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IDENTIFICATION, STRUCTURAL ELUCIDATION AND VALIDATION OF HPLC METHOD FOR THE DETERMINATION OF NEW IMPURITIES IN CEFTAZIDIME DRUG SUBSTANCE

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ABSTRACT

Ceftazidime is a third generation semi-synthetic cephalosporin antibiotic drug. During the analysis of ceftazidime commercial batches, three unknown impurities other than those specified in European Pharmacopeia were detected in HPLC analysis at levels ranging from 0.05 to 0.2%. These unknown impurities were isolated by preparative HPLC and co-injected with ceftazidime sample to confirm the retention times in HPLC. Structural elucidation of these impurities [Impurity-I ((6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)-3-cephem-4-carboxylate sulfoxide), Impurity-II ((6*R*,7*R*)-7-[(*Z*)-2-(2-N-Formylaminothiazol-4-yl)-2-(2-carboxy-prop-2-oxyimino)-acetamido]-3-(1-pyridiniummethyl)-3-cephem-4-carboxylate), and Impurity-III ((6*R*, 7*R*)-7-[(*Z*)-2-(2-Amino-thiazol-4-yl)-2-(2-carboxyprop-2-oximino)acetamido]-3-hydroxymethyl-3-cephem-4- car boxylic acid lactone)] by spectral data (^{1}H NMR, ^{13}C NMR, IR and MS) has been discussed. The developed RP-LC method was validated with respect to linearity, accuracy, precision and high sensitivity with detection limits and quantification limits ranging from 0.13 μg/mL to 0.142 μg/mL and 0.260 μg/mL to 0.284 μg/mL, respectively.

Key words: Ceftazidime, New impurities, Identification, Structural elucidation, Reversed-phase HPLC, Validation.

INTRODUCTION

Ceftazidime is a third generation semi-synthetic cephalosporin antibiotic having

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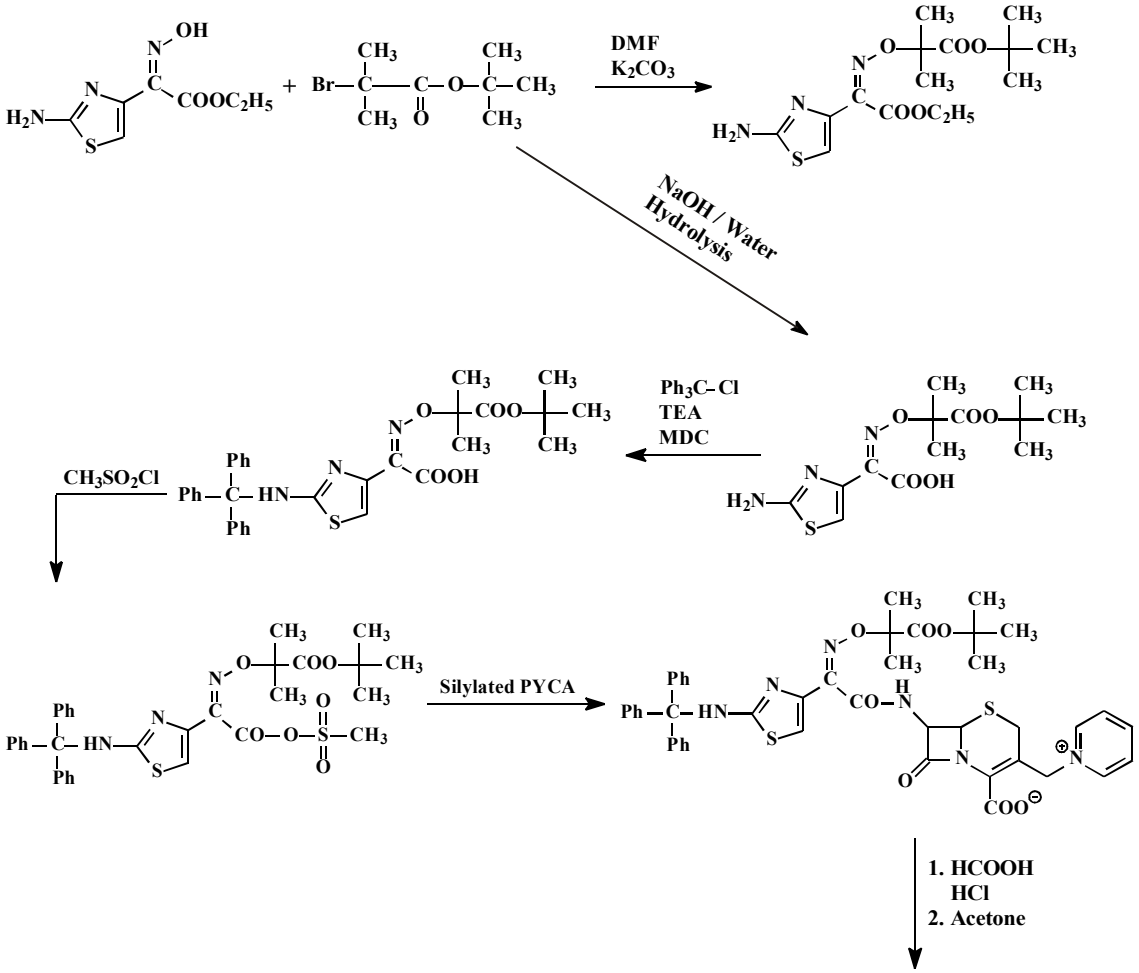
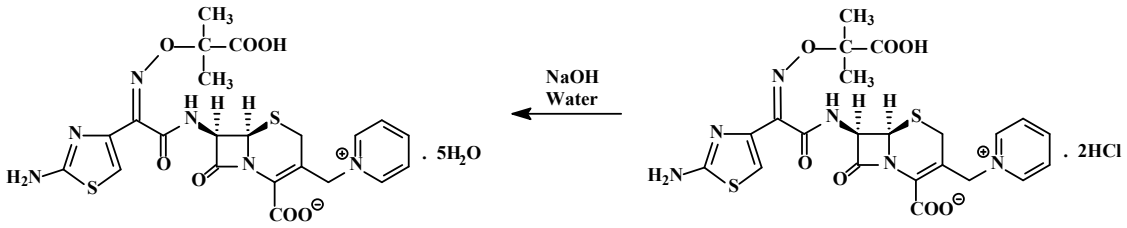
broad spectrum of activity with enhanced activity against pseudomonas aeruginosa[1] and chemically designated as (6*R*, 7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino) acetamido]-3-(1-pyridiniummethyl)-3-cephem-4-carboxylate pentahydrate. Several methods have been reported in the literature for the determination of ceftazidime[2-9]. During the preparation of ceftazidime in our laboratory as per the Scheme given in Fig. 1, three unknown impurities [Impurity-I, Impurity-II and Impurity-III] were detected consistently in HPLC along with known impurities reported in European Pharmacopoeia[10]. However, there were no reports for these three unknown impurities i.e. Impurity-I, Impurity-II and Impurity-III in the literature.

