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Research Article

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Preparation, Identification, Isolation and Characterization of Impurity in Cefoxitin

Jun Yu[1], Luo-bin Jiang[1], Wei-dong Feng[1], Chuan-meng Zhao[1]*, Fu-li Zhang[1]*

[1]*Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, Pudong, Shanghai, China*

ABSTRACT

The preparation and isolation for impurities (2R,6R,7S)-3-((carbamoyloxy)methyl)-7-methoxy-8-oxo-7-(2-(thiophen-2-yl)acetamido)-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid (A) , (6R,7S)-3-((carbamoyloxy)methyl)-7-methoxy-7-((R)-2-methoxy-2-(thiophen-2-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (B) , (6R,7S)-3-((carbamoyloxy)methyl)-7-methoxy-7-((S)- 2-methoxy-2-(thiophen-2-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (C) of cefoxtin were firstly described. These impurities are characterized by NMR and HRMS spectral data. These findings could be important for quality control and validation of the analytical method in the manufacture of cefoxitin.

Keywords: Cefoxitin; Impurity; Preparation; Identification; Characterization

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INTRODUCTION

Cefoxitin (Figure 1) is a second- Preparation, identification, isolation and characterization generation cephalosporin antibiotic with long-acting, broad-spectrum antibacterial properties, which has a slight advantage in its lack of potential nephrotoxicity and ototoxicity [1,2]. The antibacterial action of cefoxitin is similar to that of the second generation cephalosporins, but due to the fact that the structure of cefoxitin contains a methoxy group, the hydrolysis and destruction of it by β-lactamase in bacteria is greatly reduced [3]. So the drug resistance problem is much smaller than other second generation cephalosporins.

Since the treatment of bacterial infections will require large dose injection (Adult daily dose 3g), the impurities in the API is a significant concern [4]. There are seven impurities of Cefoxitin in the European Pharmacopoeia 9 Edition 2016 Cefoxitin quality standard [5]. Vundavilli and co-workers also reported an impurity in cefoxitin drug substance resulting from stress stability studies [6]. Due to the poor stability of cephalosporins, the directional synthesis of impurities is more difficult. Impurity A, impurity B and impurity C are not available commercially and no preparation method is reported (Figure 1). According to the guidelines on impurities in drug substances suggested by the International Conference on Harmonization (ICH) [7], it is necessary to isolate these impurities in pure form for the analytical method developments. Therefore, the practical synthetic methods were developed for the preparation of impurity A, impurity B and impurity C. In additional, exact structure of these impurities were confirmed by 1H NMR, 13C NMR, HRMS, HMBC, HSQC. This work will be useful for quality control and the validation of the analytical method in the manufacture of Cefoxitin.

