Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1476–1482
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Isolation, structural elucidation and characterization of impurities in Cefdinir

K.V.V. Prasada Rao [a], A. Rani [a], A.V. Raghava Reddy [a], C.H. Bharathi [a], Ramesh Dandala [a,*], A. Naidu [b]

[a] *Department of Chemical Research, Aurobindo Pharma Research Centre, 313 Bachupally, Quthubullapur Mandal, Hyderabad 500072, India*
[b] *Department of Chemistry, J.N.T. University, Kukatpally, Hyderabad 500072, India*

Received 28 June 2006; received in revised form 5 October 2006; accepted 10 October 2006
Available online 15 December 2006

Abstract

Three unknown impurities in Cefdinir bulk drug at levels below 0.2% (ranging from 0.05 to 0.2%) have been detected by high performance liquid chromatography (HPLC). These impurities were isolated from crude sample of Cefdinir using preparative HPLC. Based on the spectral data (NMR, IR and MS) the structures of these impurities were characterized as (6R, 7R)-7-[(z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid-5-oxide (I). (6R, 7R)-7-[(z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-8-oxo-3-vinyl-5-thia-1-azabi-cyclo [4.2.0] oct-3-ene-2-carboxylic acid (II). (6R, 7R)-7-[(z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-8-oxo-3-methyl-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid (III), respectively. The origin and structural elucidation of all impurities have been discussed.

Keywords: Cefdinir; Impurities; Preparative HPLC; Spectroscopy; Identification; Characterization

1. Introduction

Cefdinir, Syn 7-[2-(2-aminothiazol-4-yl)-2-hydroxyimino-acetamido]-3-vinyl-3-cephem-4-carboxylic acid, is a third generation cephalosporin antibiotic for oral administration [1]. Cefdinir was first synthesized in the New Drug Research Laboratories of Fujisava Pharmaceutical Company Ltd., in 1985 [2]. It is used to reduce the infection caused by both Gram-positive and Gram-negative bacteria. Cefdinir had a broader spectrum than those of Cefixime, Cefaclor and Amoxicillin. The most remarkable feature of Cefdinir is the excellent activity against Staphylococcus species [3]. Many analytical methods have been reported in the literature for the determination of Cefdinir. High performance liquid chromatographic (HPLC) methods have been reported in the literature [4,5] for the determination of Cefdinir using UV detection. Okamoto has carried out degradation kinetics and isomerization study of Cefdinir in aqueous solutions [6,7].

The HPLC analysis of Cefdinir bulk drug has been performed as per the method described in Section 2.2. During this analysis of different batches of Cefdinir, three unknown impurities have been detected whose area percentage ranged from 0.05 to 0.2%. A comprehensive study has been carried out to isolate and characterizes these impurities. This paper aims at the isolation, structure elucidation and characterization of the potential impurities that are present at a level of $\leq$0.2% in the bulk drug of Cefdinir. The impurity profile has to be carried out for any final product to identify and characterize all the unknown impurities that are present at a level of even below 0.05% [8]. An in-house HPLC method was developed and validated for the analysis of Cefdinir. This LC method was able to detect impurities, which ranged from 0.05% and above in the presence of parent compound.

2. Experimental

2.1. Samples

The investigated samples of Cefdinir bulk were synthesized (Fig. 1) in Chemical Research Department of Aurobindo

* Corresponding author. Tel.: +91 40 23040261; fax: +91 40 23042932.
E-mail address: rdandala@aurobindo.com (R. Dandala).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.10.031

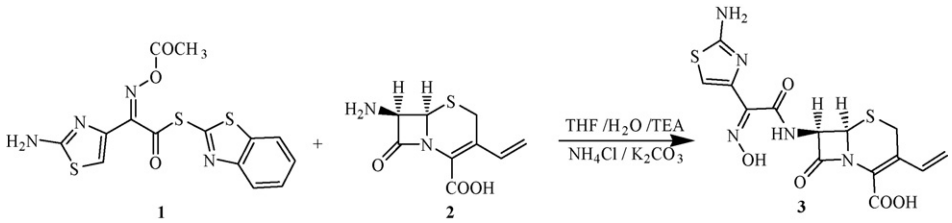


Fig. 1. Scheme for the synthesis of Cefdinir.

