

## 分析质控与制剂

## 头孢唑肟钠有关物质的研究

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**摘要:** **目的** 采用液相色谱法和液相色谱-质谱联用法对不同批次的头孢唑肟钠粉针中的杂质进行全面深入研究。方法 对头孢唑肟钠进行加速稳定性试验, 采用液相色谱、液质联用仪检测头孢唑肟钠中的杂质类型, 采用制备型液相色谱制备有关杂质, 经波普分析确定各杂质的结构。**结果** 共检出12种杂质, 其中2种为新检出的杂质, 且在制剂中含量较高, 本次报道的杂质为B (6*R*,7*R*)-7-((*Z*)-2-(2-氨基噻唑-4-基)-2-(甲氧基亚氨基)乙酰胺-8-氧代-5-噻-1-氮杂双环[4,2,0]辛-3-烯-2-羧酸, C 2-(*R*)-((*Z*)-2-(2-氨基噻唑-4-基)-2-(甲氧基亚氨基)乙酰胺(羧基)甲基)-3,6-二氢-2*H*-1,3-噻嗪-4-羧酸, D 2-((*R*)-(*Z*)-2-(2-氨基噻唑基)-2-(甲氧基亚氨基)乙酰胺(羧基)甲基)-3,6-二氢-2*H*-1,3-噻嗪-4-羧酸, F (*Z*)-2-((2-(2-氨基噻唑-4-基)-2-(甲氧基亚氨基)乙酰胺)甲基)-3,6-二氢-2*H*-1,3-噻嗪-4-羧酸, G 2-((*R*)-((*E*)-2-(2-氨基噻唑-4-基)-2-(甲氧基亚氨基)乙酰胺基(羧基)甲基)-3,6-二氢-2*H*-1,3-噻嗪-4-羧酸, H 2-((*R*)-(*E*)-2-(2-氨基噻唑-4-基)-2-(甲氧基亚氨基)乙酰胺基(羧基)甲基)-3,6-二氢-2*H*-1,3-噻嗪-4-羧酸, I (6*R*,7*R*)-7-((*E*)-2-(2-氨基噻唑-4-基)-2-(甲氧基亚氨基)乙酰胺基)-8-氧代-5-噻-1-氮杂双环[4,2,0]辛-3-烯-2-羧酸, J (6*R*)-7-((*Z*)-2-(2-氨基噻唑-4-基)-2-(甲氧基亚氨基)乙酰胺)-8-氧代-5-噻-1-氮杂双环[4,2,0]辛-2-烯-2-羧酸。**结论** 本文报道的分析方法能较好的检测分析头孢唑肟钠中的各种可能的常见杂质, 本实验室开发的分离纯化方法能够分离得到纯度合乎结构确证要求的杂质, 用NMR、HRMS、NOE和CD等分析方法能准确推测这些杂质的结构。

**关键词:** 头孢唑肟钠; 杂质分析检测; 结构分析

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## Study on related substances in ceftizoxime sodium

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**Abstract Objective** To study the impurities in different batches of ceftizoxime sodium by HPLC and LC-MS. **Methods** The accelerated stability test of ceftizoxime sodium was carried out. The impurities in ceftizoxime sodium were detected by HPLC and LC-MS. The related impurities were prepared by preparative HPLC. The structure of each impurity was determined by pop analysis. **Results** A total of 12 impurities were detected, of which 2 were newly reported for the first time, and the content was high in the preparation. The impurities reported in this report are B (6*R*,7*R*)-7-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-3-ene-2-carboxylic acid, C 2-(*R*)-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid, D 2-((*R*)-((*Z*)-2-(2-aminothiazoyl)-2-(methoxyimino)acetamido)(carboxyl)methyl)-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid, F (*Z*)-2-((2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)

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methyl)-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid, G 2-((*R*)-((*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid, H 2-((*R*)-((*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid, I (6*R*,7*R*)-7-((*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-3-ene-2-carboxylic acid, and J (6*R*)-7-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid. **Conclusion** The analytical method reported in this paper can detect and analyze all kinds of possible common impurities in ceftizoxime sodium. The separation and purification method developed by our laboratory can separate the impurities with purity meeting the requirements of structure confirmation. The structures of these impurities can be accurately characterized by NMR, HRMS, NOE, CD and other analytical methods.

