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Characterization of Impurities in Cefdinir Bulk Material by Online Column-Switching Liquid Chromatography and Tandem Mass Spectrometry

Li Jin, Wang Li-Xin, Yao Shang-Chen and Hu Chang-Qin*

National Institutes for Food and Drug Control, Beijing 100050, People's Republic of China

Abstract: Column switching LC/MS methods were developed for rapid identification of impurities in cefdinir. Based on the mechanism by which cephalosporins are degraded, stress tests were designed and performed. It was found that eight main impurities were degradation products and five impurities originated from the synthesis process. Cefdinir bulk material was eluted as gradient on a C18 column, with 0.25% tetramethyl ammonium hydroxide solution (pH5.5)-acetonitrile-methanol as mobile phase. According to the retention time of different impurities, the target impurities were separated and enriched in another chromatographic column using column switching technology. Then the target impurity was desalted using an elution of 0.5% formic acid solution. Mass analysis was performed by elution with a mobile phase consisting of 1.0% formic acid-acetonitrile (3:7). The structures of ten related impurities were characterized on the bases of MS/MS data, general mass fragmentation pathway of cefdinir, and UV spectra. This study provides the material basis for the related substances of cefdinir in the *Chinese Pharmacopeia* 2010 edition. It also provides an effective means for the rapid identification of impurities in chromatographic systems containing non-volatile salt.

Keywords: Cefdinir, cephalosporin, column switching, impurities, liquid chromatography-tandem mass spectrometry.

1. INTRODUCTION

Impurity control is one of the key factors in drug quality control. Currently the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) requires that impurities whose apparent contents are 0.1% or above in new drugs should be identified [1]. The third-generation cephalosporin cefdinir acts against Gram-positive and Gram-negative bacteria. It is used clinically for the treatment of skin infections, postoperative wound infections, and respiratory tract infections [2].

This drug is included in the *Chinese Pharmacopoeia* (ChP) 2010 Edition and the *United States Pharmacopoeia* 34th Edition (USP 34) [3, 4]. Its related substances are controlled by HPLC method. There is obvious difference in the control method of cefdinir related substances between ChP and USP. For example, 8 species of cefdinir impurities are identified by relative retention time (RRT) and controlled separately by response factors in USP 34 [4], while only the contents of single impurity and total impurities are controlled in *Chinese Pharmacopoeia* 2010 Edition, besides the difference in chromatographic conditions. Therefore, it is necessary to evaluate the separation ability of the HPLC methods described in these two pharmacopoeias and identify those impurities detected by the HPLC method in the *Chinese Pharmacopoeia*. The cefdinir impurities listed in USP34 could not be directly matched to the peaks in the chromatogram because those eight impurities were not at that time available from USP. LC-MS is a useful on-line means of characterizing the structures of impurity [5–11]. However, this chromatographic system cannot be applied to LC-MS because an ion pair reagent called tetramethyl ammonium hydroxide and a non-volatile salt called EDTA disodium are dissolved in the mobile phase.

Cefdinir has been analyzed by LC/MS by application of ammonium acetate-acetonitrile as a mobile phase system [12]. However, the impurities were not well separated and it was difficult to attribute the impurities identified under LC/MS method to the peaks detected using other chromatographic systems.

Column-switching LC/MS, a combined system consisting of column switching technology and the LC/MS method, can perform on-line sample enrichment, separation, purification and analysis [13]. It is currently used in the field of *in-vivo* drug metabolism, food composition analysis, pesticide, and antibiotic polymer analysis [14–22]. However, there is no report of this technique being used to characterize the impurities in chromatographic systems containing non-volatile salts.

This paper describes the use of column-switching LC/MS technology to identify the cefdinir impurities detected in the *Chinese Pharmacopoeia* system. With reference to the impurity structure information included in USP 34 and relevant literature, ten related impurities in cefdinir were characterized using the *Chinese Pharmacopoeia* system, including seven impurities found in USP34 [4, 23, 24]. The chemical structures of cefdinir and related impurities are given in Fig. (**1**).

*Address correspondence to this author at the National Institutes for Food and Drug Control, No.2, Tiantan Xili, Chongwen District, Beijing,100050, P.R. China; Tel: + 8610-67095308 ; Fax: +8610-65115148;
E-mail: hucq@nicpbp.org.cn