Scheme





Ceftazidime pentahydrate

Fig. 1: Synthesis of ceftazidime

The HPLC analysis of ceftazidime bulk drug has been performed (as per the method mentioned in method development) that revealed the presence of these unknown impurities at levels ranging from 0.05 to 0.2%. 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[11-13]. This paper describes identification, structural elucidation and validation of unknown impurities, which were present in ceftazidime. Identification and structural elucidation of these new impurities were not reported till date to the best of our knowledge.

EXPERIMENTAL

Materials and reagents

Disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, hydrochloric acid, sodium hydroxide and hydrogen peroxide were all of AR grade, and acetonitrile (HPLC grade) were procured from E. Merck Limited, Mumbai, India. HPLC grade water, obtained from Millipore system (Millipore Inc., USA), was used throughout the analysis. Orthophosphoric acid was obtained from Fluka Chemicals, Switzerland. The investigated samples of ceftazidime and its known impurities were prepared in APL Research Centre (A Division of Aurobindo Pharma Limited, Hyderabad, India).

Analytical procedure

A Waters HPLC (MILFORD, MA01757, USA) equipped with Alliance 2695 separations module and 2996 photodiode array detector was used. A mixture of phosphate buffer and acetonitrile in the ratio of 95 : 5 (v/v) was used as diluent in the preparation of analytical solutions. Ceftazidime working standard solution (12.5 μg/mL) spiked with Impurity-II at a level of 1% (w/w) was used as system suitability solution. Ceftazidime working reference standard at 12.5 μg/mL and cefazidime drug substance at 1250 μg/mL concentrations were used as diluted standard solution and sample solution, respectively. The

specification limits used for validation studies were 0.1%. 10 µL of system suitability solution, blank, six replicate injections of diluted standard solution and sample solutions were separately chromatographed. A resolution of not less than 2.0 between impurity-II and ceftazidime was set as system suitability requirement. The R.S.D. of not more than 5.0% for ceftazidime peak areas obtained from six replicate injections of diluted standard solution is used to verify the system precision. These new impurities were determined against mean area obtained from replicate injections of diluted standard solution and correction factors (CF). The CF for new impurities was calculated w.r.t. ceftazidime from the ratio of slope of ceftazidime to slope of individual new impurities obtained from the regression analysis.

