 mL) of CEF standard solution (100 µg/mL), equivalent to 2 – 25 µg/mL, were transferred into a series of 10mL volumetric flasks. The volumes were then adjusted with the mobile phase. An aliquot of 20 µL was injected into the chromatographic

system and processed according to the previously described conditions.

Pharmaceutical sample solutions

(a) Cefdin Capsules: Six capsules were emptied and the powder was accurately weighed. An amount of powder equivalent to 10 mg CEF was transferred into a 100 mL volumetric flask, the volume was completed to the mark using methanol. The solution (100 µg/mL) was shaken for 10 min using ultrasonic bath. The solution was then filtered, different aliquots (0.4, 0.8, 1 mL) were transferred into a series of 10mL volumetric flasks and the volumes were completed to the mark with the mobile phase to produce solutions of concentration equivalent to 4, 8, 10 µg/mL, respectively. The general procedure for HPLC method described in this work was followed and the concentrations of the drug were calculated. The accuracy of the analytical method was also checked by applying the standard addition technique.

(b) Dinar Suspension: An accurately weighed amount of the powder equivalent to10 mg CEF was transferred into a 100 mL volumetric flask, the volume was completed to the mark using methanol. The solution (100 µg/mL) was shaken for 15 min using ultrasonic bath. The solution was then filtered on a dry funnel and a dry filter paper, different aliquots (0.4, 0.8, 1 mL) were transferred into a series of 10ml volumetric flasks and the volumes were completed to the mark with the mobile phase to produce solutions of concentration equivalent to 4, 8, 10 µg/mL, respectively. The general procedure for HPLC method described in this work was followed and the concentration of the drug was calculated. The accuracy of the analytical method was also checked by applying the standard addition technique.

RESULTS AND DISCUSSION

Simple, precise, and selective HPLC method for the determination of CEF in pure form and in presence of its degradation products without prior separation was developed and validated according to the ICH guidelines[9]. The method was successfully applied for the determination of the drug in its pharmaceutical dosage forms.

In order to optimize the proposed HPLC method, all the experimental conditions were investigated. Several trials were carried out to obtain good and optimum separation of CEF from its degradation products. Different composition mobile phases with different ratios were tried such as sodium dihydrogen phosphate : acetonitrile (80 : 20, v/v), sodium dihydrogen phosphate : acetonitrile (85 : 15, v/v), sodium dihydrogen phosphate : methanol (80 : 20, v/v), and sodium dihydrogen phosphate: acetonitrile : methanol (80 : 10: 10, by volume). Best resolution was obtained upon using sodium dihydrogen phosphate : acetonitrile : methanol (80 : 10 : 10, by volume, 0.5% triethylamine, pH adjusted to 4.5 using O-phosphoric acid) with a flow rate of 1mL/min and a detection wavelength 285 nm. Triethylamine was added in order to decrease the tailing problem.

Upon applying the previously described HPLC optimum experimental conditions, good and efficient separation was observed between CEF and its degradation products. Representative chromatograms showing successful separation of all compounds of interest are shown in Figure 1.

Relative peak areas of CEF were plotted versus CEF concentrations and linear regression analysis was performed on the resultant curve. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value, which was not statistically ($p < 0.05$) different from zero (TABLE 1). Characteristic parameters for regression equation for the HPLC method obtained by least squares treatment of the results were given in TABLE 1. Typically, the regression equation for the calibration curve was found to be $Y = 0.1092C - 0.0806$, where C is the concentration in µg/mL and y is the relative peak area.

TABLE 2 shows the precision of the analytical procedure for both intra- and inter-day variations expressed as the coefficient of variance (CV%). Repeatability (intra-day CV%, n = 5) was excellent being in range of 0.420 – 0.781. Reproducibility (inter-day CV%, n = 5) was in range of 0.212 – 0.559 (TABLE 2).

The method was applied for the determination of the drug in laboratory prepares mixture with its acidic degradation products (TABLE 3) (10% up to 80% for degradate), in Cefdin capsule and Dinar suspension. The validity of the method was assessed by applying the standard addition technique and good recoveries have been obtained (TABLE 4, 5).

The results of the presented HPLC method were

compared with those of the reported HPLC method[5]. Statistical comparison between the results was per-