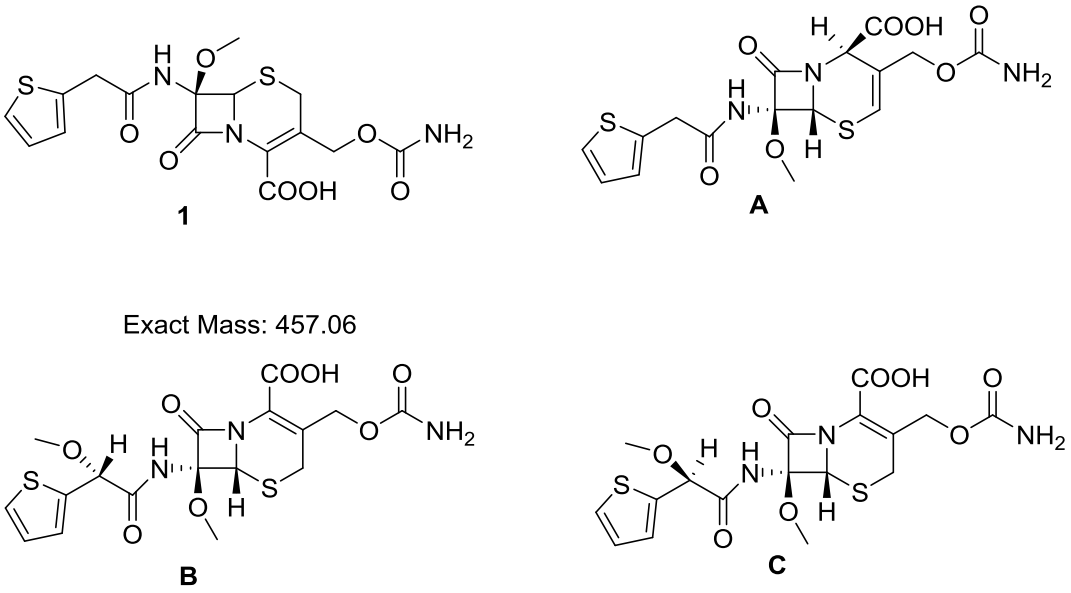


Figure 1: Chemical structures of cefoxitin and impurities A, B, C

EXPERIMENTAL SECTION

General

Reagents and solvents used in this study were commercially available. All reactions were monitored by HPLC on a Dionex Ultimate 3000 HPLC instrument using Shiseido ACR C18 (5 um 4.6 mm × 250 mm). The mobile phase consisting of A (100 mmol / L NH4OAc, pH 5.9, adjusted by acetic acid) and B (acetonitrile: ethanol = 15:7) were used with the gradient mode at the flow rate of 1.0 mL / min. The UV detection at 254 nm was used. Initial gradient started with 10% of mobile phase B and at 10 min it was set to 15%. The ratio had been set to 20% at 15 min, and then was set to 35% at 30 min. followed the ratio of mobile phase B was set to 10% at 40 min, which was continued at 50 min. HPLC purity is reported in area %. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Arance Ⅲ 600 MHz spectrometer. The solvents used were CDCl3 or CD3OD. The 1H-NMR chemical shift values were reported as δ ppm relative to tetramethylsilane (TMS) and the 13C-NMR chemical shift values were reported on δ ppm relative to CDCl3 or CD3OD. The MS spectra and high resolution mass spectrum were recorded on Aglient 6210B series single quadrupole LC-MS and Q-Tof micro YA019 instrument.

(2R,6R,7S)-3-((carbamoyloxy)methyl)-7-methoxy-8-oxo-7-(2-(thiophen-2-yl)acetamido)-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid (A)

Triethylamine (4.7 g, 46.5 mmol) was added to a solution of cefoxitin (10.0 g, 23.4 mmol) in methanol (100 mL) and stirred for 24 hours at room temperature. HPLC showed about 15% of 1 converted to A. The above-obtained mixture was further purified by preparative HPLC and obtained A 800 mg (8% yields) as a white solid. 1H NMR (600 MHz, CDCl3): δ 7.29 (dd, 1H, J1 = 1.2 Hz, J2 = 5.4 Hz), 7.01 (m, 1H), 6.97 (dd, 1H, J1 = 3.6 Hz, J2 = 5.4 Hz), 6.25 (s, 1H), 5.50 (s, 1H), 4.80 (s, 1H), 4.77 (d, J = 12.6 Hz), 4.62 (d, J = 12.6 Hz), 3.87 (q, 2H, J = 15.6 Hz), 3.50 (s, 3H). 13C NMR (150 MHz, CDCl3): δ 174.7, 174.0, 162.6, 160.0, 137.5, 128.5, 128.3, 126.5, 124.3, 120.5, 97.5, 67.9, 61.5, 54.9, 54.3, 38.0. HRMS m/z: C17H19N3O8S2 (M+Na) + calcd for 450.0400, found 450.0404.

General procedure for the preparation of impurities B and C

Methylene chloride (150 mL), methanol (15 mL), compound 4 (30 g, 75.7 mmol) were added in a 500-mL three-necked flask. The reaction mixture was cooled down to -70±5^{o}C on a liquid nitrogen bath, and then 5.4 mol/L sodium methoxide solution (84.1 mL, 454.1 mmol) was added into the solution at -70±5^{o}C. Next, tert-butyl hypochlorite (20.5 g, 188.8 mmol) was slowly added dropwise to control the temperature at -70±5^{o}C. After the reaction is completed, the reaction mixture was added sodium metabisulfite (28.7 g, 107.8 mmol) and stirred for 10 min at -70^{o}C. The pH value of the solution was adjusted to 6-7 at -60^{o}C with acetic acid and then further adjusted to pH = 1-2 with 10% hydrochloric acid at 0 - 5^{o}C. The aqueous layer was separated and was extracted again with dichloromethane (100 mL). The combined organic phase is washed once with water (100 mL) and the organic phase was added with saturated sodium bicarbonate solution to adjust aqueous phase pH = 7-8. The organic phase is discarded. The obtained aqueous phase was adjusted carefully to pH = 2.0 with 10% hydrochloric acid and exacted with ethyl acetate (200 mL). The organic phase was concentrated to generate light brown oil (24.6 g), which is not purified for the next step.

