Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 733–740
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Structural identification and characterization of impurities in ceftizoxime sodium

Ch. Bharathi [a], Ch.S. Prasad [a], D. Vijaya Bharathi [a], Rama Shankar [a], V. Janardhana Rao [a], Ramesh Dandala [a,*], A. Naidu [b]

[a] *A.P.L. Research Centre, 313, Bachupally, Hyderabad 500072, India*
[b] *Department of Chemistry, J.N.T. University, Kukatpally, Hyderabad 500072, India*

Received 17 February 2006; received in revised form 17 July 2006; accepted 18 July 2006
Available online 6 September 2006

Abstract

Ceftizoxime sodium is a parenteral ⟨beta⟩-lactamic antibacterial drug. In the synthesis of ceftizoxime sodium, eight process related impurities were detected in HPLC analysis. Pure impurities obtained by both synthesis and preparative HPLC were co-injected with ceftizoxime sample to confirm the retention times in HPLC. The impurities were characterized as, (6*R*,7*R*)-7-amino-3-cephem-4-carboxylic acid (impurity I); (6*R*, 7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-1-oxo-4-carboxylic acid (impurity II); (4*RS*,6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino) acetamido]-3,4-dihydro-3-cephem-4-carboxylic acid (impurity III); (6*R*,7*R*)-7-[(*E*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylic acid (impurity IV); (6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-*N*-(3-cephem-4-carboxy-7-yl)-4-carboxamide (impurity V); (6*R*,7*R*)-7-[(*Z*)-2-[[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetylamino]thiazol-4-yl]-2-methoxyiminoacetamido]-3-cephem-4-carboxylic acid (impurity VI); 2-mercaptobenzothiazole (impurity VII) and 2-mercapto benzothiazolyl [(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyimino] acetate (impurity VIII). Structural elucidation of all impurities by spectral data (^{1}H NMR, ^{13}C NMR, MS and IR) has been discussed.

Keywords: Ceftizoxime; Impurities; Isolation; Preparative HPLC; Characterization

1. Introduction

Ceftizoxime is a third generation cephalosporin antibiotic and is used to reduce the infection caused by both gram-negative and gram-positive bacteria. Ceftizoxime sodium is chemically designated as mono sodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-meth oxyiminoacetamido]-3-cephem-4-carboxylate. Its molecular formula is $C_{13}H_{12}N_5NaO_5S_2$ and molecular weight is 405.39.

Many analytical methods have been reported in the literature for the determination of ceftizoxime. They are HPLC determination of cephalosporin antibiotics using 0.3 mm i.d. column [1], determination of third generation cephalosporins by HPLC in connection with pharmacokinetic studies [2], determination of ceftizoxime in pharmaceutical formulations by spectrophotometric method [3], HPLC assay methods [4,5], analysis by HPLC-APCI mass spectrometry using bromoform [6], quantitative determination of some thiazole cephalosporins through complexation with palladium(II) chloride [7], spectro-photometric determination of selected cephalosporins in drug formulations using flow-injection analysis [8] and quantitation and evaluation of cephalosporins by densitometry on a hydrocarbon-impregnated silica gel HPTLC plate [9].

The HPLC analysis of ceftizoxime bulk drug has been performed as per the method mentioned in Section 2.2 that revealed the presence of eight impurities, up to 0.1%. The impurity profile study has to be carried out for any final product as per the regulatory requirements to identify and characterize all the unknown impurities [10]. This paper describes the identification, isolation and characterization of impurities present in ceftizoxime. The impurity profile study by HPLC, detection of masses by LC-MS, isolation and characterization of the detected impurities were not reported till date to the best of our knowledge.

* 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.2006.07.031

2. Experimental

2.1. Samples

The investigated sample, ceftizoxime sodium was synthesized in APL Research Centre (A unit of Aurobindo Pharma Limited, Hyderabad, India). Impurities I, II, IV, VII and VIII were synthesized in the laboratory after identification by HPLC and detection of masses by LC-MS. Impurities III, V and VI were synthesized and isolated by preparative HPLC. Reagents used for analysis, i.e., ammonium acetate (GR grade), potassium dihydrogen orthophosphate (AR grade), disodium hydrogen orthophosphate anhydrous (AR grade), orthophosphoric acid (∼85%, w/w, AR grade), sodium hydroxide (AR grade), acetonitrile (HPLC grade) were procured from Merck (India) Limited. Water used was Milli-Q grade.