Research Centre, Hyderabad, India. All the Cefdinir samples contains impurity I, II and III consistently in the range between 0.05 and 0.20%. Origin of these impurities was identified. The impurity I was originates from the oxidation of cephem sulfur with traces of peroxides present in tetrahydrofuran. Impurity II results from the isomerization of doble bond from C-2 position to C-3 position due to presence of base used in the acylation reaction for the preparation of Cefdinir. Similarly impurity III was originates from the acylation of desacetoxy 7-aminocephalosporanic acid (7-ADCA) with compound 1. 7-ADCA present as an impurity in the key raw material 7-amino-3-vinyl-3-cephem-4-carboxylic acid (2). Presence of 7-ADCA in compound 2 has been confirmed by LCMS analysis and it was synthesized and co-injected with compound 2.

2.2. High performance liquid chromatography (analytical)

A Water Model Alliance 2695 separation module equipped with a waters 2996 Diode array detector was used. A Hypersil BDS C18 column with the dimension of 150 mm × 4.6 mm i.d. (Thermo-Electron) was used for the separation. The column eluent was monitored at a wavelength of 254 nm and the data was recorded using Empower Pro Software. The 3.12 g of sodium dihydrogen orthophosphate and 15 mg of EDTA disodium dihydrate in 100 ml of water (Mobile phase A) and 20% methanol in acetonitrile (Mobile phase B) mixture was used as mobile phase

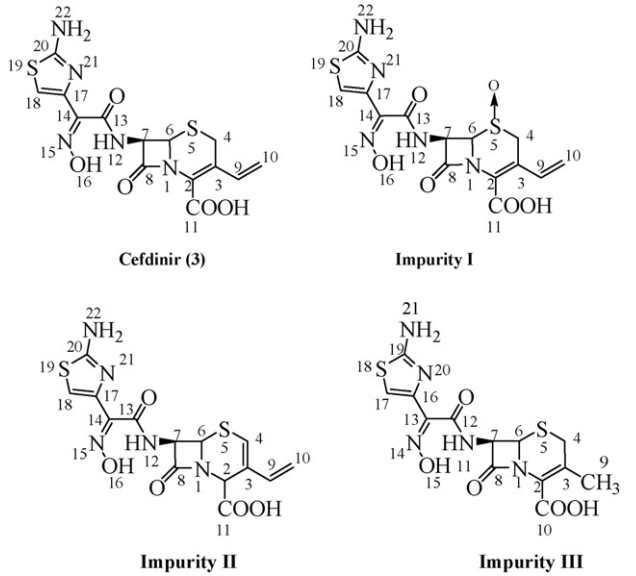


Fig. 2. Structures of Cefdinir (3), impurities I, II and III with numbering.

Table 1
Gradient program for LC method (analytical)

Time (min)	Mobile phase A (%, v/v)	Mobile phase B (%, v/v)
0.01	98	2
10	92	8
30	70	30
40	30	70
50	30	70
50	98	2
60	98	2

at a flow rate of 1.0 ml/min. The gradient program was given in Table 1.

2.3. High performance liquid chromatography (preparative)

A Shimadzu LC-8A Preparative Liquid Chromatograph equipped with SPD-10 A VP, UV–vis detector was used. Hyper Prep. HS C-18 (250 mm long × 21.2 mm i.d.) Preparative column packed with 10 μm particle size was employed for isolation of impurities. The mobile phase consisted of (A) 1% ammonium acetate and (B) acetonitrile. Flow rate was 30 ml/min and detection was carried out at 254 nm. The gradient program was given in Table 2.

2.4. NMR spectroscopy

NMR measurements were performed on a Bruker–Avance 300 MHz instrument (both for ^{1}H and ^{13}C) at 25 °C in DMSO-d_6. The ^{1}H NMR and ^{13}C chemical shift values were reported on the δ scale in ppm, relative to TMS ($\delta = 0.00$) and DMSO-d_6 ($\delta = 39.5$ ppm) as internal standard, respectively.

2.5. FT-IR spectroscopy

The IR spectra for Cefdinir, impurity-I, II and III were recorded in the solid state as KBr dispersion using Perkin-Elmer spectrum on FT-IR spectrophotometer.

Table 2
Gradient program for LC method (preparative)

Time (min)	Mobile phase A (%, v/v)	Mobile phase B (%, v/v)
0.01	100	0
30	95	5
45	70	30
50	50	50

2.6. Mass spectrometry

Mass spectra were recorded on API 2000 Perkin-Elmer (PE SCIEX) mass spectrometer.