Procedure for forced degradation study

Forced degradation of ceftazidime drug substance was carried out under acid / base hydrolytic, oxidative, thermolytic, photolytic and humidity stress conditions. Solutions were prepared by dissolving drug substance in diluent and then treating with aqueous 1M hydrochloric acid, aqueous 1M sodium hydroxide and aqueous 3% hydrogen peroxide. After the degradation, these solutions were diluted with diluent and analyzed in the proposed method. For thermal stress, sample of drug substance was placed in a controlled temperature oven at 105°C for 168 h. For photolytic stress, the sample was exposed for 168 h to attain 10 K lux and for degradation under humidity, samples were prepared by dissolving respective samples in diluent and diluted to the desired concentration and subjected for analysis using the proposed method.

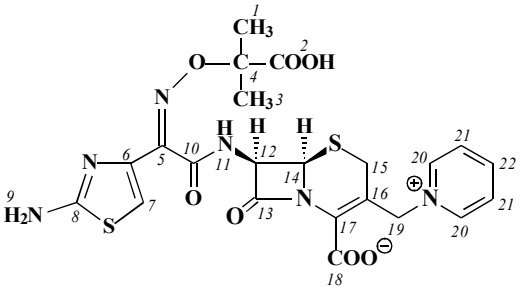
High performance liquid chromatography (Preparative)

A Shimadzu LC-8A Preparative liquid chromatograph equipped with SPD-10A VP, UV-Vis detector (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) was used. Hypersil BDS C18 (250 mm long x 21.2 mm i.d.) preparative column packed with 8 µm particle size (Thermo Electron Corporation, UK) was employed for isolation of impurities. The mobile phase consisted of (A) 1M ammonium acetate solution and (B) acetonitrile. Flow rate was kept at 40 mL / min and detection was carried out at 255 nm. The analysis was carried out under gradient conditions as follows, time (min)/A (v/v): B (v/v); $T_{0.01}$/97:03, $T_{20.0}$/90:10, $T_{30.0}$/80:20 and $T_{40.0}$/70:30.

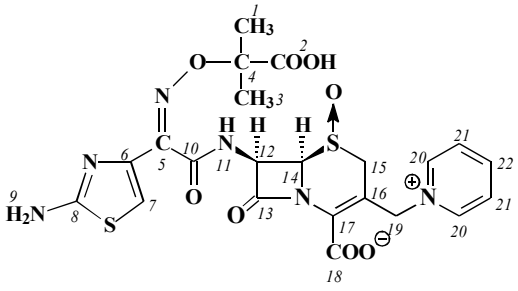
LC-MS / MS Analysis

LC-MS / MS Analysis was carried out using Perkin-Elmer triple quadrupole mass spectrometer (API 2000, PE SCIEX, Foster city, CA) coupled with a Shimadzu HPLC (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) equipped with SPD 10 A VP UV-vis detector and LC 10 AT VP pumps. Analyst software was used for data

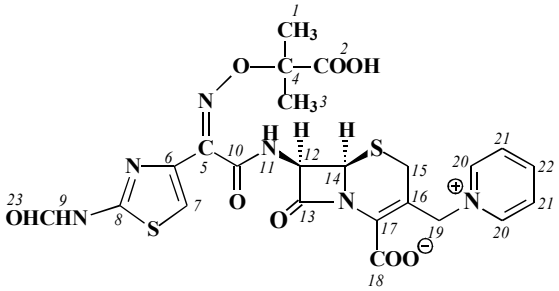
acquisition and processing. The turbo ion spray voltage was maintained at 5.5 Kv and temperature was set at 375°C. High pure nitrogen gas was used as auxiliary gas and curtain gas. Zero air was used as nebulizer gas. LC-MS / MS Spectra were acquired from m/z 100 to 1000 in 0.1 amu steps with 2.0 s dwell time. Ceftazidime sample was subjected to LC-MS / MS analysis. The analysis was carried out using Hypersil BDS C18, 250 mm x 4.6 mm, 5-µm. Mobile phase A used was 0.01M ammonium acetate solution and mobile phase B was acetonitrile. Detection was carried out at 255 nm and flow rate was 1.0 mL/min. Mobile phase was used as diluent. Data acquisition time was 55 min. The analysis was carried out under gradient conditions as follows, time (min)/A (v/v): B (v/v); $T_{0.01}$/100:0, $T_{15.0}$/75:25, $T_{25.0}$/30:70, $T_{45.0}$/30:70, $T_{47.0}$/100:0 and $T_{55.0}$/100:0. Known impurities were detected in laboratory batch samples. The masses of detected peaks were identical to the values of the known impurities. Additionally, three impurities corresponding to m/z 562, 574 and 467 were also observed in sample of ceftazidime. Based on these mass values, the structures given in Fig. 2 were suggested. Based on the mass spectral information, tentative structures of all the three impurities were proposed. To confirm the proposed structures, sample of ceftazidime containing all the target impurities was subjected to preparative LC to isolate the impurities and carry out further spectroscopic experiments.