2.2. High performance liquid chromatography

A Waters 2695 separation module equipped with 2996 photo diode array detector with Empower pro data handling system [Waters Corporation, MILFORD, MA01757, USA] was used. The analysis was carried out on YMC Pack-ODS AQ, 250 mm long, 4.6 mm i.d., 5 μm particle diameter column. Mobile phase A was phosphate buffer (pH 7.0) and acetonitrile in the ratio of 98:2 [phosphate buffer (pH 7.0), prepared by dissolving 1.15 g of disodium hydrogen orthophosphate anhydrous and 0.85 g of potassium dihydrogen orthophosphate in 1000 ml of water, pH adjusted to 7.0 with dilute orthophosphoric acid or dilute sodium hydroxide solution if required]. Mobile phase B was acetonitrile. UV detection was at 254 nm and flow rate was kept at 1.0 ml/min. Column oven temperature was 30 °C and data acquisition time was 50 min. Pump mode was gradient and the program was as follows, time (min)/A (v/v):B (v/v); $T_{0.01}$/98:2, $T_{10.0}$/85:15, $T_{20.0}$/70:30, $T_{30.0}$/50:50, $T_{40.0}$/40:60, $T_{50.0}$/40:60.

2.3. Preparative liquid chromatography

A Shimadzu LC-8A Preparative Liquid Chromatograph equipped with SPD-10A VP, UV–vis detector [Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan] was used. YMC Pack ODS AQ (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) 0.1 M ammonium acetate solution, pH adjusted to 5.0 with glacial acetic acid and (B) acetonitrile. Flow rate was kept at 20 ml/min and detection was carried out at 254 nm. The gradient program was as follows, time (min)/A (v/v):B (v/v); $T_{0.01}$/100:0, $T_{20.0}$/98:2, $T_{30.0}$/96:4, $T_{40.0}$/94:6, $T_{60.0}$/92:8, $T_{90.0}$/90:10, $T_{100.0}$/80:20, $T_{120.0}$/50:50.

2.4. LC-MS/MS analysis

LC-MS/MS analysis was carried out using Perkin-Elmer triple quadrupole 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 [Foster city, CA]. Analyst software was used for data acquisition and data processing. The turbo ion spray voltage was maintained at 5.5 kV and temperature was set at 375 ^{O}C. High pure nitrogen gas was used as auxillary gas and curtain gas. Zero air was used as nebulizer gas. LC-MS spectra were acquired from *m/z* 100 to 1000 in 0.1 amu steps with 2.0 s dwell time. Ceftizoxime sodium sample was subjected to LC-MS/MS analysis. The analysis was carried out using Hypersil BDS, 250 mm × 4.6 mm column with 5 μm particle dia. Mobile phase A used was 0.01 M ammonium acetate and Mobile phase B was methanol. Detection was carried out at 254 nm and flow rate was kept at 1.0 ml/min. 0.01 M ammonium acetate:acetonitrile (50:50) was used as diluent. Data acquisition time was 40 min. The gradient program was as follows: time (min)/A (v/v):B (v/v); $T_{0.01}$/98:2, $T_{20.0}$/85:5, $T_{30.0}$/20:80, $T_{35.0}$/20:80. Five impurities were detected in laboratory batch sample. The masses of detected peaks were 400 [$(MH)^+$], 384 [$(MH)^+$] (Ceftizoxime), 384 [$(MH)^+$], 566 [$(MH)^+$], 567 [$(MH)^+$], 168 [$(MH)^+$], respectively. Additionally, impurities correspond to *m/z* 386 [$(MH)^+$], 351 [$(MH)^+$] were also observed in another laboratory batch sample. From mass values and MS/MS fragmentation pattern, structures given in Table 1 were suggested. Mass fragmentation pattern is shown in Fig. 3.

2.5. NMR spectroscopy

The ^{1}H, ^{13}C NMR (proton decoupled) spectra were recorded on Bruker 300 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard.

2.6. Mass spectrometry

Mass spectra were recorded on Perkin-Elmer PE SCIEX-API 2000 mass spectrometer equipped with a Turboionspray interface at 375 °C. Detection of the ions was performed in electrospray ionization, positive ion mode.

2.7. FT-IR spectroscopy

FT-IR spectra were recorded as KBr pellet on Perkin-Elmer instrument model-spectrum one.