3. Results and discussions

3.1. Detection of impurities

A typical LC chromatogram (Fig. 4a) of a laboratory batch of Cefdinir bulk recorded using the LC method as described in Section 2.2. The target impurities under study are marked as Imp-I, Imp-II and Imp-III, which eluted at retention times of about 6.6, 9.9 and 11.4 min, respectively, while Cefdinir eluted at about 14.5 min.

3.2. LC/MS analysis

LC–MS analysis of crude samples of Cefdinir was carried out using Perkin-Elmer triple quadruple mass spectrometer (API 2000, PE sciex) coupled with a Shimadzu HPLC equipped with SPD 10 A VP UV–vis detector and LC 10 AT VP pumps. Analyst software was used for data acquisition and data processing. The turbo ion spray voltage was maintained at 5.5 kV. MS capillary voltage and temperature were set at 75 V and 375 °C. The auxillary gas and sheath gas used was high pure nitrogen. Zero air was used as Neubilizer gas. LC–MS spectra were aquired from *m/z* 100–1000 in 0.1 amu steps with 2.0 s dwell time. Cefdinir crude sample was subjected to LC–MS/MS analysis. The analysis was carried out using Hypersil BDS C 18, 150 mm × 4.6 mm column with 5 μm particle dia, Mobile phase A used was 0.01 M ammonium acetate of pH 4.6 adjusting with acetic acid and 1:1 mixture of acetonitrile and methanol mixture was used as a Mobile phase B. The 0.01 M ammonium acetate was used as diluent. Detection was at 254 nm and flow rate was 1.0 ml/min. Data acquisition time was 50 min. The gradient program was given in Table 3. Flow was diverted away from the MS source during elution of Cefdinir peak to reduce Cefdinir background in subsequent scans. Three unknown impurities were detected in this sample. The masses of these impurities were identified as 412$[M+H]^+$, 396$[M+H]^+$ and 384 $[M+H]^+$, respectively. The structures of these impurities and retention times of Cefdinir and the target impurities are given in Table 4. Based on the

Table 3
Gradient programme for LC/MS

Time (min)	Mobile phase A (%, v/v)	Mobile phase B (%, v/v)
0.01	95	5
20.0	85	15
35.0	75	25
45.0	30	70
46.0	95	5
55.0	95	5

Table 4
Retention times of Cefdinir, impurity I, II and III in analytical HPLC and in LC/MS

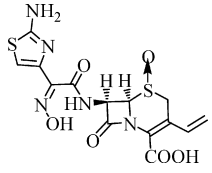
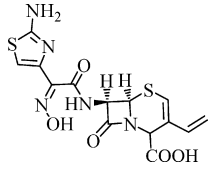
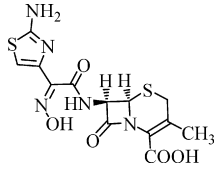
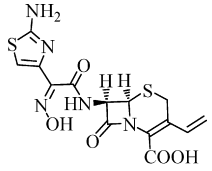
S.No	Retention time HPLC (min)	Compound	Structure	LC/MS analysis	
				~RT	M^+
01	6.6	Impurity I		4.85	412.2
02	9.9	Impurity II		7.15	396.2
03	11.4	Impurity III		9.61	384.0
04	14.5	Cefdinir		12.63	396.0

Table 5
Comparative ^{1}H and ^{13}C NMR assignments for Cefdinir and its impurities