**Key words** Ceftizoxime sodium; Impurity analysis and detection; Structural analysis

Ceftizoxime sodium is a third-generation semi-synthetic cephalosporin developed by Fujisawa Pharmaceutical Co., Ltd., which can inhibit the biosynthesis of peptidoglycan of the bacterial cell wall<sup>[1]</sup>. Ceftizoxime sodium is stable to a broad spectrum of  $\beta$ -lactamases including penicillinase and cephalosporinase, which has good bactericidal activity against many Gram-positive and Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, etc.. Clinically, it is mainly used to treat acute or moderate respiratory infections, sepsis, pneumonia, etc., with the advantages of high curative effect and few adverse reactions<sup>[2-4]</sup>. However, cephalosporins tend to produce impurities during storage. Moreover, some process impurities and intermediates also tend to remain as impurities in the drug substances and drug products. In view of these problems, a series of studies on impurities of ceftizoxime were carried out, which greatly promoted the quality control of products<sup>[5-7]</sup>. Yao Lei<sup>[8]</sup> reported the test and verification of high molecular weight impurities in ceftizoxime sodium. An HPLC method for the determination of related substances in ceftizoxime sodium was developed by Yang Qian<sup>[9]</sup>. The type and structure of impurities produced by the forced degradation of ceftizoxime sodium were also reported<sup>[10-11]</sup>. However, some high content of ceftizoxime injection powder impurities were not included in the literature and reports, and the fine structure of some impurities is insufficient. To further improve the research of the related substances in the raw material and the preparations of ceftizoxime sodium, impurities of ceftizoxime sodium powder injection from

Bai Yun Shan Pharmaceutical Factory with different batches have been detected by LC-MS. The detected ceftizoxime impurities were prepared and compared by forced degradation and purification. These impurities obtained in this study are pure products, which can be used as the reference standard for impurities, quality inspection, and quality control of raw materials and ceftizoxime sodium preparations.

## 1 Materials and methods

### 1.1 Samples

Cefazoxime sodium was synthesized in Wuhan Zeshancheng biotechnology Co Ltd., (China), impurities B, C, D, F, G, H, I, J were prepared in the laboratory and identification by MS and NMR. hydrogen peroxide, trifluoroacetic acid, citric acid, sodium hydrogen phosphate, sodium hydroxide and hydrochloric acid were purchased from Tianjin FuChen Chemical Reagent Factory (Analytically pure). DMSO, methanol and acetonitrile were purchased from Merck Co (Chromatographically pure and Special Mass pure).  $C_{18}$  separation material was purchased from Jinan Bona Biotech Co., Ltd. PIPO-02 material was provided by Guangzhou PI & PI Biotech Inc.

### 1.2 High performance and liquid chromatography(HPLC)

The Thermo U3000 HPLC was used to detect the ceftizoxime sodium raw material and impurities, ThermoHypersil GOLD  $C_{18}$  was used as the columns in this experiment. The mobile phase A (buffer salt) was made up by 1.42 g citric acid and 2.31 g sodium dihydrogen phosphate diluting into the water, the mobile phase B was acetonitrile, the mobile phase for the detection was A:B=90:10, UV detection was set to

254 nm and the flow rate was kept at 1 mL/min, the temperature of column oven was set to 30°C and the data acquisition time was 40 min.