Compound 3 1H NMR (600 MHz, CDCl3): δ 7.63 (d, J = 21.6 Hz, 1H), 7.27-7.25 (m,1H), 7.11 (dd, J1 = 3 Hz, J2 = 17.4 Hz, 1H), 6.95-6.92 (m, 1H), 5.06-5.01 (m, 3H), 4.95 (d, J = 7.8 Hz, 1H), 4.88 (d, J = 14.4 Hz, 1H), 3.52-3.35 (m,

7H), 3.29-3.19 (m, 1H), 2.02 (d, 3H, J = 4.2 Hz, 3H); 13C NMR (150 MHz, CDCl3): δ 170.0, 169.8, 162.1, 159.7, 130.1, 129.2, 126.8, 126.3, 126.0, 125.9, 125.5, 125.4, 124.6, 124.5, 93.9, 78.7, 78.4, 63.4, 63.3, 61.8, 56.7, 56.4, 52.9, 52.7, 25.8, 19.7. MS (ESI) m/z: 474.14 [M+H2O].

To a mixture of water (50 mL) and methanol (100 mL), compound 3 crude (24.0 g) was cooled to -30°C. To the reaction mixture, sodium hydroxide solution (6.3 g sodium hydroxide dissolved in 40 mL water) was added at -30°C and the solution was stirred for 3 hours. After completion of the hydrolysis reaction, pH was adjusted to 6-7 using acetic acid at -30°C. The reaction temperature was raised to 30°C. The solvent was distilled off under reduced pressure, followed by a solvent switch to tetrahydrofuran. Anhydrous magnesium sulfate is used to dry the tetrahydrofuran solution of compound 2. The solvent is concentrated under reduced pressure to obtain a solid and the obtained solid (30.0 g) is directly transferred to the next step without isolation and purification.

Crude compound 2 (30.0 g) in tetrahydrofuran was cooled to -40°C under liquid nitrogen, followed slow added solution of chloro sulphonyl isocynate (22.3 g) in tetrahydrofuran at -40°C. The reaction was monitored by HPLC. When the reaction was completed, the reaction mixture was added into ice water and stirred for 3 h. Tetrahydrofuran was remove under reduced pressure. The product was extracted with ethyl acetate and discarded the aqueous phase. The organic layer was washed with saturated sodium chloride solution. The organic phase treated with sodium bicarbonate solution to adjust aqueous phase pH = 7 - 8. The obtained aqueous phase was adjusted carefully to pH = 2.0 with 10% hydrochloric acid and exacted in ethyl acetate. Ethyl acetate is distilled off under reduced pressure and a crude mixture of impurities B and C (11.1 g yield 32%) was obtained. The mixture B and C (2 g) are further separated by semi-preparative liquid chromatography to obtain B (900 mg) and C (750 mg), respectively.

(6R,7S)-3-((carbamoyloxy)methyl)-7-methoxy-7-((R)-2-methoxy-2-(thiophen-2-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (B)

1H NMR (600 MHz, CD3OD): δ 1H NMR (600 MHz, CD3OD): δ 7.45 (dd, 1H, J1 = 1.2 Hz, J2 = 5.4 Hz), 7.24 (m, 1H), 7.03 (dd , J1 = 3.6 Hz, J2 = 4.8 Hz, 1H), 5.13 (s, 1H), 5.08 (s,1H), 4.90 (d, J = 12.6 Hz, 1H), 4.78 (d, J = 12.6 Hz, 1H), 3.56 (d, 1H, J = 18.0 Hz), 3.25 (d, 1H, J = 17.4 Hz), 3.47 (s, 3H), 3.41 (s, 3H). 13C NMR (150 MHz, CD3OD): δ 172.1, 166.7, 159.7, 158.3, 139.0, 131.5, 127.2, 126.3, 126.2, 116.2, 95.1, 78.6, 64.2, 63.6, 63.5, 56.3, 52.2, 25.3.HRMS m/z: C17H19N3NaO8S2 (M+Na)+ calcd for 480.0506, found 480.0511.