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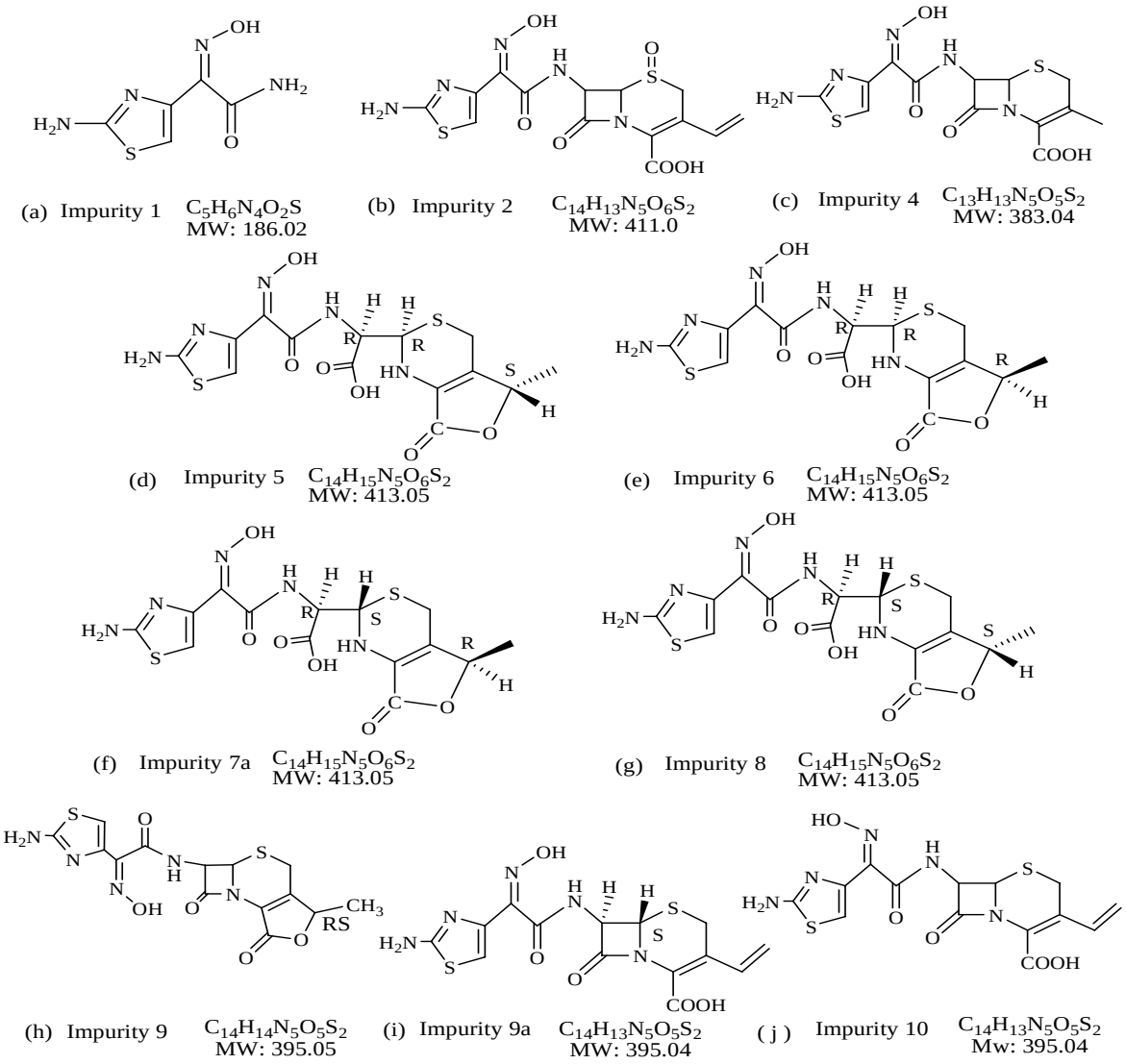

Fig. (1). Structures of ten related substances in cefdinir bulk material.

2. EXPERIMENTAL PROCEDURES

2.1. Reagents and Materials

Methanol, acetonitrile, and formic acid were of chromatographic grade, (Fisher Company, U.S.); 25% tetramethyl ammonium hydroxide aqueous solution, phosphoric acid, sodium hydroxide, potassium hydrogen phosphate, potassium dihydrogen phosphate, and EDTA disodium were analysis grade (Beijing Chemical Plant, China). The water used in mobile phase mixtures was purchased from WAHAHA Company, China. Cefdinir bulk material (Batch No. DN100601) was provided by Zhong Lian Pharmaceutical Co. Ltd. in China's Jiangsu Province.

2.2. Preparation of Sample Solutions

Phosphate buffer (pH 7.0): About 7.1 g of anhydrous dibasic sodium phosphate was dissolved in 500 ml of water (Solution A). About 6.8 g of monobasic potassium phosphate was dissolved in 500 ml of water (Solution B). Appropriate amounts of Solutions A and B (approximately 2:1 v/v) were added to obtain a mixture with a pH of 7.0.

Cefdinir stock solution (T-1): About 37.5 mg of cefdinir bulk material was transferred into a 25 ml of volumetric flask, and the sample was dissolved with 1.6 ml of phosphate buffer (pH 7.0) and diluted with mobile phase A.

2.3. Stress Tests

The stock solution (T-1) was transferred and degraded under acidic, basic, 100°C water bath, oxidation, UV, and high-temperature conditions.