### 1.3 LC-MS/MS analysis

All mass spectrometry measurements were performed on a Thermo Scientific QE Orbitrap LC-MS. Instrument operating in positive electrospray ion mode. Analysis conditions, column: Thermo Scientific ODS Hypersil(3  $\mu$ m, 150 mm $\times$ 2.1 mm), scan type: full MS, scan range: 150.0 to 2000  $m/z$ ; resolution: 35000; polarity: positive, maximum inject time: 50 ms; sheath gas flow rate: 30 arb; aux gas flow rate: 10arb; spray voltage:3.8 kV; capillary temperature: 350°C; aux gas heater temperature: 320°C. mobile phase A was 1% formic acid water( $V/V$ ) and mobile phase B was acetonitrile. Detection was carried out at 254 nm and the flow rate was kept at 0.4 mL/min. 0.1% formic acid water: acetonitrile (50:50) was used as diluent. Data acquisition time was 25min. The gradient program was as follows: time (min)/A( $V/V$ ):B( $V/V$ );  $t_0$ /93:7,  $t_{8.0}$ /93:7,  $t_{14}$ /70:30,  $t_{22}$ /70:30,  $t_{23}$ /93:7, and  $t_{25}$ /93:7.

### 1.4 NMR analysis

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker NMR Avance III 500MHz superconducting spectrometer with TMS as an internal standard (DMSO- $d_6$  as the solvent, actual exposure frequency: 125.76 MHz, Decoupling field frequency: 500.13 MHz,  $\delta$ : 0~210ppm, analysis of the results by Mnova software)

### 1.5 ECD(Electronic circular dichroism) calculation of D(OP1) and B(OP2)

ECD calculation for the optimized conformers was carried out utilizing time-dependent DFT methods at the [B3LYP/6-311+g(2d,p)] level in the acetonitrile-water (50:50) solution by Gaussian 09 program, the calculated ECD spectra of D and B were compared with the experimental ones and the ECD curves were generated by Origin 8.0 Software.

### 1.6 preparation of the impurities

Impurities B, C, D, F, G, H, I and J were prepared in the laboratory and purified by preparative chromatography. The impurities were obtained by freeze-drying after desalination.

## 2 Results and discussions

### 2.1 Impurity detection

Impurities of ceftizoxime sodium for injection were detected by LC-MS, the results were given in Tab.1 and Fig.1. The mobile phase consisted of (A) 0.1% formic acid, and (B) acetonitrile. In the detection and analysis process, twelve ceftizoxime sodium impurities were obtained, and impurities B, H, C, I, D, F were first reported by our team.

### 2.2 Structural elucidation

The carbon and hydrogen spectrum data of various impurities are listed in Tab.2 and Tab.3.

#### 2.2.1 Impurity B and H

Impurity B (RT3.49) and impurity H(RT8.34) exhibited a molecular ion peak at  $m/z$ : 402.05  $[M+H]^+$  in positive ion mode in HRMS, its exact molecular weight is 401.05, an increase of 18 amu compared with ceftizoxime, comparison  $^1H$  NMR and  $^{13}C$  NMR spectra of impurity B and H with ceftizoxime, it is easy to see that B and H are ring opening isomeric impurities<sup>[12-14]</sup>. B was named as 2-((*R*)-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid. Impurity H (RT 8.34) can be obtained by exposing impurity B to 254nm UV lamp, impurity H was named as 2-((*R*)-((*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-3,6-dihydro-2-H-1,3-thiazine-4-carboxylic acid.

