Journal of Food and Drug Analysis

Volume 18 | Issue 5 Article 7

Identification, synthesis and spectral characterization of a potential impurity of ceftazidime

Journal of Food and Drug Analysis, Vol. 18, No. 5, 2010, Pages 346-352 藥物食品分析 第十八卷 第五期

Identification, Synthesis and Spectral Characterization of a Potential Impurity of Ceftazidime

BIN QU[1,2], BEI-LI OU[1,2], DE-YING CHEN[1,2] AND YU-ZHU HU[1,2*]

[1,] Department of Analytical Chemistry, China Pharmaceutical University, Tongjiaxiang 24, Nanjing 210009, China*

[2.] Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, Tongjiaxiang 24, Nanjing 210009, China

(Received: September, 2009; Accepted: June 23, 2010)

ABSTRACT

During the process development of ceftazidime, a new impurity which exceeded the limit of 0.1% was detected by a simple HPLC method. The molecular weight of the target impurity was determined by LC/MS. This suspected impurity was synthesized and purified for characterization. When co-injected with ceftazidime in HPLC, the retention time of the impurity was the same as the ceftazidime sample containing the impurity. The structural determination of the suspected impurity was conducted by IR, MS, ^{1}H-NMR and ^{13}C-NMR spectroscopic techniques. This new impurity was the methyl ester of ceftazidime, and its structure was determined as (6*R*,7*R*)-7-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(1-methoxycarbonyl-1-methylethoxy) imino]acetyl]amino]-8-oxo-3-[(1-pyridinio)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, with molecular formula of $C_{23}H_{24}N_6O_7S_2$ and molecular weight of 560 Da.

Key words: ceftazidime, impurity, identification, synthesis, characterization

INTRODUCTION

Ceftazidime (Figure 1a), (6*R*,7*R*)-7-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(1-carboxy-1-methylethoxy)imino]acetyl] amino]-8-oxo-3-[(1-pyridinio)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, is a third generation cephalosporin antibiotic which is widely used to treat infections from both Gram-positive and Gram-negative bacteria, especially against *Pseudomonas aeruginosa*[1,2].

Different methods of synthesizing ceftazidime were reported in literature[3,4]. Generally, ceftazidime is semi-synthesized by two main steps: synthesis of the activated intermediate of side-chain thioester and synthesis of bulk drug substance from 7-aminocephalosporanic acid (7-ACA). 7-ACA is then combined with the activated thioester to form ceftazidime. The total synthesis is a long and complex process. Side reactions and impurities are usually generated along the process. Only five impurities in ceftazidime have been identified and documented in the European Pharmacopoeia 5.0 (EP 5.0). Methods by high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC) are described for the simultaneous examination of these related compounds in EP 5.0[5] and also listed in the British Pharmacopoeia 2005[6].

The impurity profile of a drug substance is critical

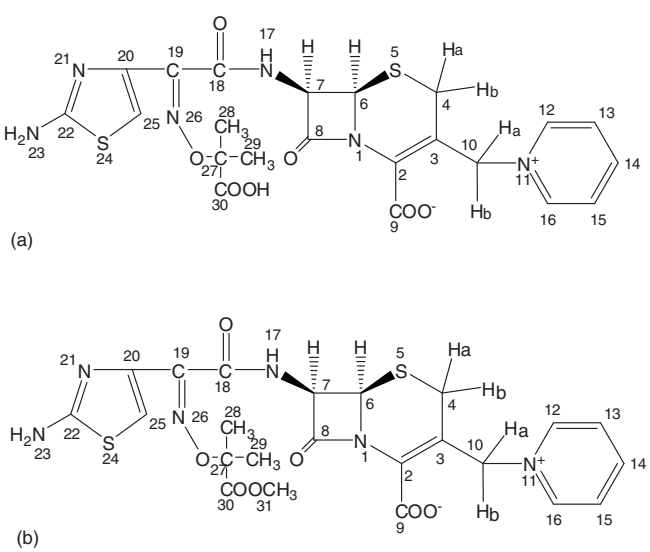

Figure 1. Structures of (a) ceftazidime and (b) the suspected impurity, with numbering.