Acid degradation solution (T-2): About 5 ml of cefdinir solution (T-1) was transferred into a 25 ml of volumetric flask. Then 2 ml of 0.1 M hydrogen chloride was added .This mixture was maintained for 2 h, and then the acidic solution was neutralized with 2 ml of 0.1 M sodium hydroxide.

Base degradation solution (T-3): About 5 ml of cefdinir solution (T-1) was transferred into a 25 ml of volumetric flask. Add 2 ml of 0.1 M sodium hydroxide and maintained

for 2 h. The basic solution was then neutralized with 2 ml of 0.1 M hydrogen chloride.

Oxidative degradation solution (T-4): About 5 ml of cefdinir solution (T-1) was transferred into a 25 ml volumetric flask. Then 1.0 ml of 10% hydrogen peroxide solution was added. This mixture was maintained for 2 h.

Water bath degradation solution (T-5): About 5 ml of cefdinir solution (T-1) was transferred into a 25 ml volumetric flask. This solution was maintained in a 100°C water bath for 45 min. Then it was allowed to cool to room temperature.

UV degradation solution (T-6): About 100 mg of cefdinir bulk material was placed under the UV light (254 nm) for 12 hours. Then 15 mg of the UV-degraded sample was transferred into a 10 ml of volumetric flask. The sample was dissolved in 1.6 ml of phosphate buffer (pH 7.0) and the volume was diluted with mobile phase A.

High-temperature degradation solution (T-7): About 100 mg of cefdinir bulk material was placed in a 100°C oven for 12 hours. Then 15 mg of the above degraded sample was transferred into a 10 ml of volumetric flask. The sample was dissolved in 1.6 ml of phosphate buffer (pH7.0) and diluted with mobile phase A.

2.4. Experimental Method

2.4.1. HPLC-UV

HPLC analysis was carried out using a Waters 2695 Chromatograph with PDA detector (Waters, U.S.). Chromatographic system I consisted of phase A (1000 ml of 0.25% tetra methyl ammonium hydroxide, adjusted to pH 5.5 using phosphoric acid, and 0.4 ml of 0.1 M EDTA disodium) and phase B (acetonitrile:methanol = 3:2 v/v, and 0.4 ml of 0.1 M EDTA disodium was added). A gradient elution procedure was performed (Table **1**). Chromatographic column 1: Diamonsil® (Dikma, U.S.) C18, Size 4.6 mm I.D. × 150 mm, 5 μm; Flow Rate: 1.0 mL·min^{-1}; Oven Temperature: 40°C; Injection Volume: 10 μL.

Table 1. Gradient Elution System Used In Chromatographic System I

Time (min)	Phase A (%)	Phase B (%)
0	97.5	2.5
2	97.5	2.5
25	87.5	12.5
37	75	25
42	75	25
43	97.5	2.5
58	97.5	2.5

2.4.2. Column-Switching LC/MS

Column-Switching LC/MS instrument: NanoSpace S1-2 HPLC (Shiseido Company JP) tandem with Q Trap 3200 MS-MS (AB Company U.S.)

Chromatographic system II: the same as Chromatographic I, with an injection volume of 50 μL.

Chromatographic system III: 0.5% formic acid solution, Flow Rate: 0.5 mL·min^{-1}.

Chromatographic system IV: a mixture of methanol and 0.5% formic acid (70:30), Flow Rate: 0.5 mL·min^{-1}

Chromatographic column 2: Diamonsil® (Dikma, U.S.) C18 250×4.6 mm I.D., 5 μm

LC-MS method (1)

+EMS and +EPI: Scanning Range: 10–500; Curtain Gas: 20 L·min^{-1}; IS: +4500V; TEM: 500.00°C; GS1: 60 L·min^{-1}; GS2: 65 L·min^{-1}; CAD: Medium; DP: +37.5 V; EP: +6.0 V; CEP: +19.00 V; CE: +15.00 V; CXP: +3.00 V.

Direct Injection MS Method (2)

Flow Rate: 10 μL·min^{-1}; +Q1 and +MS2: Scanning Range: 10–600; Curtain Gas: 10 L·min^{-1}; IS: +4500 V; TEM: 0.00°C; GS1: 10 L·min^{-1}; GS2: 0 L ·min^{-1}; CAD: Medium; DP: +50.00 V.

2.4.3. Column-Switching HPLC

Column-Switching HPLC instrument: Dionex P680 Dual Pumps system (Dionex U.S.).

Chromatographic sytem V: the same as Chromatographic I, with an injection volume of 100 μL using Diamonsil® (Dikma, U.S.) C18, 5μm, 250×4.6mmI.D. column, Flow Rate: 1.0mL·min^{-1}.

Chromatographic sytem VI: a mixture of 10 mM butylammonium hydroxide solution (pH 7.0, containing 0.04 M EDTA disodium)-acetonitrile (17: 3), using Diamonsil® (Dikma, U.S.)C18, 5μm, 250×4.6mmI.D. column, Flow Rate: 1.0mL·min^{-1}.