Position	Cefdinir			Impurity-I			Impurity-II			Impurity-III		
	^{1}H	ppm	^{13}C	^{1}H	ppm	^{13}C	^{1}H	ppm	^{13}C	^{1}H	ppm	^{13}C
2	–	–	125.7	–	–	120.3	1H	4.73/s	60.2	–	–	123.7
3	–	–	126.0	–	–	125.4	–	–	121.1	–	–	131.2
4	2H	3.55 and 3.83/ABq	24.1	2H	3.55 and 4.29/2d	43.4	1H	6.44/s	126.6	2H	3.55 and 3.55/ABq	30.0
5	–	–	–	–	–	–	–	–	–	–	–	–
6	1H	5.19/d	58.6	1H	5.04/d	58.8	1H	5.36/d	53.2	1H	5.10/d	58.2
7	1H	5.79/dd	59.5	1H	5.98/dd	67.0	1H	5.45/dd	54.3	1H	5.71/dd	59.3
8	–	–	164.8	–	–	164.5	–	–	169.0	–	–	164.4
9	1H	6.90/dd	132.6	1H	7.08/dd	133.2	1H	6.27/dd	137.0	3H	2.02/s	20.3
10	2H	5.31 and 5.59/ABq	118.6	2H	5.34 and 5.60/ABq	118.6	2H4.89 and 5.35/2d		111.4	–	–	148.6
11	–	–	149.0	–	–	148.4	–	–	149.5	–	–	–
12	1H	9.50/d	–	1H	8.59/d	–	–	–	–	–	–	164.4
13	–	–	164.0	–	–	163.3	1H	9.42/d	163.4	–	–	164.4
14	–	–	164.6	–	–	163.9	–	–	164.6	–	–	–
15	–	–	–	–	–	–	–	–	–	1H	11.46/brs	142.7
16	1H	11.32/brs	–	1H	11.67/brs	–	1H	11.61/brs	–	–	–	108.1
17	–	–	143.9	–	–	143.1	–	–	144.6	1H	6.69/s	–
18	1H	6.67/s	108.4	1H	6.81/s	108.0	1H	6.70/s	107.8	–	–	169.4
19	–	–	–	–	–	–	–	–	–	–	–	–
20	–	–	169.2	–	–	169.2	–	–	169.9	–	–	–
21	–	–	–	–	–	–	–	–	–	2H	7.40/brs	–
22	2H	13.62/brs	–	2H	7.30/brs	–	2H	7.38/s	–	–	–	–

Refer structural formula for numbering (Fig. 2). s, singlet; d, doublet; dd, doublet of doublet; brs, broad singlet.

Table 6
FT-IR spectral data

S. no.	Compound	IR (KBr)
1	Cefdinir (3)	3302, 3176 (N—H stretching vibrations), 2980 (aliphatic C—H stretching), 1784 (β-lactam C=O stretch), 1668 (amide C=O stretch, amide I band) 1611, 1429 (C=O stretch, asymmetric and symmetric in COOH), 1545 (N—H bending amide II band) 1350, 1334 (NH_2 bending vibrations), 1050, 1017 (C—C, C—O and N—O stretching vibrations)
2	Impurity I	3320, 3150 (N—H stretching vibrations), 2900 (aliphatic C—H stretching), 1777 (β-lactam C=O stretch), 1666 (amide C=O stretch, amide I band) 1633, 1425 (C=O stretch, asymmetric and symmetric in COOH), 1525 (N—H bending amide II band) 1348, 1303 (NH_2 bending vibrations), 1115, 1023 (C—C, C—O and N—O stretching vibrations)
3	Impurity II	3300, 3196 (N—H stretching vibrations), 3000 (aliphatic C—H stretching), 1760 (β-lactam C=O stretch), 1674 (amide C=O stretch, amide I band) 1614, 1401(C=O stretch, asymmetric and symmetric in COOH), 1533 (N—H bending amide II band) 1370, 1310 (NH_2 bending vibrations), 1113, 1046 (C—C, C—O and N—O stretching vibrations)
4	Impurity III	3297, 3200 (N—H stretching vibrations), 2980 (aliphatic C—H stretching), 1760 (β-lactam C=O stretch), 1658 (amide C=O stretch, amide I band) 1622, 1400 (C=O stretch, asymmetric and symmetric in COOH), 1534 (N—H bending amide II band) 1380, 1365 (NH_2 bending vibrations), 1015 (N—O stretching vibrations)

mass spectral information, tentative structures of all the three impurities were proposed. To confirm the proposed structures, crude sample of Cefdinir containing all the target impurities was subjected to preparative LC to isolate the impurities in pure form and to carry out further spectroscopic experiments.

3.3. Isolation of impurities by preparative HPLC

The retention times of Cefdinir and impurities detected in HPLC are shown in Table 4. All three impurities were isolated by preparative HPLC as per the method discussed under Section 2.3. Fractions collected were analyzed by HPLC as per the conditions described in Section 2.2. Collected fractions of these impurities were pooled together, concentrated on rotavapour to remove acetonitrile. Concentrated fractions were passed through the preparative column by using water to remove ammonium acetate. Again the eluate was lyophilized using freeze dryer (Virtis Advantage 2XL). The chromatographic purity of these impurities I, II and III was tested by HPLC and found to be 97.2, 99.1 and 98.7%, respectively, indicating that these impurity fractions are quite stable during and after isolation. The isolated solids were used to generate spectral data. The details of the elucidation of structures of these impurities are presented in the following sections (Tables 5 and 6).