#### 2.2.2 Impurity C and I

Impurity C (RT 3.97) and impurity I(RT 10.62) exhibited a molecular ion peak at  $m/z$ : 384.04  $[M+H]^+$  in positive ion mode in HRMS, which is equal to the mass of ceftizoxime, the exact molecular weight of them are 383.03, which indicating that impurity C, I and ceftizoxime are isomers. Comparison the  $^1H$  NMR and  $^{13}C$  NMR spectra data of ceftizoxime sodium with impurity C and I the chemical shifts of C-3 / C-4 in impurity C, I and its neighboring C-2 / C-6 / C-7 are significantly different from those of ceftizoxime, one  $CH_2$  in the  $^1H$  NMR of the impurity C, I becomes  $=CH$ , the carbon-carbon double bond of the six-membered ring in ceftizoxime molecule was transferred from C-2/C-3 to C-3/C-4, the impurity C was characterized as (6*R*,7*R*)-

**Tab. 1** Chemical structure of ceftizoxime sodium and their impurities

**表1** 头孢唑肟钠及其杂质列表

Impurity(RT)	Structure	Chemical name
A(1.54)		(6 <i>R</i> ,7 <i>R</i> )-7-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid 5-oxide
B(3.49)		2-(( <i>R</i> )-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-3,6-dihydro-2 <i>H</i> -1,3-thiazine-4-carboxylic acid
C(3.97)		(6 <i>R</i> ,7 <i>R</i> )-7-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-3-ene-2-carboxylic acid
D(4.6)		2-(( <i>R</i> )-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxyl)methyl)-3,6-dihydro-2 <i>H</i> -1,3-thiazine-4-carboxylic acid
E (API 5.17)		(6 <i>R</i> ,7 <i>R</i> )-7-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid
F (5.85)		( <i>Z</i> )-2-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)methyl)-3,6-dihydro-2 <i>H</i> -1,3-thiazine-4-carboxylic acid
G (6.33)		2-(( <i>R</i> )-(( <i>E</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-3,6-dihydro-2 <i>H</i> -1,3-thiazine-4-carboxylic acid
H (8.34)		2-(( <i>R</i> )-(( <i>E</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-3,6-dihydro-2 <i>H</i> -1,3-thiazine-4-carboxylic acid
I (10.62)		(6 <i>R</i> ,7 <i>R</i> )-7-(( <i>E</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-3-ene-2-carboxylic acid

续表1

J (10.62)		(6 <i>R</i> )-7-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid
K (11.78)		(6 <i>R</i> ,7 <i>R</i> )-7-(( <i>E</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid
L (15.39)		(6 <i>R</i> ,7 <i>R</i> )-7-((6 <i>R</i> ,7 <i>R</i> )-7-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxyamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid
M (17.85)		(6 <i>R</i> ,7 <i>R</i> )-7-(( <i>Z</i> )-2-(( <i>Z</i> )-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)thiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid

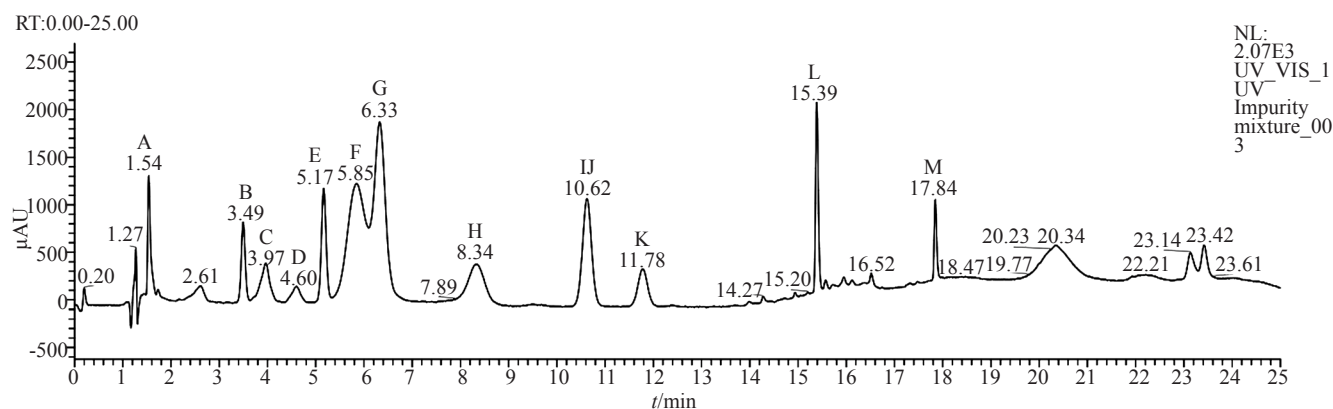


Fig. 1 Localization of each impurity of ceftizoxime sodium HPLC-UV

图1 头孢唑肟钠中杂质的液相色谱定位

7-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid, impurity I was characterized as (6*R*,7*S*)-7-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