2.5. Column-Switching LC/MS Procedure

The LC/MS instrument was equipped with two switching valves, as shown in Fig. (**2**). The impurities were separated and analyzed using the following procedures.

Step 1: Switching valves 1 and 2 are positioned in A, and then the sample is delivered to chromatographic column 1 using chromatographic system II through an auto-sampler by pump 1. The impurities are separated and detected using a PDA detector.

Step 2: When the target impurity peak flows out from column 1, it is detected by the UV detector. Valve 1 switch from position A to position B. It is maintained in this position for 0.5 min, and then switched back to position A. Valve 2 is kept unchanged in Position A. The target impurity peak at Rt±0.25 min is switched to the 500 μL loop;

Step 3: Valves 1 and 2 both keep at position A, and the target impurity is transported from 500 μL loop to column 2 using chromatographic system III by pump 2 for impurity enrichment and desalination. This lasts for about 5 min; meantime, pump 3 continues to clean the mass spectrometry using chromatographic system IV.

Step 4: After desalination, valve 2 is switched from position A to position B and valve 1 is kept at position A. Then

the desalinated impurities in chromatographic column 2 are transported to the mass spectrometer using chromatographic system IV by pump 3, and mass spectrometric data is acquired.

3. RESULTS AND DISCUSSION

3.1. Analysis of Impurities in Cefdinir by HPLC

Cefdinir bulk material was analyzed using the HPLC-UV method, as shown in Fig. (**3**). In the chromatographic system I, a total of 13 impurity peaks were isolated from the sample.

3.2. Stress Test Result

In order to determine the source of the impurities, cefdinir was degraded under alkaline, acid, 100°C water bath, oxidation, high–temperature, and UV irradiation conditions, according to the degradation reaction mechanism of cephalosporins [25,26], listed in Table **2**.

The stress test suggested that impurities 1, 2, 4, 5, 6, 8, 9, and 10 originated from degradation and that impurities 4, 11, 12, 13, and 14 were introduced from the synthesis process.

3.3. Sample Preparation for Column-Switching LC/MS Analysis

The stress test results indicated that the concentrations of impurities 1, 5, 6, 8, and 10 increased significantly under water bath conditions. Another two impurities, 7a and 9a, which were absent from the sample, were detected simultaneously. The water bath degradation solution (T-5) was used for the analysis of impurities 1, 5, 6, 8, 10, 7a, and 9a.

Under oxidative conditions, the concentration of impurity 2 increased significantly. For this reason, oxidative degradation solution was used for mass analysis of impurity 2. Under high-temperature degradation conditions, impurity 9 was significantly increased, so the high temperature degradation solution was applied for impurity 9 by mass spectrometric analysis. Because impurities 3, 4, 11, 12, 13, and 14 come from synthetic sources, they cannot be enriched through the degradation pathway. We decided to analyze those impurities using cefdinir bulk materials, as shown in Fig. (**4**).

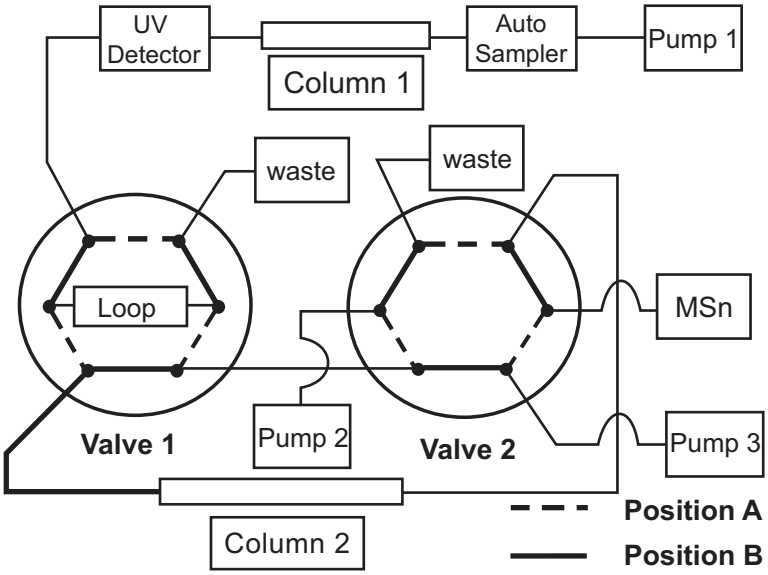

Fig. (2). Schematic diagram of column switching LC/MS system.