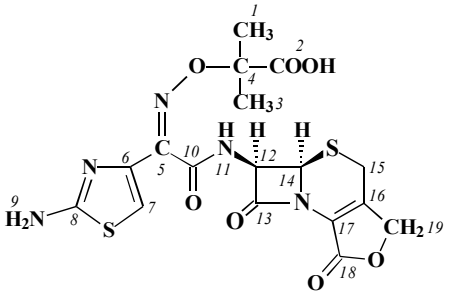
Ceftazidime



Impurity-I, RRT ~ 0.63



Impurity-II, RRT ~ 1.40



Impurity-III, RRT ~ 1.99

Fig. 2: Chemical structures of ceftazidime and its unknown impurities

NMR Spectroscopy

The ^{1}H NMR and ^{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.

FT-IR Spectroscopy

The IR spectra for ceftazidime and its impurity-I, impurity-II and impurity-III were recorded in the solid state as KBr dispersion using Perkin-Elmer instrument, model-spectrum one (Perkin Elmer Ltd, Beaconsfield, UK).

Mass Spectrometry

Mass spectra were recorded on Perkin-Elmer PE SCIEX-API 2000 mass spectrometer equipped with a Turboion spray interface at 375°C.

RESULTS AND DISSCUSION

Method development

In the method for related substances given in European pharmacopoeia, impurity-I was not eluting in the specified runtime and peak shape of impurities impurity-II and impurity-III were broad affecting the detection and quantification limits. Method development for quantification of related substances was initiated with impurities mentioned in European pharmacopoeia (i.e., Ph.Eur.Impurity-A, Ph.Eur.Impurity-B, Ph.Eur.Impurity-C, Ph. Eur. Impurity-D, Ph. Eur. Impurity-E, Ph. Eur. Impurity-F, Ph. Eur. Impurity-G and Ph. Eur, Impurity-H) and identified impurities (Impurity-I, Impurity-II and Impurity-III). Ceftazidime samples, spiked with these impurities were co-eluted by gradient elution using different combinations of following chromatographic parameters

(a) Different stationary phases like C18, C8 and Phenyl,

(b) Different buffers like phosphate, sodium and acetate with different pH (2-7) and

(c) Different organic modifiers like acetonitrile and methanol.

Satisfactory separations were achieved on YMC pack ODS-A, 250 mm long, 4.6 mm i.d and 5µ particle diameter column thermostated at 30°C. Mobile phase A was phosphate buffer (pH 7.0 ± 0.05) and acetonitrile in the ratio of 98 : 2 (phosphate buffer pH 7.0 prepared by dissolving 2.3 g of disodium hydrogen orthophosphate anhydrous and 1.75 g of potassium dihydrogen orthophosphate in 1000 mL of water, pH adjusted to 7.0 ± 0.05

with dilute orthophosphoric acid). Acetonitrile was used as mobile phase B. The flow rate was 1.0 mL / min and injection volume was 10 µL. The analysis was carried out under gradient conditions as follows, time (min)/A (v/v): B (v/v); $T_{0.01}$/95:05, $T_{15.0}$/75:25, $T_{25.0}$/30:70, $T_{40.0}$/30:70, $T_{42.0}$/95:05 and $T_{50.0}$/95:05. Detraction was optimised at 255. In the optimized conditions, all impurities were separated from each other with resolution greater than 2.5 and typical relative retention times of *impurity-I, impurity-II and impurity-III* were about 0.63, 1.40 and 1.99, respectively (Ceftazidime retention time at about 5.89 min). A representative HPLC chromatogram of ceftazidime spiked with impurities is given in Fig. 3.