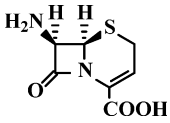
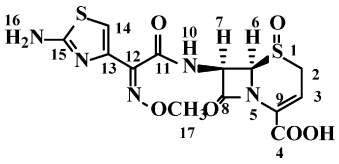
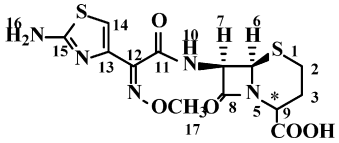
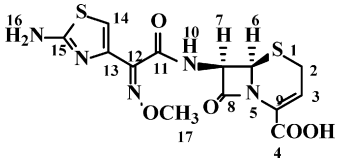
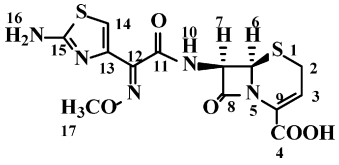
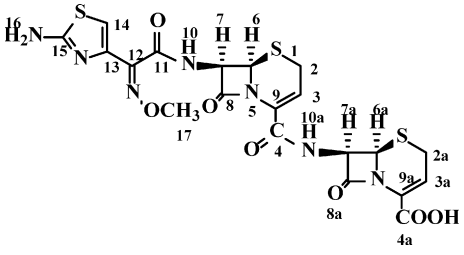
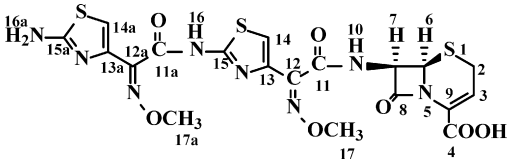
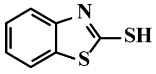
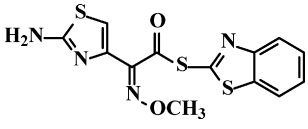
2.8. Synthesis of impurities

Impurities III, V and VI were synthesized in the laboratory as per the scheme given in Fig. 1. The chromatographic purities were 25, 17 and 6%, respectively, by HPLC analysis. These were isolated by preparative HPLC (details given in Section 2.9).

2.9. Isolation of impurities by preparative HPLC

Impurities III, V and VI which were prepared synthetically showed 25, 17 and 6% purity, respectively, by HPLC analysis. All three were isolated by preparative HPLC as per the conditions described in Section 2.3. Fractions collected were analyzed by analytical HPLC as per the conditions mentioned in Section 2.2. Fractions of >95% were pooled together, concentrated on rotavapour to remove acetonitrile. Concentrated fraction was passed through the preparative column using water:acetonitrile (50:50) as mobile phase to remove ammonium acetate. Again

Table 1
Chemical structures of impurities

S. no.	RRT	Compound	Molecular weight	Structure
1	0.39	Impurity I	200	
2	0.76	Impurity II	399	
3	0.91	Impurity III	385	 * asymmetric centre
–	1.0	Ceftizoxime	383	
4	1.46	Impurity IV	383	
5	1.51	Impurity V	565	
6	1.73	Impurity VI	566	
7	2.31	Impurity VII	167	
8	3.74	Impurity VIII	350	

the eluate was concentrated using rotavapour to remove acetonitrile and the aqueous solution was lyophilized using freeze dryer (Virtis advantage 2XL). Impurity III was obtained as a white powder with chromatographic purity of 96%. Impurity V was obtained as an off-white powder with chromatographic purity of 90%. Impurity VI was obtained as an off-white powder with chromatographic purity of 98%.

3. Results and discussion

3.1. Detection of impurities

Laboratory batches of ceftizoxime sodium were analyzed for their impurities identification using the HPLC method described in Section 2.2. These samples were subjected to LC-MS/MS