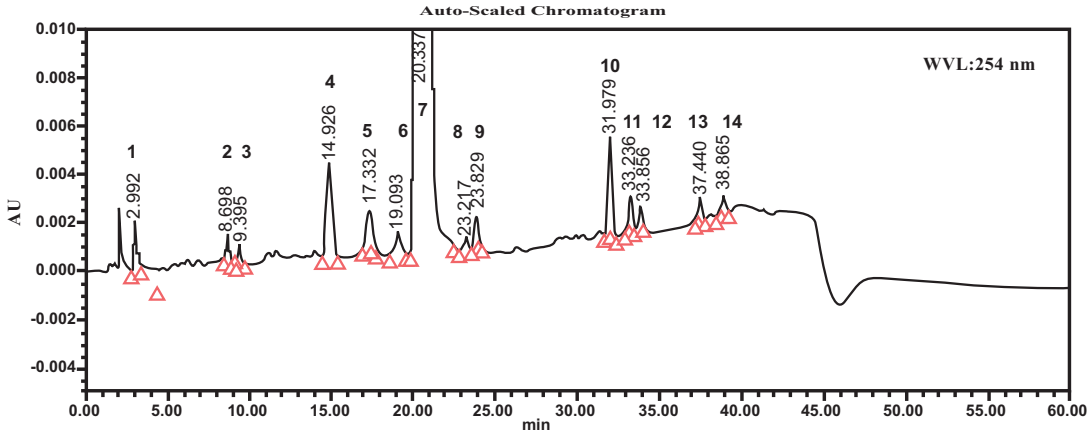

Fig.(3). Typical chromatogram of cefdinir under chromagraphic system I: Peak 7 is cefdinir.

Table 2. Resources of Impurities in Cefdinir Bulk Material

Peak No.	1	2	3	4	5	6	7
Retention Time（min）	2.99	8.90	9.40	14.93	17.33	19.09	20.34
Name	Impurity 1	Impurity 2	Impurity 3	Impurity 4	Impurity 5	Impurity 6	Cefdinir
Resource	a, c	d	g	g	a, c	a, c	--
Peak No.	8	9	10	11	12	13	14
Retention Time（min）	23.22	23.83	31.98	33.24	33.66	37.44	38.87
Name	Impurity 8	Impurity9	Impurity 10	Impurity 11	Impurity 12	Impurity 13	Impurity 14
Resource	a, c	e	c, f	g	g	g	g

Notes: a: base degradation, b: acid degradation, c: 100℃water bath degradation, d: oxidative degradation, e: high-temperature degradation, f: UV degradation, g: synthesis origin.