### 2.2.3 Impurity D and G

Impurity D (RT 4.6) and impurity G (RT 8.34) exhibited a molecular ion peak at  $m/z$ : 402.05  $[M+H]^+$  in positive ion mode in HRMS, the exact molecular weight of D, G was 401.05, an increase of 18 amu compared with ceftizoxime, this is equivalent to adding a molecule

of water. Comparison  $^1H$  NMR and  $^{13}C$  NMR spectra of impurity D, G with ceftizoxime, the value of C-8 chemical shift of impurity D and G increased obviously, it may be related to the hydroxyl added to the carbonyl group (C-8) after the cleavage of the amide bond (C-8/N-1), in addition,  $^1H$  NMR spectrum show one hydrogen atom was added on N-1, in addition the chemical shift values of H-6 / H-7 / H-9 were significantly decreased, this also indicates that the  $\beta$ -lactam ring in the ceftizoxazole molecule has been opened. Therefore D was characterized as 2-(*R*)-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)(carboxy)methyl)-

Tab. 2 <sup>13</sup>C NMR chemical shift of the impurities  
表2 杂质的碳谱数据

Position	B	C	D	F	G	H	I	J	TBZW
2	133.2	62.39	133	133.63	133.4	133.53	128.83	63.25	135.61
3	103.7	114.36	109.44	110.54	115.43	115.69	118.37	119.67	112.92
4	23.9	120.49	24.27	24.43	24.5	24.56	25.07	138.82	24.27
6	57.8	53.64	56.69	62.4	54.95	56.93	62.16	57.32	58.7
7	54.3	49.74	54.73	42.23	63.31	63.25	55.5	53.64	57.71
8	171	169.64	170.06		171.03	170.64	162.64	169.56	164.89
10	165.9	163.42	165.32	166.03	165.98	165.96	162.48	164.27	163.7
11	149.6	149.45	147	150.14	146.43	146.88	148.76	163.25	149.56
12	143.4	142.93	139	143.28	138.73	138.9	142.31	146.75	143.73
13	110.9	109.47	104.24	103	104.04	103.75	109.15	116.05	109.47
14	163.2	168.84	168	168.75	166.96	167	168.45	166.93	168.89
16	62.4	60.75	62.01	56.38	58.38	56.93	63.44	59.67	62.34
17	168.7	163.66	162.21	163.2	163.36	163.54	160.21	164.16	163.12