3.4. Structural elucidation

3.4.1. Impurity-I

The electrospray ionization mass spectrum of impurity-I showed a protonated molecular ion peak at *m/z* 412, which is 16 amu more than that of Cefdinir, which indicates the possible incorporation of oxygen in the molecule. The major fragmentation peaks in MS/MS were observed at *m/z* 227, 185 and 368, confirm the oxygen addition on sulphur of cephem ring. The fragmentation pattern is shown in Fig. 3. The S-CH_2 carbon was shifted to 43.4 ppm from 24.1 ppm of cefdinir in ^{13}C NMR spectrum is also confirming the oxygen addition to the molecule is on sulphur in impurity-I.

3.4.2. Impurity II

The specific ABq of S-CH_2 protons of Cefdinir in ^{1}H NMR spectrum of impurity-II was absent and appearance of two CH protons at 4.73 and 6.44 ppm is an indicative for the shifting of double bond from C-2 to C-3. ^{13}C NMR signal at 24.1 ppm corresponds to S-CH_2 was disappeared and new signals at 60.2 and 126.6 ppm correspond to two CH protons of position 2

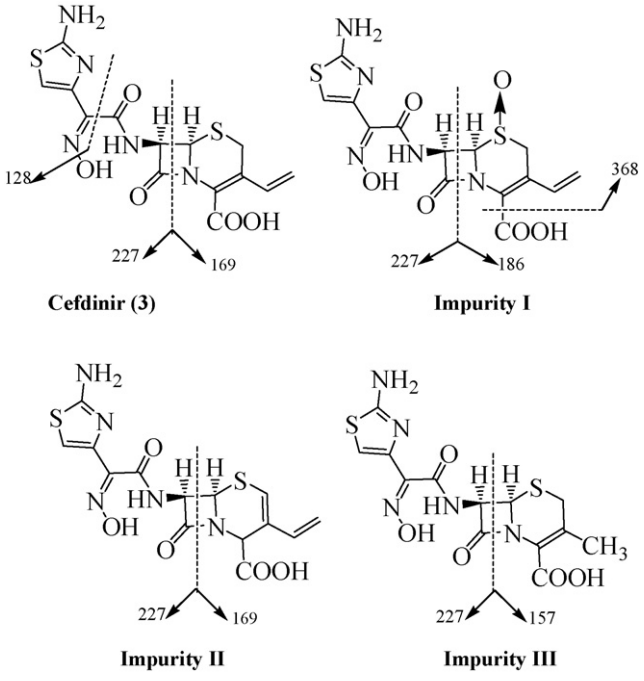


Fig. 3. MS/MS fragmentation pattern of Cefdinir and its impurities I, II and III.