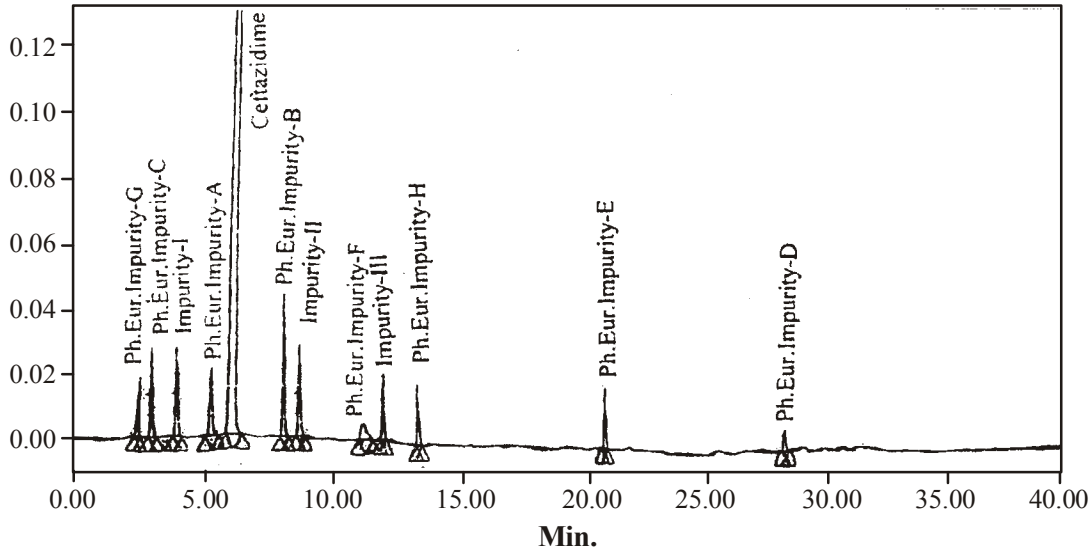


Fig. 3: LC Chromatogram of ceftazidime sample spiked with known and unknown impurities

Detection of impurities

The samples were subjected to LC-MS / MS analysis using conditions as described under section (LC-MS / MS) to identify the mass of the impurities. The masses of the impurities were 561, 575 and 468 for impurity-I (RRT-0.64), impurity-II (RRT-1.40) and impurity-III (RRT-1.99) respectively.

Isolation of impurities by preparative HPLC

Three new impurities were isolated by preparative HPLC as per the method discussed under Section (*High Performance Liquid Chromatography (Preparative).* 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 *impurity-I, impurity-II and impurity-III* was tested by HPLC and found to be 97.1, 99.0 and 98.2%, respectively.

Structural elucidation

The chemical structures of ceftazidime and its unknown impurities are given in Fig. 2. ^{1}H and ^{13}C NMR spectral assignments for ceftazidime and its unknown impurities are given in Table 1. FT-IR spectral assignments are given in Table 2.

Impurity-I

The electrospray ionization mass spectrum of impurity-I showed a molecular ion peak m/z 561.2 in the negative mode indicating the molecular weight of impurity-I as 562, which is 16 amu more than that of ceftazidime. This suggested the possible incorporation of oxyzen in the molecule. The major fragmentation peaks in MS / MS were observed at m/z 338.2 and 482.1, which confirm the oxyzen addition on sulfur of cephem ring. In the ^{1}H NMR spectrum, S-CH_2 carbon at position-15 as shifted to down field to δ 3.66 and 3.93 ppm from δ 3.07 and 3.53 ppm with respect to ceftazidime. The S-CH_2 carbon at position-15 was shifted to 45.1 ppm from 24.8 ppm of ceftazidime in ^{13}C NMR spectrum confirming the oxyzen addition to the cephem sulfur atom. These spectral data indicated that this impurity was (6*R*, 7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)-3-cephem-4-carboxylate sulfoxide.

Impurity-II

The electrospray ionization mass spectrum of impurity-II showed a molecular ion peak m/z 575 in the positive mode indicating the molecular weight of impurity-II as 574, which is 28 amu more than that of ceftazidime. The major fragmentation peaks in MS / MS were observed at 496 and 468 confirm the formyl addition on the molecule. In the ^{1}H NMR spectrum, no significant change is observed but the broad signal at δ 7.28 corresponds to NH_2 in ceftazidime disappeared and a singlet at δ 8.49 ppm corresponds to CHO appeared. In ^{13}C NMR, CHO signal at δ 159.9 was observed. From these spectral data, this impurity was identified as (6*R*, 7*R*)-7-[(*Z*)-2-(2-N-Formylaminothiazol-4-yl)-2-(2-carboxy-prop-2-oxyimino)-acetamido]-3-(1-pyridiniummethyl)-3-cephem-4-carboxylate.