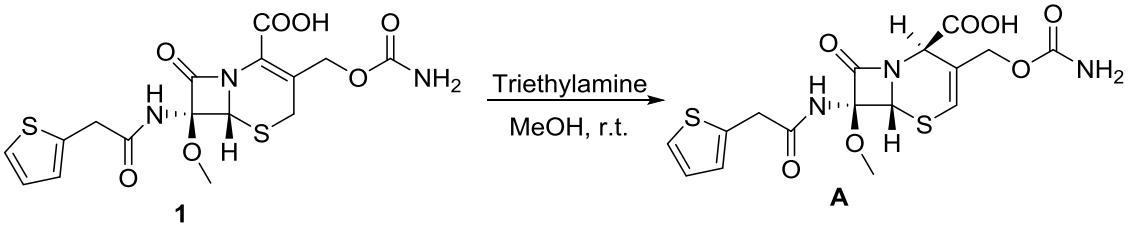
(6R,7S)-3-((carbamoyloxy)methyl)-7-methoxy-7-((S)-2-methoxy-2-(thiophen-2-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (C)

The preparation method is the same as impurity B. 1H NMR (600 MHz, CD3OD): δ 7.45 (dd, 1H, J1 = 1.2 Hz, J2 = 5.4 Hz), 7.24 (m, 1H), 7.03 (dd , J1 = 3.6 Hz, J2 = 4.8 Hz, 1H), 5.09 (s, 1H), 5.08 (s, 1H), 4.90 (d, J = 12.6 Hz, 1H), 4.78 (d, J = 12.6 Hz, 1H), 3.55 (d, 1H, J = 17.4 Hz), 3.24 (d, 1H, J = 17.4 Hz), 3.51 (s, 3H), 3.48 (s, 3H). 13C NMR (150 MHz, CD3OD): δ 172.1, 166.3, 159.8, 158.2, 139.1, 130.8, 127.0, 126.3, 126.2, 119.4, 95.3, 79.4, 63.7, 63.4, 56.5, 52.4, 25.4. HRMS m/z: C17H19N3NaO8S2 (M+Na) +calcd for 480.0506, found 480.0510.

RESULTS AND DISCUSSION

Preparation and characterization of impurity A

According to the previous literature reported, the rearrangement of the Δ3 to Δ2-double bond in the cephem substrate would require a base [8]. The conversion of compound 1 to Δ2-isomers A was in the presence of triethylamine in methanol at room temperature (Scheme 1). About 15% of Compound 1 was converted to A for 24 hours (Figure 2). In addition, prolonging reaction time could not significant increase in conversion of compound A. Impurity A and Compound 1 have similar polarities and are difficult to separate by column chromatography. The above-obtained mixture was further purified by preparative HPLC. The exact structure of A was isolated, identified, and characterized using HPLC, HRMS, 1H NMR, 13C NMR, 2D NMR (HSQC, HMBC).