Tab. 3 <sup>1</sup>H NMR chemical shift of the impurities  
表3 杂质的氢谱数据

Position	B	C	D	F	G	H	I	J	TBZW
1			5.39~5.40 (1H, d)	3.32~3.40 (1H, b)	5.54~5.56 (1H, d)	5.61~5.62 (1H, d)			
2		4.93~4.94 (1H, dd)						4.78~4.82 (1H, d)	
3	5.81~5.83 (1H, t)	5.89~5.92 (1H, dd)	5.85~5.87 (1H, t)	5.75~5.76 (1H, t)	5.81~5.83 (1H, t)	5.80~5.82 (d, 1H)	6.41~6.43 (1H, dd)	5.90~5.93 (1H, dd)	6.04~6.06 (1H, dd)
4		6.55~6.58 (1H, dd)	3.41~3.51 (2H, m)	3.46~3.47 (2H, d)	3.36~3.37 (1H, d)	3.42~3.43 (2H, d)	3.52~3.72 (2H ,qq)	6.49~6.51 (1H, d)	3.34~3.53 (2H, qq)
6	4.60~4.64 (1H, t)	5.12~5.13 (1H, d)	4.62~4.64 (1H, t)	5.51~5.53 (1H, d)	4.62~4.65 (1H, t)	4.59~4.62 (1H, t)	4.74~4.75 (1H, d)	5.12 (1H, d)	4.97~4.98 (1H, d)
7	4.71~4.74 (1H, t)	5.56~5.59 (1H, q)	4.68~4.71 (1H, t)	4.49~4.50 (1H, d)	4.75~4.79 (1H, t)	4.64~4.66 (1H, t)	4.79~4.81 (1H, q)	5.53~5.56 (1H, dd)	5.63~5.66 (1H, q)
8				8.74~8.77 (1H, t)					
9	9.16~9.18 (1H, d)	9.6~9.68 (1H, d)	9.23~9.40 (1H, s)		8.82~8.84 (1H, d)	8.84~8.86 (d, 1H)	9.69~9.71 (1H, d)	9.39~9.41 (1H, d)	9.52~9.53 (1H, d)
12				6.87(1H, s)					
13	6.82(1H, s)	6.78(1H, s)	6.84(1H, s)		7.34(1H, s)	7.371(1H, s)	6.78(1H, s)	7.44(1H, s)	6.73(1H, s)
14				7.16 (2H, s)					
15	7.22 (2H,b)	7.24 (2H, s)	7.23 (2H, s)	3.83 (3H, s)	7.0(1H, s)	6.989 (2H,s)	7.23 (2H, s)	7.08 (2H, s)	7.24(2H, s)
16	3.81 (3H, s)	3.85 (3H, s)	3.84 (3H, s)	12.79 (1H, b)	3.97 (3H, s)	3.97 (3H, s)	3.85(3H, s)	3.06 (3H, s)	3.84(3H, s)
17	12.96 (2H, b)		13.04 (2H, b)			12.88 (1H, b)	13.26 (1H, s)		

3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.Impurity G can be obtained by exposing impurity D to 254 nm UV lamp. G was characterized as 2-((*R*)-((*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)cetamido)(carboxy)methyl)-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid<sup>[13-14]</sup>.

2.2.4 Impurity F

Impurity F(RT 5.85) exhibited a molecular ion peak at *m/z*: 358.05 [M+H]<sup>+</sup> in positive ion mode in HRMS, its molecular weight was 357.06, which was 26 amu less than that of ceftizoxime. Comparison <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of F with ceftizoxime, the impurity



F reduced a carbonyl group and added two hydrogen atoms, indicating that the carbonyl group on the  $\beta$ -lactam ring was lost and N-1 / C-7 in the molecule were both added one hydrogen atom, impurity F was characterized as (Z)-2-((2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)methyl)-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.

### 2.2.5 Impurity J

Impurity J (RT 10.62) exhibited a molecular ion peak at  $m/z$ : 384.04  $[M+H]^+$  in positive ion mode in HRMS, its exact molecular weight is 383.03, which is the same as ceftizoxime, revealing that they are isomers. Comparison the chemical shifts of  $^1H$  NMR and  $^{13}C$  NMR of the impurity J with ceftizoxime, only the chemical shift value of H-7 in the impurity J decreased more significantly from 5.63 to 4.79, the H-6 / H-7 coupling constant of impurity J became smaller (H-6: from 5.0Hz to 2.5Hz, H-7: from 5.0Hz to 2.0Hz), showing that the correlation between H-6 / H-7 in J was reduced, there was no significant correlation between H-6 / H-7 in the compound molecule (Fig.2), which demonstrated that the structure of impurity J is (6*R*,7*S*)-7-((Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