* Author for correspondence. Tel: +86-25-83271280;
E-mail: njhuyuzu@126.com

Journal of Food and Drug Analysis, Vol. 18, No. 5, 2010

to its safety assessment and the optimization of the manufacturing process. It is thus of great importance to identify the impurities during drug manufacture development and quality control. The impurity profile of the final product needs to be studied, as the regulatory requires to identify and characterize all unknown impurities[7].

An unknown impurity was observed in the process development of ceftazidime bulk drug substance. This new impurity could not be detected by HPLC, as described in EP 5.0. The TLC examination revealed its presence and its amount exceeded the single impurity limit (0.5%) for ceftazidime in EP 5.0. A modified HPLC method was employed to detect this compound and the area percentage analysis of the ceftazidime sample showed that the content of this impurity could reach up to 0.2%, which exceeded the value (0.1%) of identification threshold according to the International Conference on Harmonization (ICH) Guideline Q3A[7].

This report describes the identification, synthesis and characterization of the new impurity present in ceftazidime. To the best of our knowledge, the impurity profile of this compound has not been reported yet. The impurity was synthesized and characterized by liquid chromatography-mass spectrometry (LC/MS), HPLC and other spectroscopic techniques.

MATERIALS AND METHODS

I. *Materials and Reagents*

Ceftazidime bulk drug substance was obtained from Harbin Pharmaceutical Group Co., Ltd. (Harbin, China). The impurity was synthesized in the laboratory and isolated by preparative HPLC after identification by LC/MS. Acetonitrile was of HPLC grade (Merck KGaA). Acetic acid, hydrochloric acid, ammonium acetate, phosphoric acid, ammonium dihydrogen phosphate and diethyl ether were of analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Dimethylsulfoxide (DMSO) was of analytical grade and purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China).

II. *Analytical HPLC*

A Shimadzu LC-10ADvp separation module equipped with Shimadzu SPD-M10Avp diode array detector and Class-vp data handling system (Shimadzu, Kyoto, Japan) were used. The analysis was carried out on a Kromasil C_{18} (250 mm × 4.6 mm id, 5 μm) column. The absorbance of eluate was monitored at 255 nm. The column temperature was maintained at 35°C. A mixture of 200 mM ammonium dihydrogen phosphate in water (pH adjusted to 3.9 with diluted phosphoric acid) and acetonitrile (85 : 15, v/v) was used as the mobile phase in an isocratic elution mode at a flow rate of 1.0 mL/min.

III. *Preparative HPLC*

An Agilent 1100 series preparative liquid chromatography system, equipped with an Agilent G1361A prep-pump and an Agilent G13658 Multiple Wavelength Detector (Agilent, Palo Alto, CA), was used to isolate the impurity from the synthetic product. A Shim-pack PREP-ODS (250 mm × 20 mm id, 10 μm) preparative column and a mobile phase consisting of water (pH adjusted to 3.9 with hydrochloric acid) and acetonitrile (85 : 15, v/v) were employed, with a flow rate of 10.0 mL/min. The UV absorbance detection was set at 255 nm.

IV. *LC/MS Conditions*

All LC/MS mass spectral data were acquired on an Agilent 1100 Series LC/MSD VL system (Agilent, Palo Alto, CA, U.S.A.). The sample components were fractionated over a Kromasil C_{18} (250 mm × 4.6 mm id, 5 μm) column. The mobile phase was 1 mM ammonium acetate (pH adjusted to 3.9 with diluted acetic acid) and acetonitrile (85 : 15, v/v). Forty micromilliliter of sample was injected into the LC/MS system with a flow rate of 1.0 mL/min. A quadrupole mass spectrometer equipped with an electrospray ion (ESI) source was employed. The ESI source was operated in positive mode and the acquired *m/z* ranged from 105 to 1000. The mass spectrometry conditions were set as follows: capillary temperature of 350°C, capillary voltage of 4 kV, drying gas (N_2) flow of 4.0 L/min, nebulizer pressure of 30 psi, drying gas temperature of 300°C and the fragmentation voltage of 70 V.