Scheme 1: Simple synthetic route of impurity A

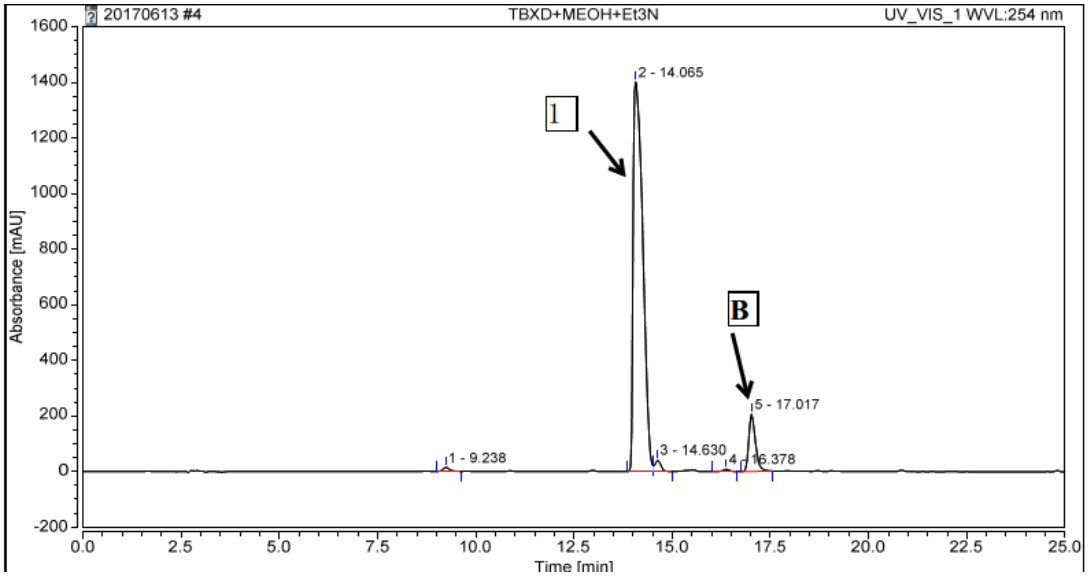
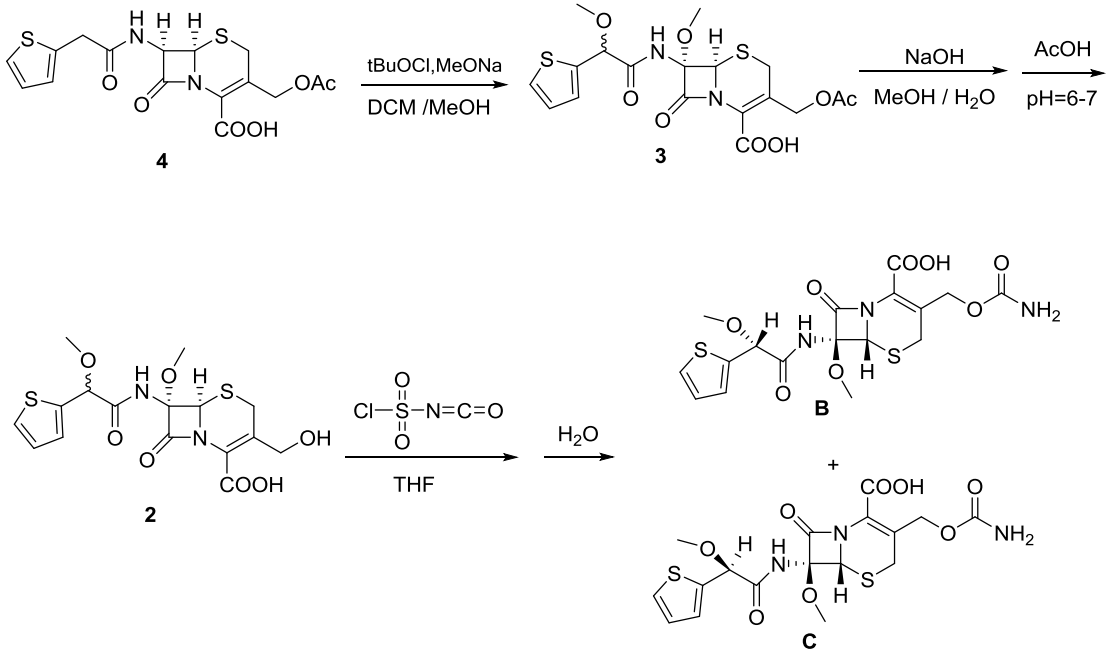


Figure 2: The typical HPLC spectral of cefoxitin and impurity A

Preparation and characterization of impurity B and C

Inspiration for the synthesis of cefoxitin [9], impurity B and C were prepared in three steps starting from cephalothin (4). Compound 4 was methoxylated with 2.5 equivalents of tert-butyl hypochlorite and 6 equivalents of sodium methoxide to give (6R,7S)-3-(acetoxymethyl)-7-methoxy-7-(2-methoxy-2-(thiophen-2-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (3) at -75 oC. Intermediate 3 was directly used for the next hydrolysis without purification to afford (6R,7S)-3-(hydroxymethyl)-7-methoxy-7-(2-methoxy-2-(thiophen-2-yl) acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2). Since the intermediate 2 is extremely unstable under acidic conditions, which can transform to the ring-forming lactone forms. After the complete hydrolysis, adjust the pH = 6 - 7 by acetic acid to control the formation of lactone. Removing methanol and water under reduced pressure, followed by a solvent switch to tetrahydrofuran. and then chlorosulfonyl isocyanate underwent a nucleophilic addition with tetrahydrofuran solution of intermediate 2 to yield the corresponding N-chlorosulfonyl carbamate, further hydrolysis of gave the mixture of impurities **B** and **C** (Scheme 2). The crude **B** and **C** is obtained by preparative separation in a purity of 95%. The preparation of impurities B and C had not been reported in the literature.