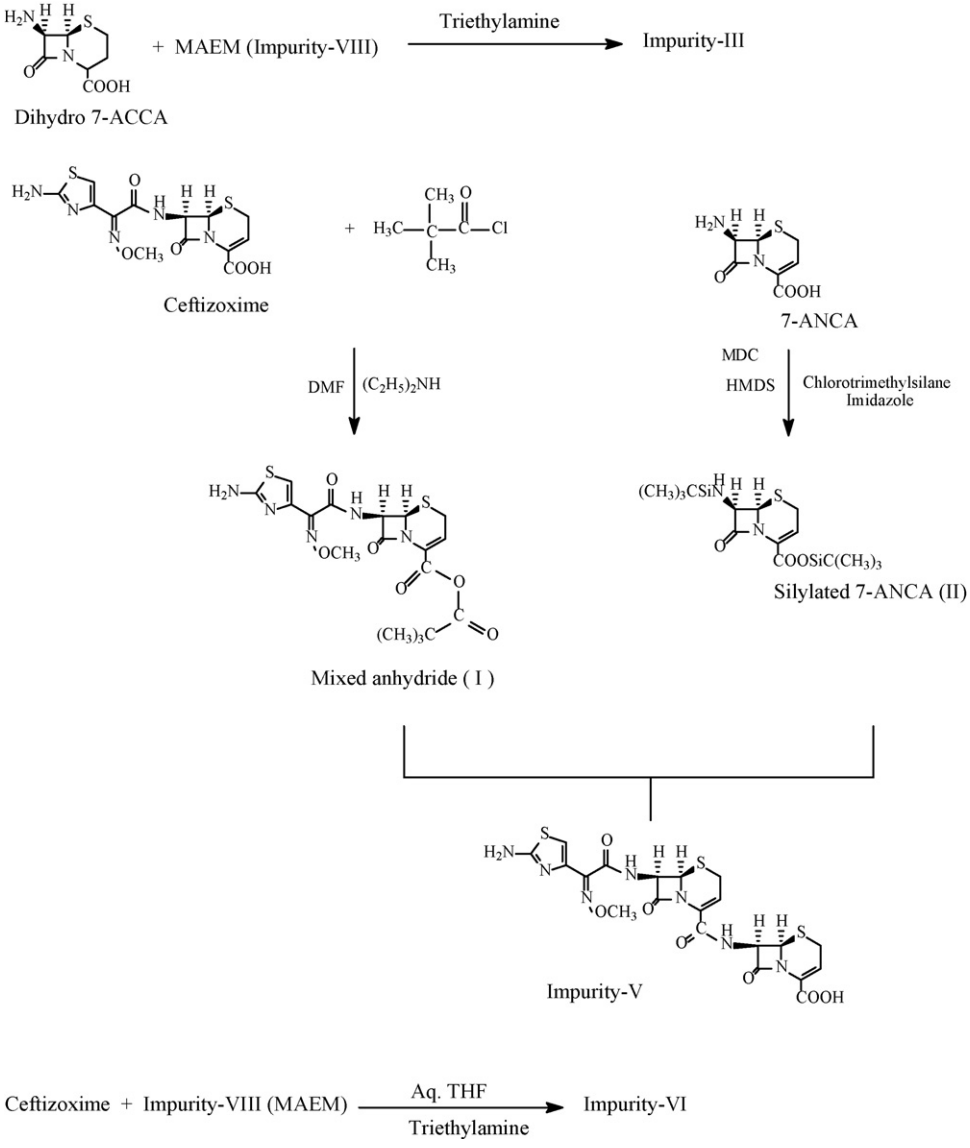


Fig. 1. Scheme for the synthesis of impurities III, V and VI.

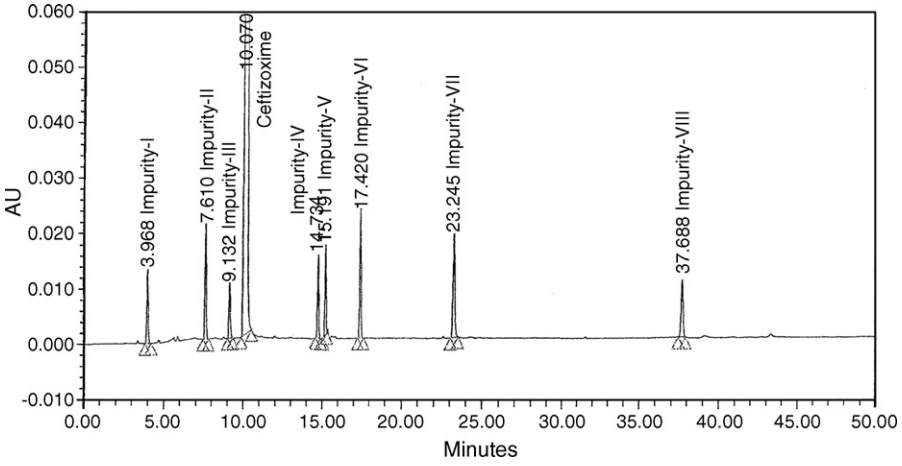


Fig. 2. Typical LC-chromatogram of ceftizoxime sample spiked with impurities. Column: YMC Pack-ODS AQ, 250 mm × 4.6 mm, mobile phase (A): phosphate buffer (pH 7.0) and acetonitrile (98:2), mobile phase (B): acetonitrile, pump mode: gradient (time (min)/A (v/v)/B (v/v); $T_{0.01}$/98:2, $T_{10.0}$/85:15, $T_{20.0}$/70:30, $T_{30.0}$/50:50, $T_{40.0}$/40:60, $T_{50.0}$/40:60), flow rate: 1.0 ml/min, wavelength at UV 254 nm.