V. *Synthesis and Isolation of the Suspected Novel Impurity*

The suspected novel impurity was synthesized in the laboratory according to the scheme shown in Figure 2. A freshly prepared solution of diazomethane in diethyl ether was slowly added to a solution of ceftazidime (0.5 g) in dimethylsulfoxide (10 mL) with the help of a mechanical stirrer[8,9]. The reaction was stopped before the solution turned yellow by diluting the mixture with 5 volumes of water. The reaction mixture was filtered and subjected to preparative HPLC for the isolation and purification of the impurity.

The fractions collected were analyzed by analytical HPLC. Fractions of > 95% were pooled and concentrated under vacuum in a rotary evaporator (Rotavap) to remove acetonitrile. The aqueous solution was then lyophilized in a freeze dryer (Labconco, Kansas, MO, U.S.A.). The suspected novel impurity was obtained as an off-white powder.

VI. *NMR Spectroscopy*

The ^{1}H- and ^{13}C-NMR spectra were recorded at 303 K on a Bruker Avance AV-300 spectrometer (300 MHz for ^{1}H)

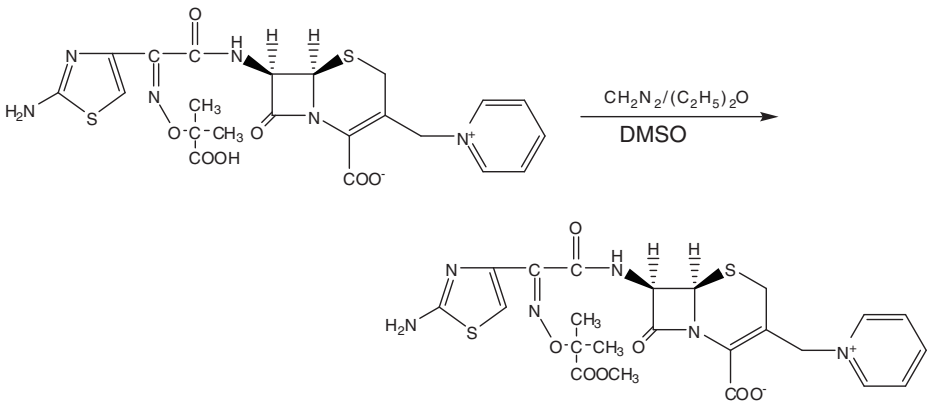

Figure 2. Scheme for the synthesis of the suspected impurity of ceftazidime.

equipped with a 5-mm probe, using dimethylsulfoxide-d_6 as solvent and tetramethylsilane (TMS) as internal standard.

VII. *FTIR Spectroscopy*

The FTIR spectrum of the impurity in the range of 4000 to 400 cm^{-1} was recorded on a Shimadzu 8400-S FT-IR spectrophotometer in the solid state using potassium bromide (KBr) pellet technique.

RESULTS AND DISCUSSION

I. *Detection of the Suspected Novel Impurity*

The suspected novel impurity was not detected by the HPLC method as described in EP 5.0[(5)], since it was tightly retained in the column. The impurity was eluted at a longer retention time (58 min) than the recommended data acquisition time of 35 min. The retention time of ceftazidime was 10 min, as shown in Figure 3(a). The method was thus modified, as described above in Section II of Materials and Methods, in which the proportion of the organic phase was increased, leading to shorter retention times for both ceftazidime and the impurity, as well as a better resolution between the peaks of the impurity and ceftazidime. The improved method shortened the analysis time for the detection of the impurity. The chromatogram for the ceftazidime sample is shown in Figure 3(b), where the target impurity was present at a level of 0.2%, thus requiring a detailed structure characterization[(7)]. To be compatible with LC/MS, the mobile phase was modified by substituting the phosphate buffer with volatile acetate buffer. The sample was then subjected to LC/MS analysis using the conditions described in Section IV of Materials and Methods. And the MS spectra for ceftazidime and the impurity are shown in Figure 4. The synthesized impurity was co-injected with ceftazidime into the HPLC system and its identity was