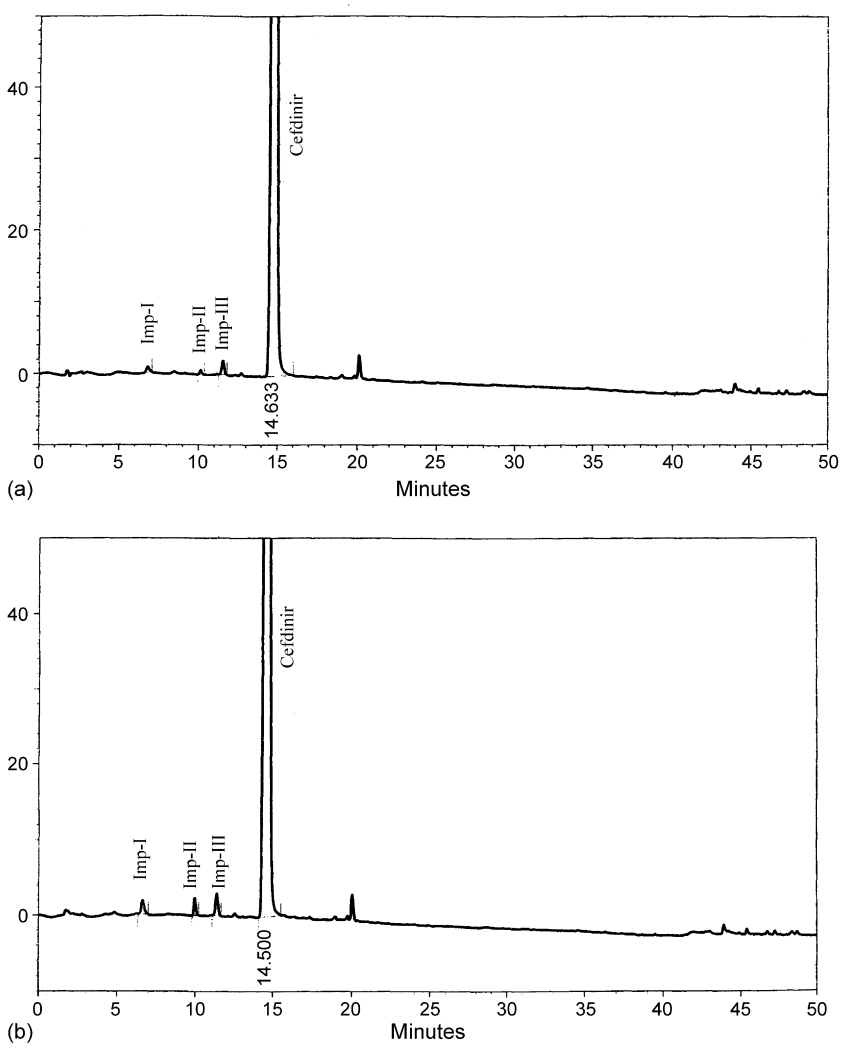


Fig. 4. LC chromatogram of Cefdinir (a) and Cefdinir with spiked impurities (b).

and 4, respectively, have appeared. From ^{1}H and ^{13}C NMR values, it was confirmed that the double bond between C-2 and C-3 position was shifted to C-3 and C-4 positions. The electro spray ionization mass spectrum of impurity-I showed a protonated molecular ion peak at *m/z* 384, which is identical to Cefdinir molecular weight. The fragmentation pattern (Fig. 4) is also similar to Cefdinir. From this data, impurity-II was assigned as (6R, 7R)-7-[(z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetylamino]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-3-ene-2-carboxylic acid. Pure synthesized impurity-II was co-injected with Cefdinir sample in HPLC to confirm the retention time.

3.4.3. Impurity III

The electro spray ionization mass spectrum of impurity-III showed a protonated molecular ion peak at *m/z* 384 indicating the molecular weight of impurity-III as 383, which is 12 amu less than that of Cefdinir. The major fragmentation (Fig. 3) peaks in MS/MS were observed at *m/z* 227, and 157 is suggesting that the absence of vinyl group at C-3 position and an additional methyl group at the same position. ^{1}H NMR signals assigned to vinyl group of Cefdinir at 5.3, 5.6 and 6.9 ppm have disappeared and a new signal at 2.0 ppm corresponds to CH_3 has appeared. ^{13}C NMR signals at 118.6 and 132.6 ppm correspond to CH_2 and CH carbons of vinyl group are disappeared. It showed a signal at 20.3 ppm corresponds to CH_3. From ^{1}H and ^{13}C NMR values, the impurity-III was confirmed as (6R, 7R)-7-[(z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetylamino]-8-oxo-3-methyl-5-thia-1-azabi-cyclo [4.2.0] oct-2-ene-2-carboxylic acid (III).

The synthetic standards of impurities I, II and III were co-injected on LC with Cefdinir and area percentage at retention times about 6.6, 9.9 and 11.4 min were enhanced and the LC chromatogram is shown in Fig. 4b.

Acknowledgements

The authors wish to thank the management of Aurobindo Pharma Research Centre for supporting this work. Cooperation from colleagues of Chemical research and analytical research is appreciated.

References

[1] R.J. Ternansky, C.L. Jordan, F.W. Bell, T.G. Skaggs, J.S. Kasher, J. Antibiot. 46 (1993) 1897–1900.
[2] T. Takaya, T. Hisashi, US Patent No. 455,334, CA 101, 130505 h.
[3] Y. Inamoto, T. Chiba, T. Kamimura, T. Takaya, J. Antibiot. 41 (1988) 828–830.
[4] Y. Okamoto, K. Itoh, Y. Namiki, J. Matsushita, Mamorujioka, T. Ysuda, J. Pharm. Biomed. Anal. 14 (1996) 739–748.
[5] T.N. Mehta, G. Subbaiah, K. Pundarikakshudu, J. AOAC Int. 88 (2005) 1661–1665.
[6] Y. Okamoto, K. Kariyama, Y. Namiki, J. Mitsishita, M. Fujioka, T. Yasuda, J. Pharm. Sci. 85 (1996) 976–983.
[7] Y. Okamoto, K. Kariyama, Y. Namiki, J. Mitsishita, M. Fujioka, T. Yasuda, J. Pharm. Sci. 85 (1996) 984–989.
[8] ICH Guideline Q3A (R), Impurities in New Drug Substances, 7 February, 2002.