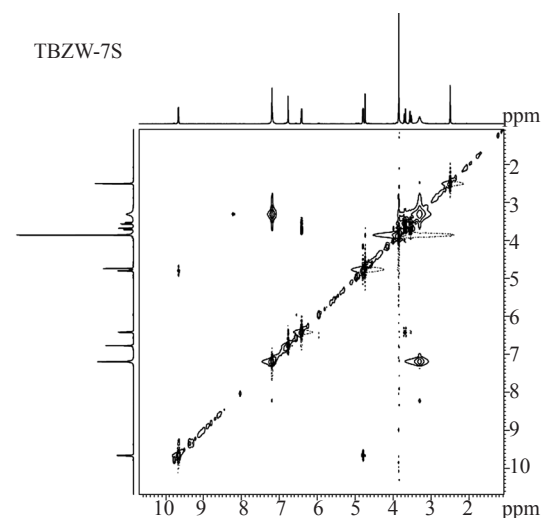


Fig. 2 The NOE spectrum of impurity J

图2 杂质J的NOE谱

### 2.2.6 Determination of C-6 / C-7 configuration in ring opening impurities

Ring opening impurities D(OP1) and B(OP2) may have two enantiomers, 6*R*7*R*, 6*S*7*S* and 6*R*7*S*, 6*S*7*R*.

the ECD calculation for the optimized conformers of 6*R*7*R*, 6*S*7*S* and 6*R*7*S*, 6*S*7*R* were carried out by means of time-dependent density functional theory (TDDFT) methods at the [B3LYP/6-311+g(2d,p)] level in the acetonitrile-water (50:50) solution by using Gaussian 09 software. All the ECD curves of D and B were weighted by a Boltzmann distribution of each conformer. The calculated ECD spectra of D and B were subsequently compared with the experiment ones<sup>[15]</sup>, the simulated curves of 7*R*6*R* and 7*S*6*S* are more similar to the ECD spectral curves of D and B than that of 7*S*6*R* and 7*R*6*S*. the ring open impurity D corresponds to the 7*R*6*R* configuration, ring open Impurity B corresponds to 7*S*6*S* configuration (Fig. 3~4).

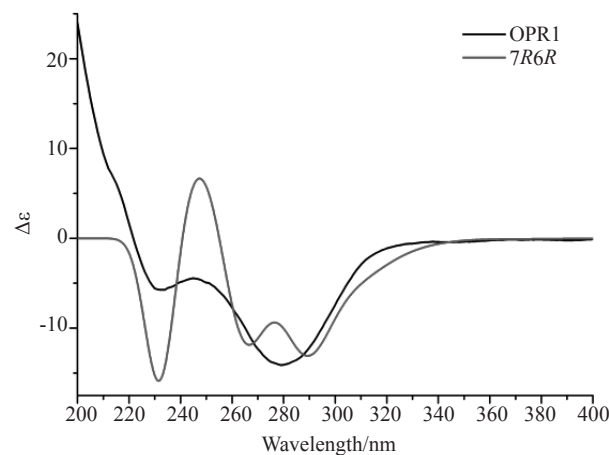


Fig. 3 CD spectrum (black) and simulated ECD spectrum (red) of impurity D

图3 杂质D的CD谱(黑色)和模拟ECD谱(红色)

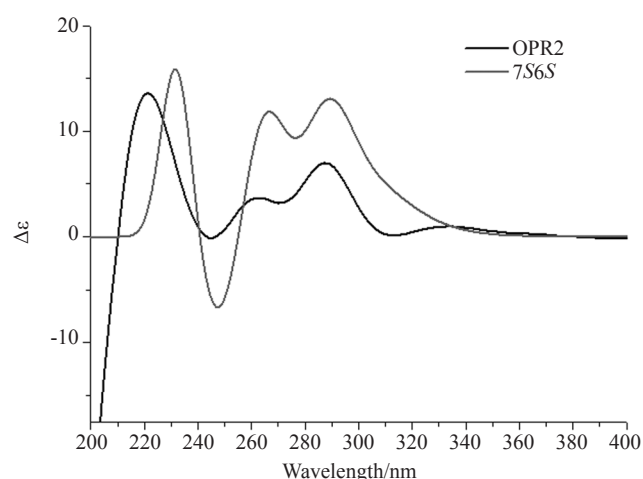


Fig. 4 CD spectrum (black) and simulated ECD spectrum (red) of impurity B

图4 杂质B的CD谱(黑色)和模拟ECD谱(红色)