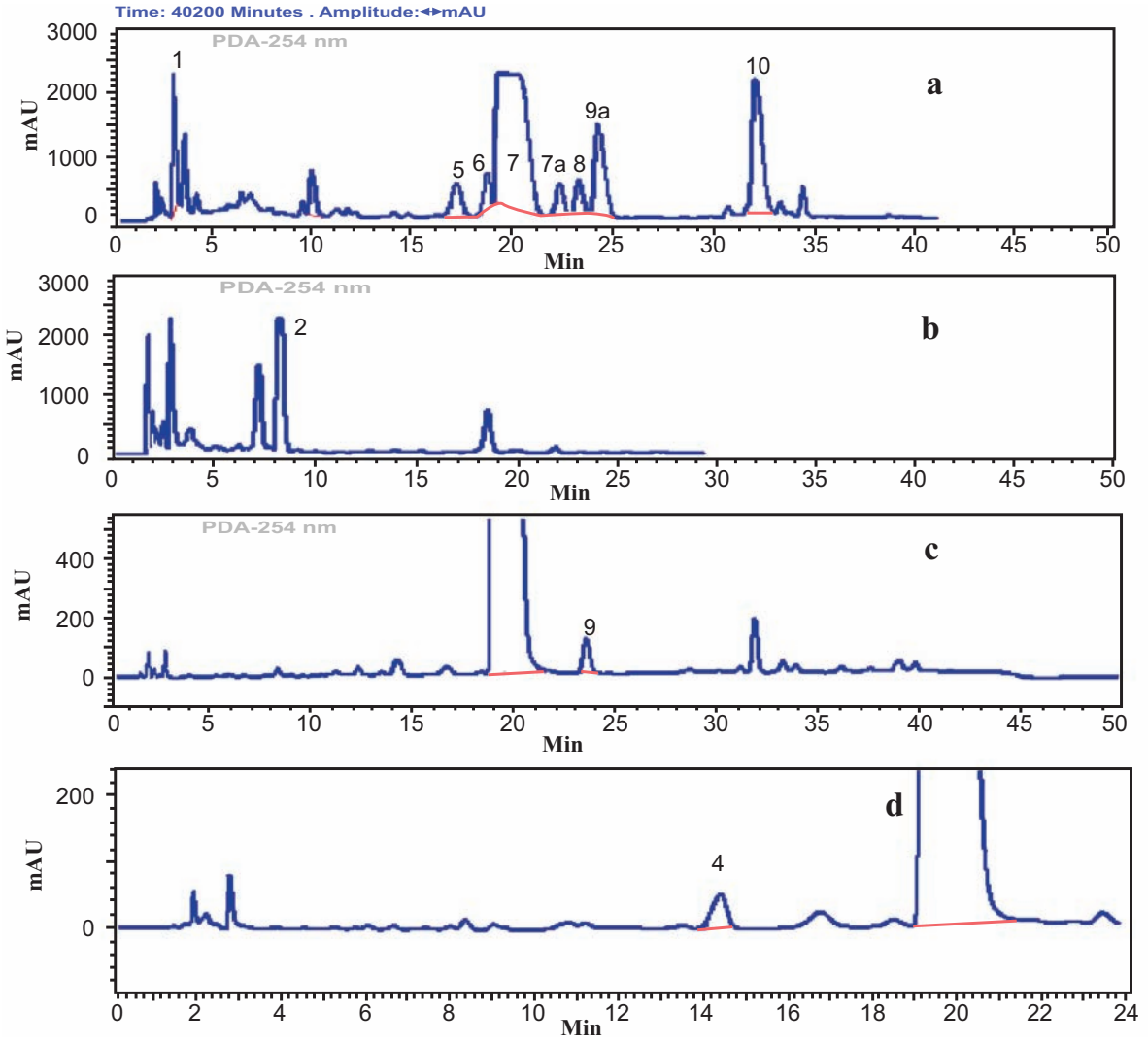

Fig. (4). Typical chromatograms of cefdinir impurities by column-switching LC/MS method including (**a**) water bath degradation solution, (**b**) oxidative degradation solution, (**c**) high-temperature degradation solution, and (**d**) stock solution T-1.

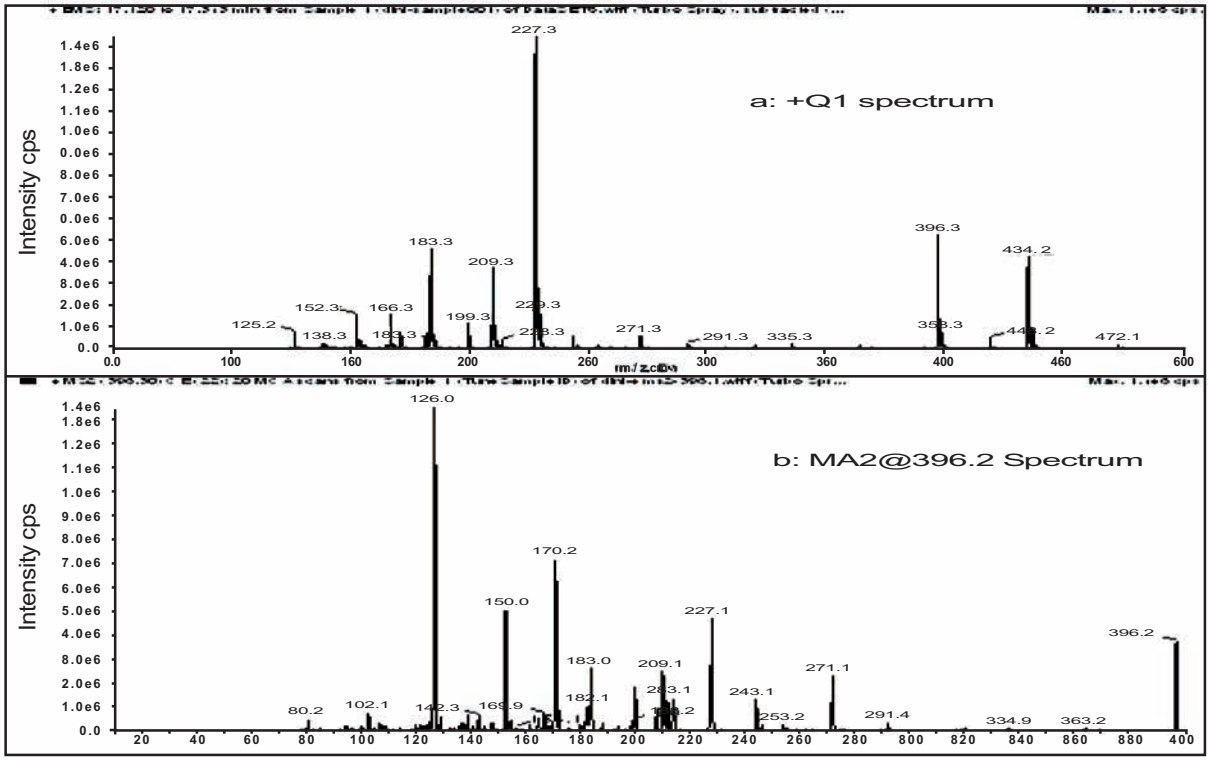

Fig. (5). Mass spectra of cefdinir including (a) +Q1 and (b) MS2@396.1 [M+H].