Impurity-III

The electrospray ionization mass spectrum of impurity-III showed a molecular ion peak m/z 468 in the positive mode indicating the molecular weight of impurity-III as 467, which is 79 amu less than that of ceftazidime. In the ^{1}H NMR spectrum, the signals

Table 1: Comparative ^{1}H, ^{13}C (Proton decoupled) and DEPT NMR assignments for ceftazidime and its unknown impurities

CEFTAZIDIME			IMPURITY-I			IMPURITY-II			IMPURITY-III		
^{1}H NMR (ppm)	^{13}C NMR (ppm)	DEPT	^{1}H NMR (ppm)	^{13}C NMR (ppm)	DEPT	^{1}H NMR (ppm)	^{13}C NMR (ppm)	DEPT	^{1}H NMR	^{13}C NMR	DEPT
1.39 (s, 3H)	23.9	CH_3	1.51 & 1.52 (2s, 3H)	23.6	CH_3	1.40 & 1.43 (2s, 3H)	24.0	CH_3	1.39 & 1.44 (2s, 3H)	24.0	CH_3
-	175.2	C	-	174.2	C	-	175.5	C	-	175.9	C
1.39 (s, 3H)	24.3	CH_3	1.51 & 1.52 (2s, 3H)	24.0	CH_3	1.40 & 1.43 (2s, 3H)	24.4	CH_3	1.39 & 1.44 (2s, 3H)	24.5	CH_3
-	81.8	C	-	83.2	C	-	82.8	C	-	82.7	C
-	162.5	C	-	162.0	C	-	-	C	-	163.6	C
-	142.7	C	-	143.6	C	-	-	C	-	142.3	C
6.68 (s, 1H)	109.8	CH	6.97 (s, 1H)	110.9	CH	7.36 (s, 1H)	115.4	CH	6.73 (s, 1H)	109.5	CH
-	163.1	C	-	163.4	C	-	-	C	-	166.6	C
7.28 (s, 2H)	-	-	-	-	-	-	-	-	7.25 (s, 2H)	-	-
-	149.3	C	-	160.2	C	-	-	C	-	149.6	C
9.46 (brs, 1H)	-	-	8.87 (d, 1H)	-	-	10.95 (brs, 1H)	-	-	11.0 (brs, 1H)	-	-
5.72 (dd, 1H)	57.5	CH	6.13 (dd, 1H)	58.1	CH	5.75 (m, 1H)	57.5	CH	6.00 (brs, 1H)	57.4	CH
-	163.1	C	-	163.1	C	-	-	C	-	164.0	C
5.08 (d, 1H)	58.6	CH	5.14 (d, 1H)	66.6	CH	5.09 (d, 1H)	58.5	CH	5.17 (d, 1H)	59.2	CH
3.07 & 3.53 (Abq, 2H)	24.8	CH_2	3.66 & 3.93 (Abq, 2H)	45.1	CH_2	3.06 & 3.50 (Abq, 2H)	24.6	CH_2	3.77 (Abq, 2H)	22.5	CH_2
-	137.9	C	-	129.6	C	-	-	C	-	130.4	C
-	109.5	C	-	113.5	C	-	108.1	C	-	122.8	C
-	168.5	C	-	170.1	C	-	-	C	-	168.3	C
5.12 & 5.66 (Abq, 2H)	61.5	CH_2	5.63 (s, 2H)	60.2	CH_2	5.13 & 5.63 (Abq, 2H)	61.7	CH_2	5.04 (Abq, 2H)	71.4	CH_2
9.47 (m, 2H)	145.1	CH	9.03 (m, 2H)	144.8	CH	9.44 (m, 2H)	145.1	CH	-	-	-
8.15 (m, 2H)	128.1	CH	8.21 (m, 2H)	128.3	CH	8.16 (m, 2H)	128.0	CH	-	-	-
8.58 (m, 2H)	145.7	CH	8.66 (m, 1H)	146.3	CH	8.58 (m, 1H)	149.1	CH	-	-	-
-	-	-	-	-	-	8.49 (s, 1H)	159.9	CH	-	-	-

[a]Refer Chemical structures in Fig. 2 for numbering of ceftazidime and its unknown impurities.

DEPT: Distortionless enhancement by polarization transfer.

s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet; ABq, AB quartet.