Based on the NMR data of the related substances, each carbon atom and hydrogen atom in the impurity molecule were assigned. The Gauss model may simulate the stable molecular configuration of ceftizoxime and several ring open impurities, and measure the relative atomic distance and dihedral angle values. It shows that the 7R6R, 7S6S is relatively high stability configuration(adjacent atoms have the largest coupling constant when the dihedral angle is 0 or 180), Maxwell-Boltzman correlation coefficient distribution curve calculated NMR data of each impurity is obtained by Gauss program. The results also support that the ring open structure is the tendency of 7R6R and 7S6S greater than 7S6R and 7R6S<sup>[16-17]</sup>. The circular dichroism of ceftizoxime, D, and B were also measured and performed circular dichroism modeling of different configurations with Gaussian calculations(Tab.4)<sup>[18]</sup>. The simulated curves of 7R6R and 7S6S are more similar to the CD spectral curves of D and B then that of 7S6R and 7R6S(Fig. 5). NMR data of impurity D and B were assigned too<sup>[19]</sup>.

### 3 Conclusions

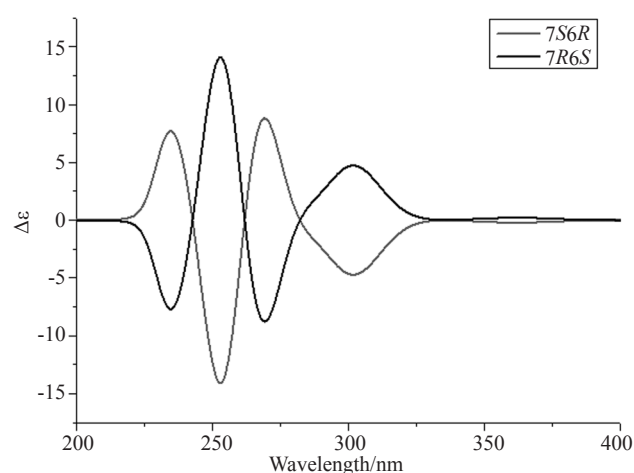
The impurities in the preparation of ceftizoxime sodium injection were tested, separated, and characterized in this study. In particular, six of these impurities were first reported, which included ring open impurities with high content in the preparations and double bond transfer impurities. In this paper, the exact molecular weight and chemical structure of these

**Tab. 4** The calculation data of the analog configuration of the ring open impurity

**表4** 开环杂质构型模拟计算数据

Configuration	7R6R	7S6S	7R6S	7S6R	TBZW
hydrogen atom position number	40,41	39,40	26,40	17,41	35,36
atomic distance (6-H/7-H)(Å)	3.07636	3.07638	2.41158	2.48042	2.43612
atomic distance (H-7/H-9)(Å)	2.96076	2.6078	2.92709	2.93322	2.96038
atomic distance (H-1/H-6)(Å)	2.35697	2.35693	2.22559	2.8211	-
two sides angle (H-6-C-6-C-7-H-7)	173.75	173.76	63.25	67.1	6.28
Maxwell-Boltzman correlation coefficient	0.9972	0.9948	0.9933	0.9922	-

"-": No calculation data



**Fig. 5** Simulated ECD spectra of ring open impurities 7S6R and 7R6S

**图5** 开环杂质7S6R and 7R6S的模拟ECD谱

impurities were confirmed by LC-MS, NMR, HRMS. The exact configurations of impurities D and B were deduced by ECD calculation.

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