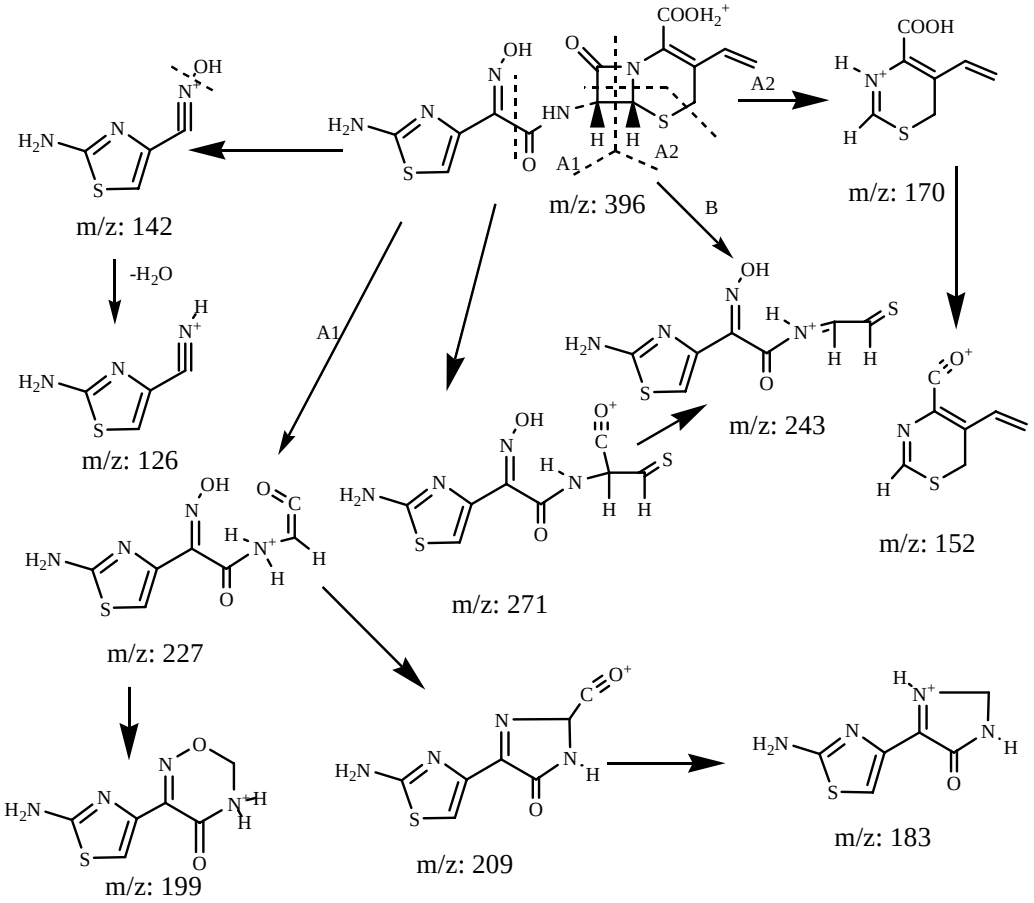

Fig. (6). Mass fragmentation pathway of cefdinir in positive ion mode.