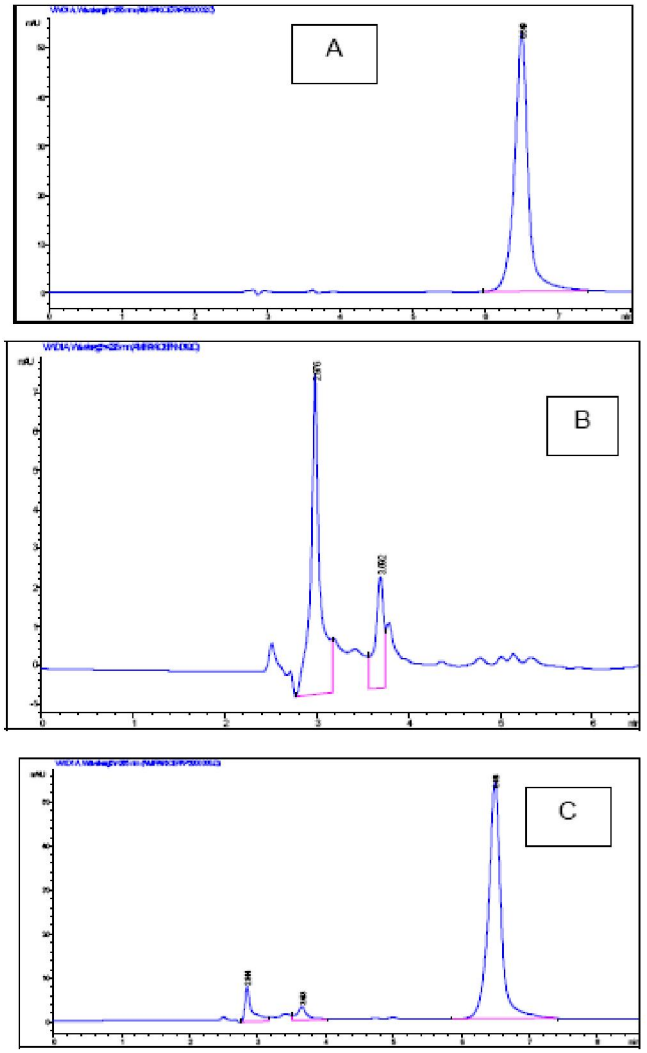


Figure 1: Typical HPLC chromatograms of: (A) intact CEF 10 µg/ml (t_r = 6.500 min): (B) acidic degradation products equivalent to 9µg/ml (t_r =2.976, 3.692): (C) intact CEF 10 µg/ml (t_r = 6.493 min) in presence of its acidic degradation products equivalent to 10 µg/ml CEF (t_r = 2.844, 3.653)

TABLE 4 : Application of standard addition technique for determination of CEF in Cefdin capsule by HPLC method

Product	Found*% ± RSD%	Standard Addition Technique	
		Added (µg mL^{-1})	Recovery%
Cefdin capsules 300mg CEF/capsule B.N.003	100.54 ± 0.527	8	99.75
		10	98.70
		12	98.00
		Mean	98.74
		RSD%	0.835

***Average of 5 determinations.**

TABLE 1 : Characteristic parameters for the regression equation of the proposed HPLC method for the determination of CEF

Parameters	Value
Calibration range (µg/ml)	2 – 25 µg/ml
Detection limit (µg/ml)	0.0598
Quantitation limit (µg/ml)	0.1813
Regression equation (Y)[a] : Slope (b)	0.1092
Standard error of the slope (S_b)	0.0007
Confidence limit of the slope[b]	0.1074 – 0.1109
Intercept (a)	-0.0806
Standard error of the intercept[b]	0.0095
Confidence limit of the intercept	-0.1039 – -0.0573
Regression coefficient	0.9999
Standard error of estimation	0.015

[a] Y = a + bC, where C is the concentration of Cefdinir in µg/ml and Y is the relative peak area.
[b] 95% confidence limit.

TABLE 3 : Determination of CEF in presence of its acidic degradation products using HPLC method

Mixture No.	Degradates %	Cefdinir (µg /ml)		
		Taken	Found	Recovery%
1	10	9	8.85	98.33
2	20	8	8.02	100.25
3	30	7	7.03	100.43
4	40	6	6.04	100.67
5	50	5	4.91	98.20
6	60	4	3.93	98.25
7	70	3	3.05	101.67
8	80	2	2.08	104.00
	Mean			100.22
	RSD%			2.001

TABLE 2 : Intra- and inter- day validation for determination of CEF by HPLC method

Concentration (µg/ml)	Intra-day assay	
	Recovery% ± SD[a]	CV%
4	99.79 ± 0.779	0.781
10	99.80 ± 0.685	0.686
20	99.51 ± 0.418	0.420
	Inter-day assay	
	Recovery% ± SD[a]	CV%
4	101.09 ± 0.373	0.369
8	99.51 ± 0.556	0.559
15	99.65 ± 0.211	0.212

[a]Mean and S.D. for five determinations

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TABLE 5 : Application of standard addition technique for determination of CEF in Dinar suspension by HPLC method

Product	Found*% ± RSD%	Standard Addition Technique	
		Added (µg mL^{-1})	Recovery%
Dinar suspension 125mg CEF/5ml B.N.100236/0348	99.92 ± 0.317	8	99.63
		10	100.70
		12	100.75
		Mean	100.36
		RSD%	0.630

***Average of 5 determinations.**

TABLE 6 : Statistical comparison between the results obtained by applying HPLC method for the analysis of Cefdinir and the reported method

Parameter	HPLC method	Reported HPLC method
Mean	99.54	100.078
SD	0.417	0.708
n	5	5
Variance	0.174	0.501
Student's t	1.453 (2.447)*	
F	2.879 (6.388)*	

formed with regards to accuracy and precision using Student's t-test and F-ratio at 95% confidence level (TABLE 6). There is no significant difference between the two methods.

CONCLUSION

The suggested method is simple, accurate, selective and sensitive. Application of the proposed method to the analysis of CEF in laboratory prepared mixtures and pharmaceutical formulation shows that neither the degradation products nor the excipients interfere with the determination, indicating that the proposed method could be applied as stability indicating method for the determination of pure CEF and in presence of its degradation products, either in bulk powder or in pharmaceutical formulations. Statistical analysis of the results obtained by the proposed method and by the reported method, revealed no significant difference within a probability of 95%.

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