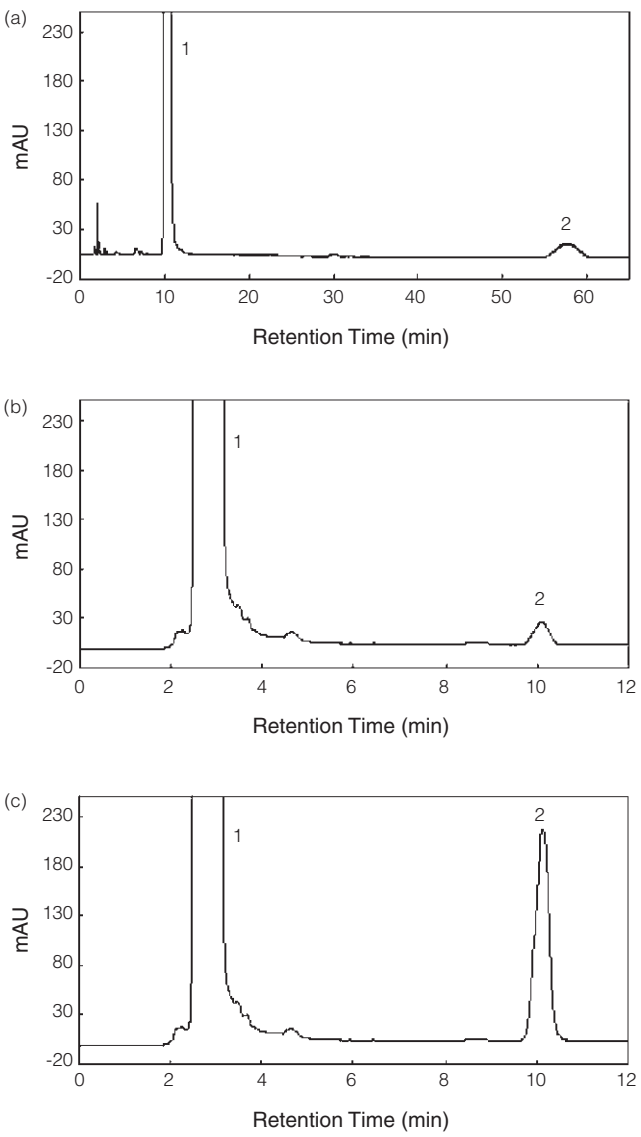

Figure 3. HPLC chromatogram of (a) ceftazidime analyzed by EP 5.0 method, (b) ceftazidime analyzed by the method described in Section II *Analytical HPLC* and (c) ceftazidime spiked with the synthetic impurity. 1: ceftazidime; 2: the suspected impurity.

confirmed by comparing its retention time with that of the impurity in ceftazidime (Figure 3c).

II. *Structural Elucidation of the Suspected Novel Impurity*

(I) *LC/MS*

The ESI mass spectrum of the suspected novel impurity exhibited a protonated molecular ion peak at *m*/*z* 561 $[M+H]^+$ in positive ion mode (Figure 4), indicating a molecular weight of 560, which is 14 amu more than that of ceftazidime (M = 546). An amu difference of 14 corresponds to the mass of N atom or CH_2 group. According to the Nitrogen Rule, a CH_2 group addition was probable because the molecular weight of ceftazidime is an even number. Other fragments obtained for the protonated impurity molecule showed three prominent peaks at *m*/*z* 482, 460 and 410. The formation of the product ion at *m*/*z* 482 could be attributed to the loss of C_5H_5N, upon which further loss of CO_2 and CO yielded the product ion at *m*/*z* 410. The proposed fragmentation scheme is illustrated in Figure 5.

(II) *FTIR*

An infrared absorption spectrum shows the functional groups which are characteristic of a particular molecule. The most important feature of the infrared spectrum of the impurity is the very strongly conjugated carbonyl group. In the FTIR spectrum of the suspected impurity (Figure 6), sharp absorption bands at 1774, 1737, 1674 and 1623 cm^{-1} corresponded to C=O stretching vibrations of beta-lactam, carboxyl acid methyl ester, amide and carboxyl group, respectively. N-H stretching, aromatic C-H stretching and C-O stretching bands were observed at 3415, 3196 and 1151 cm^{-1}, respectively. At 1533 cm^{-1}, C=N stretching vibration was recorded. Aliphatic asymmetric and symmetric C-H stretching appeared in the range 3057-2952 cm^{-1}.