Table 2
Comparative ^{1}H and ^{13}C NMR assignments for ceftizoxime and its impurities

Position[a]	Ceftizoxime		Impurity II		Impurity III		Impurity IV		Position[a]	Impurity V		Impurity VI	
	^{1}H ((ppm), multiplicity	^{13}C ((ppm)	^{1}H ((ppm), multiplicity	^{13}C ((ppm)	^{1}H ((*ppm*), multiplicity	^{13}C ((ppm)	^{1}H ((ppm), multiplicity	^{13}C ((ppm)		^{1}H ((ppm), multiplicity	^{13}C ((ppm)	^{1}H ((ppm), multiplicity	^{13}C ((ppm)
2	3.55 (m, 2H)	24.6	3.59 and 3.91 (ABq, 2H)	44.4	1.70 and 2.22 (2m, 2H)	27.8	3.48–3.66 (m, 2H)	24.3	2, 2a	3.47–3.68 (m, 4H)	24.3, 24.5	3.42–3.63 (m, 2H)	24.5
3	6.47 (m, 1H)	121.2	6.28 (m, 1H)	115.5	2.69–2.86 (m, 2H)	23.3	6.46 (m, 1H)	120.9	3, 3a	6.31 and 6.43 (2m, 2H)	118.6, 120.2	6.28 (s, 1H)	115.4
4	13.20 (brs, 1H)	169.4	13.45 (brs, 1H)	169.2	–	171.0	–	167.3	4, 4a	–	162.5, 169.2	–	169.4
6	5.09 (d, 1H) $J = 4.8$ Hz	57.8	4.93 (d, 1H) $J = 4.2$ Hz	67.3	5.16 (d, 1H) $J = 4.2$ Hz	56.3	5.08 (d, 1H) $J = 4.7$ Hz	57.7	6, 6a	5.10 (m, 2H)	57.7, 57.9	5.06 (d, 1H) $J = 4.9$ Hz	57.7
7	5.81 (dd, 1H) $J = 4.8$ and 8.1 Hz	59.8	5.88 (dd, 1H) $J = 4.2$ and 7.8 Hz	59.1	5.36 (dd, 1H) $J = 4.2$ and 8.5 Hz	59.8	5.82 (dd, 1H) $J = 4.7$ and 8.5 Hz	60.0	7, 7a	5.77 and 5.87 (2m, 2H)	59.5, 60.0	5.78 (dd, 1H) $J = 4.9$ and 8.2 Hz	59.5
8	–	164.2	–	162.7	–	169.1	–	164.7	8, 8a	–	164.4, 164.7	–	163.9
9	–	128.9	–	129.0	4.33 (m, 1H)	51.6	–	129.0	9, 9a	–	130.0	–	131.0
10	9.60 (d, 1H) $J = 8.1$ Hz	–	8.89 (d, 1H) $J = 7.8$ Hz	–	9.56 (d, 1H) $J = 8.5$ Hz	–	9.44 (d, 1H) $J = 8.5$ Hz	–	10, 10a	9.17 and 9.66 (2d, 2H) $J = 8.2$ Hz	–	9.67 (d, 1H) $J = 8.2$ Hz	–
11	–	163.6	–	163.5	–	163.6	–	163.6	11, 11a	–	163.6	–	163.5, 163.8
12	–	149.8	–	149.7	–	149.9	–	147.1	12, 12a	–	149.8	–	148.7, 149.4
13	–	143.3	–	143.3	–	143.5	–	139.1	13, 13a	–	143.3	–	142.60 and 142.62
14	6.73 (s, 1H)	110.0	6.82 (s, 1H)	109.7	6.72 (s, 1H)	109.8	7.47 (s, 1H)	116.6	14, 14a	6.73 (s, 1H)	109.7	6.90 and 7.44 (2s, 2H)	108.8
15	–	164.0	–	163.6	–	164.0	–	164.6	15, 15a	–	163.9	13.16 (brs, 1H)	162.4, 163.5
16	7.22 (s, 2H)	–	7.21 (s, 2H)	–	7.22 (brs, 2H)	–	7.12 (brs, 2H)	–	16, 16a	7.22 (brs, 2H)	–	7.24 (brs, 2H)	–
17	3.83 (s, 3H)	62.8	3.86 (s, 3H)	62.8	3.82 (s, 3H)	62.6	3.95 (s, 3H)	63.6	17, 17a	3.84 (s, 3H)	62.7	3.87 and 3.90 (2s, 6H)	62.9, 63.2

s, singlet; d, doublet; dd, doublet of a doublet; m, multiplet; brs, broad singlet; ABq, AB quartet; *J*, coupling constant.
[a] Refer structural formula for numbering (Table 1).

analysis using the method as described in Section 2.4. The prepared and isolated impurities were co-injected with ceftizoxime to confirm the retention times. All the impurities were well resolved from ceftizoxime peak and the representative resolution mixture chromatogram was shown in Fig. 2. Relative retention times of the impurities with respect to ceftizoxime and structures of those are shown in Table 1.