Scheme 2: Synthetic route of impurities B and C

The high resolution mass spectrum of B and C showed the same molecular ion peak at m/z 480.0510 (M+Na)+ in positive ion mode, indicating the mass of the two compounds to be 457.05. The identification impurities B and C were subjected to spectroscopic analysis such as 1D NMR (1H NMR, 13C NMR and DEPT, 2D NMR (HSQC and HMBC, Table 1and Table 2).

Table 1: NMR data of impurities B

Position	1H	J(Hz)	COSY	13C	DEPT	HMBC	HSQC
1	7.45	dd , J1 = 1.2 Hz, J2 = 5.4 Hz, 1H	2H	126.2	CH	C2, C3, C4	126.2
2	7.03	dd ,J1 = 3.6 Hz, J2 = 4.8 Hz, 1H	1H,3H	126.3	CH	C1, C3, C4	126.3
3	7.24	m, 1H	2H	127.2	CH	C1, C2, C4, C5	127.2
4		-		139.0		-	
5	5.13	s , 1H		78.6	CH	C1, C2, C3, C4, C6, C15	78.6
6		-		172.1		-	
7		-		95.1		-	
8		-		159.7		-	
9		-		131.5		-	
10		-		116.2		-	
11	3.56	d,J = 18.0 Hz,1H		25.3	CH2	C9, C10, C12, C13	25.3
11	3.25	d, J = 17.4 Hz, 1H		25.3	CH2	C9, C10, C12, C13	25.3
12	5.08	s , 1H		63.5	CH	C7, C8, C11	63.5
13	4.90	d, J = 12.6 Hz,1H		63.6	CH2	C9, C10, C11, C14	63.6
13	4.78	d, J = 12.6 Hz,1H		63.6	CH2	C9, C10, C11, C14	63.6
14		-		158.3		-	
15	3.47	s , 3H,		56.3	CH3	C5	56.3
16	3.41	s , 3H,		52.2	CH3	C7	52.2
17		-		166.7		-	

Table 2: NMR data of impurities C

position	1H	J(Hz)	COSY	13C	DEPT	HMBC	HSQC
1	7.45	dd , J1 = 1.2 Hz, J2 = 5.4 Hz, 1H	2H	126.2	CH	C2, C3, C4	126.2
2	7.03	dd , J1 = 3.6 Hz, J2 = 4.8 Hz, 1H	1H,3H	126.3	CH	C1, C3, C4	126.3
3	7.24	m, 1H	2H	127.1	CH	C1, C2, C4, C5	127.1
4		-	-	139.1		-	
5	5.09	s , 1H	-	79.4	CH	C1, C2, C3, C4, C6, C15	79.4
6		-	-	172.1		-	
7		-		95.1		-	
8		-	-	159.7		-	
9		-	-	131.5		-	
10		-	-	116.2		-	
11	3.55	d, J = 17.4 Hz, 1H	-	25.4	CH2	C9, C10, C12, C13	25.4
11	3.24	d, J = 17.4 Hz, 1H	-	25.4	CH2	C9, C10, C12, C13	25.4

12	5.08	s ,1H	-	63.4	CH	C7, C8, C11	63.4
13	4.90	d, J = 12.6 Hz,1H	-	63.7	CH2	C9, C10, C11, C14	63.7
13	4.78	d, J = 12.6 Hz,1H	-	63.7	CH2	C9, C10, C11, C14	63.7
14		-	-	158.3		-	
15	3.48	s ,3H,		56.5	CH3	C5	56.5
16	3.51	s ,3H,	-	52.4	CH3	C7	52.4
17		-	-	166.3		-	-

CONCLUSION

The preparation and isolation for three impurities of cefoxitin are firstly described. In addition, these impurities are characterized by NMR and HRMS spectral. This work will be useful for quality control in the manufacture of cefoxitin.

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NOTES

The authors declare no competing financial interest

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