(III) *NMR*

In order to confirm the identity of the suspected novel impurity, ^{1}H- and ^{13}C-NMR spectra of ceftazidime and the impurity were recorded. In the ^{1}H-NMR spectrum, the suspected novel impurity showed signals similar to those of ceftazidime, except for a singlet signal at 3.62 ppm corresponding to the proton of $COOCH_3$ with three proton integration. The ^{13}C-NMR spectrum, which was complementary to the ^{1}H-NMR, showed signals similar to those of ceftazidime, except for a new signal at 52.5 ppm corresponding to the methyl carbon of $COOCH_3$ group. These two signals are the typical resonance peaks of the methyl group of $COOCH_3$ and these additional signals confirmed that the OH group of the carboxyl of ceftazidime at 30-position of the structural skeleton is replaced by an OCH_3 group. The ^{1}H- and ^{13}C-NMR chemical shift values and their assignments for the suspected novel impurity are given in Table 1, in comparison with those of ceftazidime.

CONCLUSIONS

Based on the above spectral data, the molecular formula of the suspected novel impurity was confirmed as $C_{23}H_{24}N_6O_7S_2$ and the corresponding structure was characterized as (6*R*,7*R*)-7-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(1-methoxycarbonyl-1-methylethoxy)imino]acetyl]amino]-8-oxo-3-[(1-pyridinio)methyl]-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate (Figure 1b), with a molecular weight 560.

In this paper, a new impurity in ceftazidime bulk drug substance was identified, synthesized and characterized by HPLC (analytical and preparative), LC/MS, IR and NMR (^{1}H and ^{13}C) techniques. Ceftazidime products from other manufacturers will be collected to check whether the impurity is specific to the synthetic method. Up till now, no pharmacological/toxicological information has been provided for the impurity. As to the significance of this finding, more systematic research has to be carried out by pharmacological or toxicological scientists. Although this impurity has not been reported in any pharmacopoeia, it should be listed as a suspected impurity in the pharmaceutical manufacturing process and quality control of ceftazidime. In

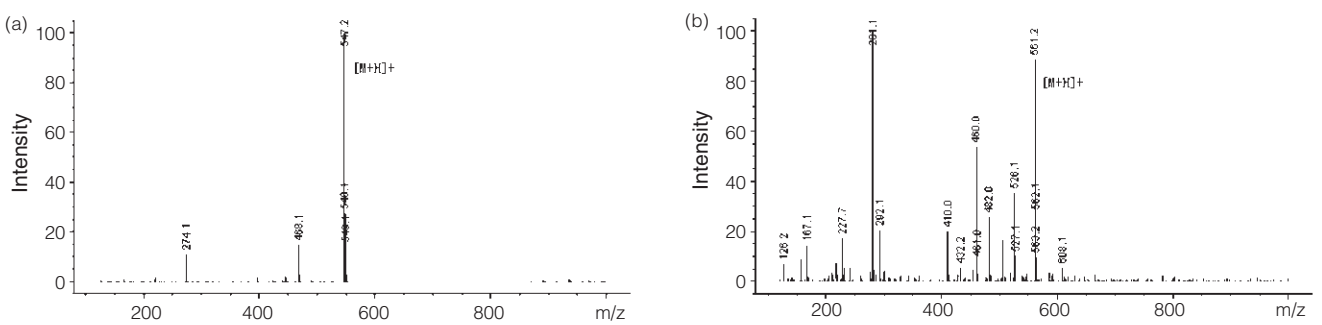

Figure 4. MS spectra of (a) ceftazidime and (b) the suspected impurity.