Impurities I, VII and VIII were starting materials of the process. The presence of these impurities was confirmed by co-injection with ceftizoxime in HPLC.

3.2. Structural elucidation

3.2.1. Impurity II

Impurity II (RRT-0.76) exhibited a molecular ion peak at *m/z*; 399.9 [$(MH)^+$] in positive ion mode in LC-MS analysis indicating a molecular weight of 399, which is 16 amu more than that of ceftizoxime acid. Being more polar than ceftizoxime, it was suggested that oxygen addition happened to the molecule. MS/MS fragmentation pattern supported that the oxygen addition was on sulphur of cephem ring. Major fragmentation peaks were at *m/z* 301 and 274. To confirm the proposed structure, pure synthesized (6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino) acetamido]-3-cephem-1-oxo-4-carboxylic acid (ceftizoxime sulfoxide) was co-injected with ceftizoxime sample in HPLC.

3.2.2. Impurity III

The electrospray ionization mass spectrum of Impurity III (RRT-0.91) exhibited a molecular ion peak at *m/z*; 386.0 [$(MH)^+$] in positive ion mode, indicating the molecular weight of the compound as 385, which is 2 amu more than that of ceftizoxime acid. The major fragment ion peaks observed at *m/z* 241 and 146. In ^{1}H NMR spectrum of ceftizoxime the signal assigned to CH at 3-position has disappeared and new signals at 1.70 and 2.23 ppm corresponding to CH_2 at 3-position and 4.32 ppm corresponding to CH at 4-position have appeared. In ^{13}C NMR spectrum signal at 114.5 ppm assigned to 3 CH has disappeared and new signals at 23.3 and 51.6 ppm corresponds to CH_2 and CH have appeared. This observation indicates that the double bond between 3 and 4 positions was removed with addition of two hydrogens. Based on this data, the Impurity III was characterized as (4*RS*,6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)-acetamido]-3,4-dihydro-3-cephem-4-carboxylic acid (dihydroceftizoxime), with molecular formula $C_{13}H_{15}N_5O_5S_2$ and molecular weight 385.

3.2.3. Impurity IV

Impurity IV (RRT-1.46) exhibited a molecular ion peak at *m/z* 384.0 [$(MH)^+$] in LC-MS analysis, indicating the molecular weight of the impurity as 383, which is equivalent to the mass of ceftizoxime acid. So it was suggested as an isomer of ceftizoxime. The mass fragmentation pattern was similar to ceftizoxime. The major fragmentation peaks are at *m/z* 285, 241, 227 and 144. Based on the fragmentation data the structure was proposed as (*E*) isomer. In ^{1}H NMR spectrum the shift of thiazole proton from 6.73 to 7.47 ppm is observed. The corresponding signal in ^{13}C NMR spectrum shifted from 110 to 117 ppm. Pure synthesized (*E*) isomer was co-injected with ceftizoxime sample in HPLC to confirm the retention time.

3.2.4. Impurity V

ESI mass spectrum of this impurity (RRT-1.51) exhibited a molecular ion peak at *m/z*; 566.1 [$(MH)^+$] in positive ion mode indicating that the impurity is having molecular weight of 565. This molecular weight is suggesting the structure as condensation product of ceftizoxime and 7-ANCA with loss of 18 amu. The structure was confirmed by ^{1}H, ^{13}C NMR values and MS/MS fragmentation. The major fragment ion peaks were observed at *m/z* 423, 241. Based on the spectral data the structure was characterized as (6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyimino) acetamido]-3-cephem-*N*-(3-cephem-4-carboxy-7-yl)-4-carboxamide (ceftizoxime amide dimer) with molecular formula $C_{20}H_{19}N_7O_7S_3$ and molecular weight 565.