Table 2: FT-IR Spectral data of ceftazidime and its unknown impurities

S. No.	Compd.	IR (KBr) Absorption bands (cm^{-1})
1	Ceftazidime	3302, 3165 (N-H Stretching vibrations), 2980 (Aliphatic C-H stretching), 1758 (β-Lactam C=O stretch), 1708 (C=N Stretching), 1621 (Amide C=O stretch, amide I band), 1606, 1432 (C=O Stretch, asymmetric and symmetric in COOH), 1537 (N-H Bending amide II band), 1360, 1346 (NH_2 bending vibrations), 1044, 1018 (C-C, C-O and N-O Stretching vibrations).
2	Impurity-I	3318, 3195 (N-H Stretching vibrations), 2975 (Aliphatic C-H stretching), 1768 (β-Lactam C=O stretch), 1722 (C=N Stretching), 1633 (Amide C=O stretch, amide I band), 1616, 1435 (C=O Stretch, asymmetric and symmetric in COOH), 1524 (N-H Bending amide II band), 1380, 1358 (NH_2 Bending vibrations), 1067 (S-O Stretching), 1052, 1026 (C-C, C-O and N-O Stretching vibrations).
3	Impurity-II	3320, 3198 (N-H Stretching vibrations), 2986 (Aliphatic C-H stretching), 1775 (β-Lactam C=O stretch), 1648 (C=O, N-H Stretching), 1610, 1445 (C=O Stretch, asymmetric and symmetric in COOH), 1545 (N-H Bending amide II band), 1384, 1360 (NH_2 Bending vibrations), 1060, 1016 (C-C, C-O and N-O Stretching vibrations).
4	Impurity-III	3305, 3190 (N-H Stretching vibrations), 2985 (Aliphatic C-H stretching), 1758 (β-Lactam C=O stretch), 1790 (β-Lactam C=O stretch), 1630 (Amide C=O stretch, amide I band), 1610, 1431 (C=O Stretch, asymmetric and symmetric in COOH), 1532 (N-H Bending amide II band), 1381, 1348 (NH_2 Bending vibrations), 1040, 1022 (C-C, C-O and N-O Stretching vibrations).

corresponding to pyridine at the 3-position side chain in ceftazidime were not seen and methylene protons at position-19 upfield shift of δ 5.04 was observed. In ^{13}C NMR, the signals corresponding to pyridine at position-3 were not seen and methylene carbon at position-19 shows a downfield shift to 71.4 from 61.5 ppm of ceftazidime. These spectral data indicated that this impurity was (6*R*, 7*R*)-7-[(*Z*)-2-(2-Amino-thiazol-4-yl)-2-(2-carboxyprop-2-oximino)acetamido]-3-hydroxymethyl-3-cephem-4-carboxylic acid lactone.

Validation

The optimized method was validated for specificity, linearity, LOD/LOQ, precision, accuracy and solution stability as per ICH guidelines.

Specificity (Selectivity)

The data on degradation studies revealed that the degradation products were well separated from the ceftazidime and unknown impurities and the peak purity data (Purity angle is less than purity threshold) of ceftazidime indicated that it was spectrally pure. The data on forced degradation studies are given in Table 3.

Table 3: Forced degradation studies data

Stress condition	Degradation (%)	Observation	Peak purity	
			PA	PT
Undegraded	-	-	0.081	0.264
Acid (1M HCl/85°C /30 min)	9.8	Increase in levels of impurity-1 (1.4%) and impurity-III (1.9%) and additional peaks.	0.065	0.253
Base (1M NaOH/ Initial)	19.6	Increase in levels of impurity-II (10.2%) and impurity-III (3.8%) and additional peak.	0.064	0.255
Oxidation (3% H_2O_2/Initial)	10.3	Increase in levels of impurity-I (7.5%), impurity-III (2.2%) and additional peak.	0.073	0.259
Thermal (105°C/168 h)	1.7	Increase in levels of impurity-I (0.1%), impurity-III (0.2%) and additional peaks.	0.080	0.263
Photolytic (10 K lx /168 h)	1.1	Increase in levels of impurity-I (0.1%) and additional peak.	0.087	0.268
Humidity (92% r.h. /25°C/ 120 h)	0.1	Increase in levels of impurity-I (0.1%).	0.079	0.263

PA, Purity angle; PT, Purity threshold

Linearity

The linearity of peak areas versus different concentrations was evaluated for ceftazidime and three unknown impurities using 10 levels ranging from 0.265 μg/mL to 9.28

μg/mL equivalent to 0.0265 (% w/w) to 0.928 (% w/w) with respect to sample concentration. The regression data for all the components tested were presented in Table 4.

Limits of detection and limit of quantification (LOD and LOQ)

The limit of detection and limit of quantification were determined for ceftazidime and for unknown impurities as per ICH guideline[13] from the standard deviation of the peak areas and slope of linearity data. The values of LOD and LOQ of ceftazidime were 0.080 μg/mL and 0.244 μg/mL, respectively and for unknown impurities were in the ranges of 0.13-0.142 μg/mL and 0.262-0.284 μg/mL, respectively. The calculated LOD and LOQ concentrations of all the components were verified for precision. R.S.D was in the range of 13.2-19.0 for LOD and 6.5-6.9 for LOQ, respectively. The results were depicted in Table 4.