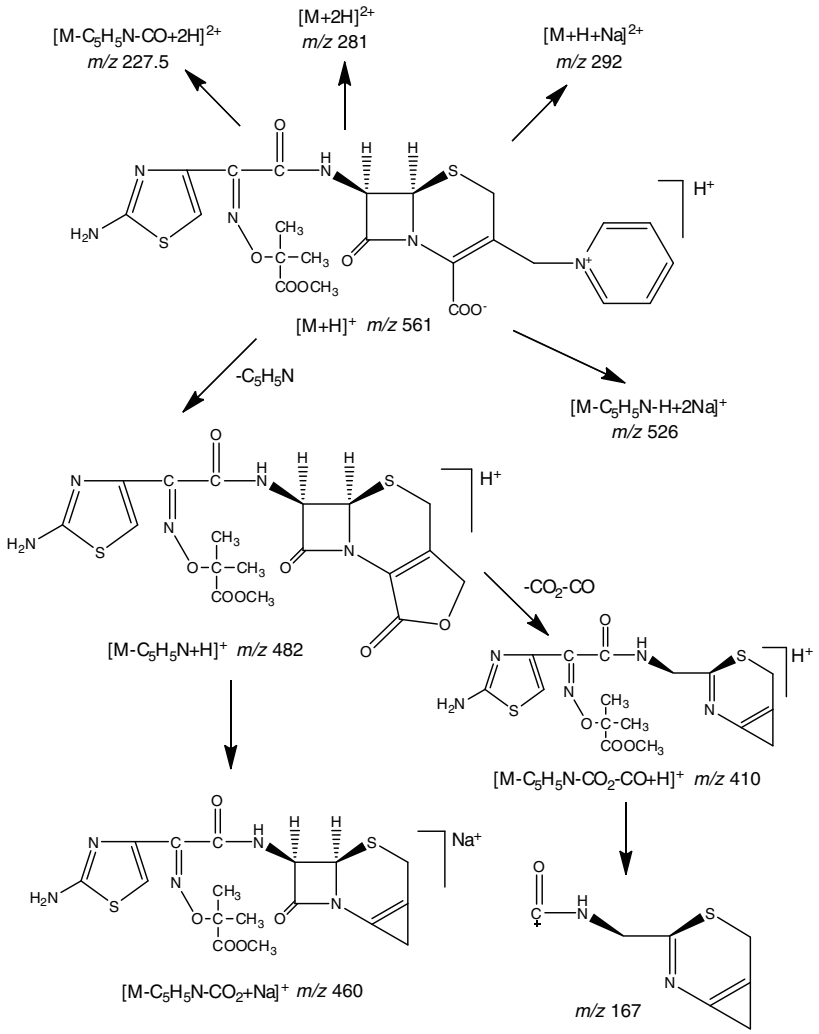

Figure 5. Fragmentation pathways of the main ions of the methyl ester impurity of ceftazidime.

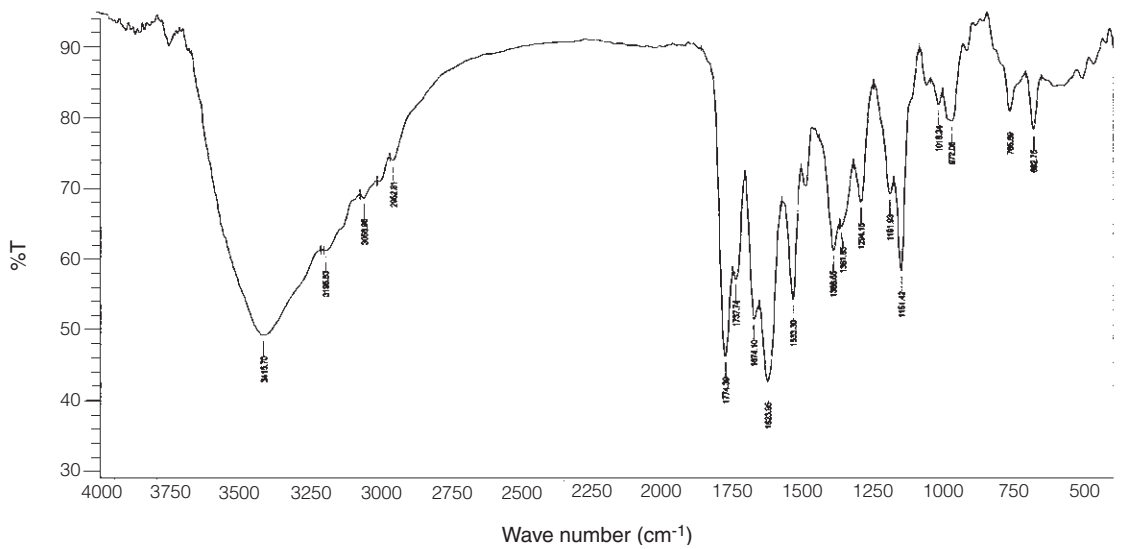

Figure 6. FTIR spectrum of the suspected impurity.