Table 3
FT-IR spectral data

S. no.	Compound	IR (KBr) absorption bands, ν (C m^{-1})
1	Ceftizoxime	3448, 3280 (s) N–H stretching, 2972, 2892 (w) aliphatic C–H stretching, 1779 (s) beta lactam C=O stretching, 1657 (s) amide C=O stretching, 1622 (s) symmetric C=O stretching (COOH), 1586 (s) C=N stretching, 1538 (s) N–H bending, 1033 (s) N–O stretching
2	Impurity II	3264 (s) N–H stretching, 2970 and 2898 (w) aliphatic C–H stretching, 1785 (s) beta lactam C=O stretching, 1661 (s) amide C=O stretching, 1621 (s) symmetric C=O stretching (COOH), 1587 (s) C=N stretching, 1540 (s) N–H bending, 1051 (s) N–O stretching
3	Impurity III	3302, 3201 (br and s) NH stretching, 2930 and 2890 (w) aliphatic C–H stretching, 1751 (s) beta lactam C=O stretching, 1662 (s) amide C=O stretching, 1621 (s) symmetric C=O stretching (COOH), 1587 (s) C=N stretching 1535 (s) N–H bending, 1038 (s) N–O stretching
4	Impurity IV	3426, 3313 (br and s) N–H stretching, 2938, 2896 (m) aliphatic C–H stretching, 1768 (s) beta lactam C=O stretching, 1674 (s) amide C=O stretching, 1619 (s) symmetric C=O stretching (COOH), 1580 (s) C=N stretching, 1525 (s) N–H bending, 1023 (s) N–O stretching
5	Impurity V	3265 (br and s) N–H stretching, 1777 (s) beta lactam C=O stretching, 1662 (s) amide C=O stretching, 1632 (s) symmetric C=O stretching (COOH), 1534 (s) N–H bending, 1041 (s) N–O stretching
6	Impurity VI	3414, 3199 (s) N–H stretching, 2981, 2941 (w) aliphatic C–H stretching, 1772 (s) beta lactam C=O stretching, 1672 (s) amide C=O stretching, 1634 (s) symmetric C=O stretching (COOH), 1536 (s) NH bending, 1040 (s) N–O stretching

w, weak; s, strong; m, medium; br and s, broad and strong.