Precision

System precision was verified using diluted standard solution, which was analyzed for six times and R.S.D. of Ceftazidime. Peak areas was evaluated and found to be 1.0 %. Precision of the method was studied for repeatability and intermediate precision. Repeatability was demonstrated by analyzing six separate ceftazidime sample solutions that were prepared by spiking the unknown impurities at specification level. The R.S.D. (0.72-1.11%, n = 6) for each unknown impurity was evaluated.

The intermediate precision of the method was determined on six separate sample solutions prepared from same lot by spiking the unknown impurities at specification level by a different analyst using fresh mobile phase and diluent preparations and on different instrument on a different day with same brand of column with different lot number. The overall R.S.D was evaluated and found to be in the range of (0.31-1.41%, n = 6) for unknown impurities, which were within the acceptance criterion of NMT 10.0% R.S.D. The results were presented in Table 5.

Accuracy (Recovery)

Accuracy of the method for unknown impurities was determined by analyzing ceftazidime sample solutions spiked with unknown impurities at three different concentration levels of 50, 100 and 150 % and each in triplicate at the specified limit. The recovery of unknown impurities was found to be in between the predefined acceptance criterion of 90 to 110% and the data is given in Table 6.

Table 4: Linearity data for ceftazidime and its unknown impurities

Component	Calibration range (µg/mL)	Calibration points	Slope	Intercept	Residual sum of squares	CC[1] (r)	CF[2]	LOD (µg/mL) (% RSD)	LOQ (µg/mL) (% RSD)
Ceftazidime	0.265 - 9.283	7	23346	-798	659	0.9999	1.00	-	-
Impurity-I	0.25 - 1.943	7	18181	-546	339	0.9996	1.28	0.142 (19.0)	0.284 (6.5)
Impurity-II	0.258 - 1.959	7	16354	156	313	0.9996	1.43	0.130 (13.2)	0.260 (6.9)
Impurity-III	0.25 - 1.945	7	15916	-154	328	0.9995	1.47	0.131 (15.6)	0.262 (6.9)

[1] CC: Correlation coefficient
[2] CF: Correction factor

Table 5: Precision results for ceftazidime and its unknown impurities (Set I - repeatability and Set II - intermediate precision)

Component	Repeatability results (n=6) (Mean result ± SD, %RSD)	Intermediate precision results (n=6) (Mean result ± SD, %RSD)	Overall results (n=12)(Mean result ± SD, %RSD)
Impurity - I	0.169 ± 0.001, 0.87	0.167 ± 0.001, 0.31	0.168 ± 0.001, 0.78
Impurity - II	0.143 ± 0.001, 0.72	0.149 ± 0.001, 0.42	0.145 ± 0.003, 2.33
Impurity - III	0.114 ± 0.001, 1.11	0.122 ± 0.001, 1.41	0.120 ± 0.004, 3.81

Table 6: Accuracy data of unknown impurities

Component	50% of specification level			100% of specification level			150% of specification level		
	Amount (% w/w)			Amount (% w/w)			Amount (% w/w)		
	Added[b]	Found[b]	% Recovery[c]	Added[b]	Found[b]	% Recovery[c]	Added[b]	Found[b]	% Recovery[c]
Impurity -I	0.049	0.051	103.4 ± 1.2	0.099	0.098	100.7 ± 2.9	0.148	0.150	102.5 ± 0.4
Impurity -II	0.052	0.054	103.8 ± 1.9	0.104	0.107	101.6 ± 1.1	0.157	0.160	101.5 ± 1.3
Impurity -III	0.051	0.053	102.0 ± 1.9	0.103	0.109	103.9 ± 1.9	0.154	0.156	100.6 ± 0.6

[a] Specification level 0.1% of , Impurity-I, Impurity-II and Impurity-III
[b] n=3, Average of three determinations
[c] Average ± R.S.D.

Stability of analytical solution

To determine the stability of sample solution, the sample solutions of ceftazidime spiked with unknown impurities at specified level were prepared and analyzed immediately after preparation and after different time intervals up to 15 h, while maintaining the sample cooler temperature at about 25°C and at about 6°C. The results from these studies indicated the sample solution was unstable at room temperature and stable for at least 15 h at 6°C temperature.

CONCLUSION

A new, accurate and selective gradient RP-HPLC method was proposed for the determination of ceftazidime unknown impurities in ceftazidime drug substance and validated as per the ICH guidelines. The method was found to be simple, selective, precise and accurate. Three unknown impurities, which were not reported earlier, were identified by this method, isolated and characterized using spectral data. Therefore, this method can be used for routine testing as well as stability analysis of ceftazidime drug substances. All statistical results for linearity, LOD/LOQ, precision and accuracy were within the acceptance criteria.

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