Table 1. Comparative ^{1}H- and ^{13}C-NMR assignments for ceftazidime and the suspected novel impurity

Position[a]	Ceftazidime				The Suspected Novel Impurity			
	^{1}H	δ (ppm)	J (Hz)	^{13}C	^{1}H	δ (ppm)	J (Hz)	^{13}C
2	-	-	-	138.0	-	-	-	143
3	-	-	-	109.3	-	-	-	110.1
4	Ha	3.12/d	17.7	24.8	Ha	3.20/d	17.5	25.4
	Hb	3.53/d	17.7	-	Hb	3.53/d	17.5	-
6	1H	5.09/d	5.0	57.4	1H	5.10/d	5.1	58.1
7	1H	5.73/dd	8.3, 5.0	58.6	1H	5.80/dd	8.0, 5.1	59.1
8	-	-	-	162.4	-	-	-	163.1
9	-	-	-	163.0	-	-	-	163.4
10	Ha	5.21/d	13.8	61.6	Ha	5.30/d	13.9	61.7
	Hb	5.65/d	13.8	-	Hb	5.65/d	13.9	-
12	1H	9.41/d	5.8	145.0	1H	9.30/d	5.4	145.5
13	1H	8.14/t	6.8	128.0	1H	8.18/t	6.9	128.6
14	1H	8.57/t	7.7	145.6	1H	8.62/t	7.8	146.3
15	1H	8.14/t	6.8	128.0	1H	8.18/t	6.9	128.6
16	1H	9.41/d	5.8	145.0	1H	9.30/d	5.4	145.5
17	1H	9.48/d	8.3	-	1H	9.45/d	8.1	-
18	-	-	-	162.9	-	-	-	163.3
19	-	-	-	149.2	-	-	-	150.0
20	-	-	-	142.6	-	-	-	143.0
22	-	-	-	168.4	-	-	-	169.0
23	2H	7.27/s	-	-	2H	7.29/s	-	-
25	1H	6.69/s	-	109.7	1H	6.70/s	-	110.1
27	-	-	-	81.8	-	-	-	82.2
28	3H	1.40/s	-	23.9	3H	1.42/s	-	24.3
29	3H	1.40/s	-	24.2	3H	1.42/s	-	24.6
30	-	-	-	175.0	-	-	-	174.0
31	-	-	-	-	3H	3.62/s	-	52.5

[a] Refer the structural formula (Figure 1) for numbering. s, singlet; d, doublet; dd, doublet of doublet; t, triplet.

addition, a simple HPLC method was developed in this study, which can be used to detect this impurity in a short duration of time. This HPLC method can be used in routine analysis and as a supplement for the method in the pharmacopoeia.

REFERENCES

1. Neu, H. C. and Labthavikul, P. 1982. Antibacterial activity and beta-lactamase stability of ceftazidime, an aminothiazolyl cephalosporin potentially active

against Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 21: 11-18.
2. Rolston, K. V., Chandrasekar, P. H., LeFrock, J. L. and Schell, R. F. 1984. The activity of ceftazidime, other beta-lactams, and aminoglycosides against Pseudomonas aeruginosa. Chemotherapy 30: 31-34.
3. Serradell, M. N., Blancafort, P. and Castaner, J. 1981. Ceftazidime. Drugs Fut. 6: 612-613. http://www.chemdrug.com/databases/8_0_peixqtrvhboaeiex.html,
4. Datta, D., Rai, B. P. and Mehre, K. 1998. Process for manufacture of cephalosporin such as ceftazidime and intermediate thereof. U.S. Patent No. 5831085.
5. European Pharmacopoeia. 2005. 5.0. pp. 1218-1220. Council of Europe. Strasbourg, France.
6. British Pharmacopoeia 2005. pp. 396-398. British Pharmacopoeia Commission. London, U.K.
7. ICH Harmonised Tripartite Guideline, Impurities in New Drug Substances (Q3A (R2)). 2006. ICH Expert Working Group.
8. Blatt, A. H. 1935. Diazomethane. Org. Syn. 15: 3-5.
9. Blatt, A. H. 1935. Nitrosomethylurea. Org. Syn. 15: 48-49.