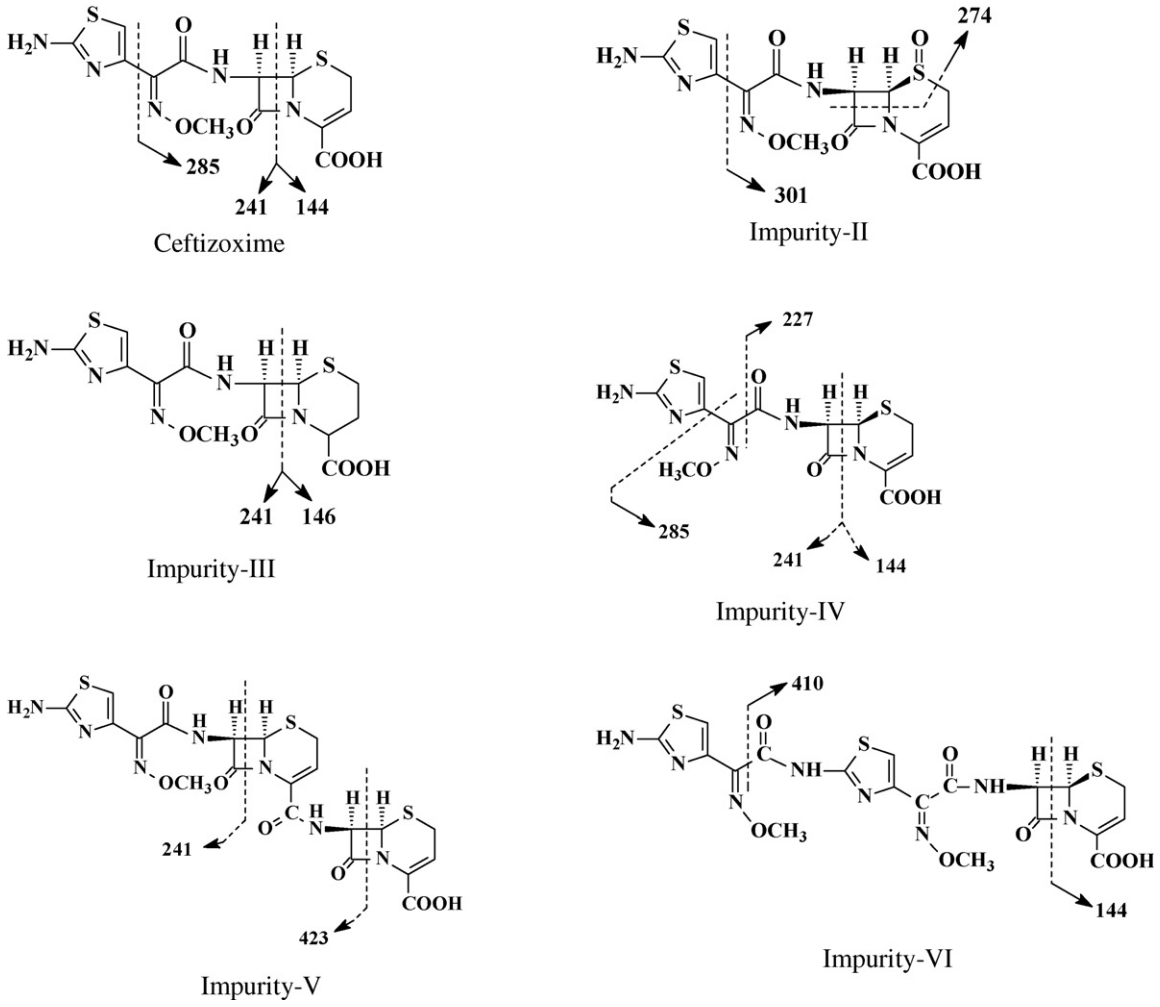


Fig. 3. MS/MS fragmentation pattern.

3.2.5. Impurity VI

The ESI mass spectrum of impurity VI (RRT-1.73) exhibited a molecular ion peak at *m/z*; 567.0 [$(MH)^+$] indicating the molecular weight of this impurity as 566.0, which was more by 183 amu than that of ceftizoxime. In MAEM structure, the removal of mercaptobenzothiazole ring gives ATMA, which is known by chemical name (*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetic acid, with molecular formula $C_6H_7N_3O_3S$ and molecular weight 201. It was likely that removal of one proton from ceftizoxime and addition of ATMA moiety with loss of 18 amu gives the desired molecular weight (566). MS–MS fragmentation showed major fragment ion peaks at *m/z* 410 and 144. ^{1}H NMR spectrum showed two N-OCH_3 groups, two thiazolyl groups, two CONHs, one NH_2 and one cephem ring. Based on this data, the molecular formula of this impurity was confirmed as $C_{19}H_{18}N_8O_7S_3$ and the structure of this impurity VI was characterized as (6*R*,7*R*)-7-[(*Z*)-2-[[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetyl amino] thiazol-4-yl]-2-methoxy imino acetamido]-3-cephem-4-carboxylic acid (ATA ceftizoxime). Pure isolated impurity was co-injected with ceftizoxime sample in HPLC.

The ^{1}H and ^{13}C NMR chemical shift values for ceftizoxime, impurities II, III, IV, V and VI are given in Table 2. The FT-IR spectral data is given in Table 3. MS/MS fragmentation pattern for ceftizoxime, impurities II, III, IV, V and VI is given in Fig. 3.

4. Conclusion

The process related impurities in ceftizoxime sodium bulk drug were identified, isolated and characterized by using HPLC (analytical and preparative), LC/MS–MS, IR and NMR (^{1}H, ^{13}C) techniques.

Acknowledgements

The authors gratefully acknowledge the management of Aurobindo Pharma Limited, for allowing to carry out the present work. The authors are also thankful to the colleagues of Analytical Research Department (ARD) and Chemical Research Department (CRD) for their cooperation.

References

[1] C.M. Moore, K. Sato, Y. Katsumata, J. Chromatogr. 539 (1991) 215–220.
[2] F. Pehourcq, C. Jarry, J. Chromatogr. A812 (1998) 159–178.
[3] F.I. Sengun, I. Fedai, Talanta 33 (1986) 366–369.
[4] M. LeBel, J.F. Ericson, D.H. Pitkin, J. Liq. Chromatogr. 7 (1984) 961–968.
[5] C.E. Fasching, L.R. Peterson, K.M. Bettin, D.N. Gerding, Antimicrob. Agents Chemother. 22 (1982) 336–337.
[6] S. Horimoto, T. Mayumi, K. Aoe, N. Nishimura, T. Sato, J. Pharm. Biomed. Anal. 30 (2002) 1093–1102.
[7] A.F.M. El-Walily, A.A. Gazy, S.F. Belal, E.F. Khamis, J. Pharm. Biomed. Anal. 22 (2000) 385–392.
[8] I.F. Al-Momani, J. Pharm. Biomed. Anal. 25 (2001) 751–757.
[9] S.C. Dhanesar, JPC—J. Planar Chromat. 12 (1999) 114–119.
[10] ICH Guideline Q3A (R), Impurities in New Drug Substances, 7 February, 2002.