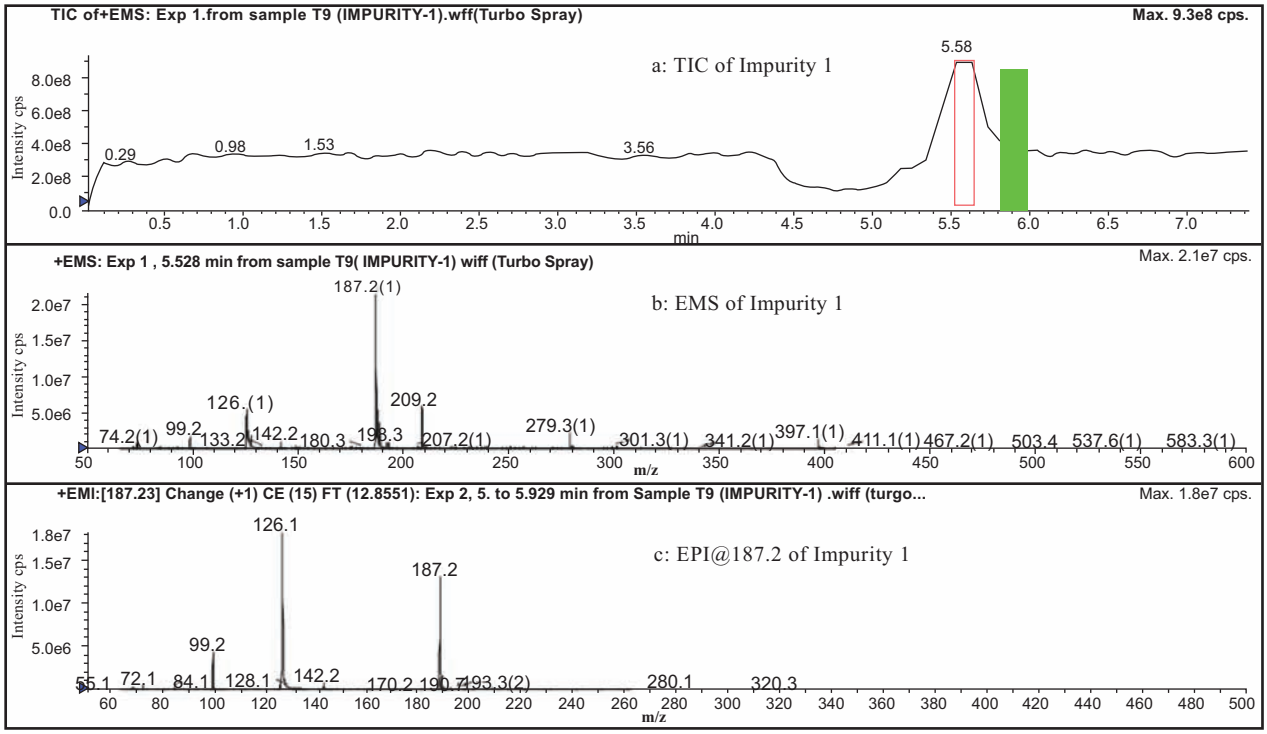

Fig. (7). Mass spectra of impurity 1: (a) TIC, (b) EMS, and (c) EPI@187.2 [M+H].

3.4. Mass Fragmentation Pathway of Cefdinir

A better understanding of cefdinir fragmentation pathways may contribute to the identification of impurities' structure. Cefdinir was analyzed in positive ion mode first by direct flow injection using a methanol/water (1:1) mixture as the solvent through multistage mass spectrometry. The mass fragmentation pathway of cefdinir was proposed successfully. Mass spectra are shown in Fig. (**5**) and the MS fragmentation pathway is shown in Fig. (**6**).

3.5. Characterization of Main Impurities

3.5.1. Impurity 1

The ions at m/z187.2 and m/z 209.2 in the EMS spectrum were hypothesized to be the [M+H] and [M+Na] ion peaks, as shown in Fig. (**7**). Therefore the molecular weight of impurity 1 was proposed to be 186.2.

Fragment ions at m/z 142.2 and m/z 126.1 were produced by EPI @m/z 187.2 [M+H], indicating that impurity 1 contained amine cefotaxime. The cefdinir synthesis process indicated that impurity 1 was identical to a cefdinir side chain at position 7, as shown in Fig. (**1a**).

3.5.2. Impurity 2

The ions at m/z 412.2 and m/z 434.2 in the EMS spectrum were hypothesized to be the [M+H] and [M+Na] ion peaks, as shown in Fig. (**8**). The molecular weight of impurity 2 was proposed to be 411.2, 16 Da more than that of cefdinir. This suggested that impurity 2 had one more oxygen atom than cefdinir.

Fragment ions at m/z287.2 and m/z 168.3 were produced by EPI @m/z 412.2 [M+H]. The former two fragment ions were 16 Da more than that of typical fragment pathways A and B of cefdinir (A: m/z271.2; B: m/z152.2). This suggested that this oxygen atom participates not only in fragmentation pathway A but also in fragmentation pathway B. It was inferred that a sulfoxide group was formed at the sulfur atom in the cefdinir parent nucleus. The structure of impurity 2 is shown in Fig. (**1b**).

3.5.3. Impurity 4

The molecular weight of impurity 4 was deduced as 383.2, based on the fragment ions at m/z384.2 and m/z406.2 which were proposed as [M+H] and [M+Na] peaks, respectively, as shown in Fig. (**9**). It was 12 Da less than the molecular weight of cefdinir, suggesting that impurity 4 had one fewer carton atom than cefdinir. The ion peaks at m/z271.2 and m/z243.2 were both detected in the EPI spectrum of impurity 4. These were the cefdinir typical ion peaks derived from B fragmentation pathway. The presence of an ion peak at m/z 227.2 suggested that impurity 4 contained the same structural group at position 7 as cefdinir. This ion peak was a typical A1 cleavage fragment of cefdinir, This suggested that the structural change was located in the A2 fragment. The ion peaks at m/z 158.2 and m/z 140.2 in the EPI spectrum of impurity 4 were 12 Da less than the A2 cleavage fragments of cefdinir at m/z 170.2 and m/z152.2 separately. This confirmed that the structural change occurred in the six-member heterocyclic portion of impurity 4. The UV spectra of impurity 4 and cefdinir indicated that the UV conjugate moiety of impurity 4 was less than that of cefdinir; a UV peak at 257 nm was detected in impurity 4 rather than the UV peak that occurred at 285 nm in cefdinir, as shown in Fig. (**10**). This indicated that impurity 4 had a methyl group at position 3 rather than a vinyl group. Fig. (**1c**) showed the structure of impurity 4, which was the same as the USP impurity C.

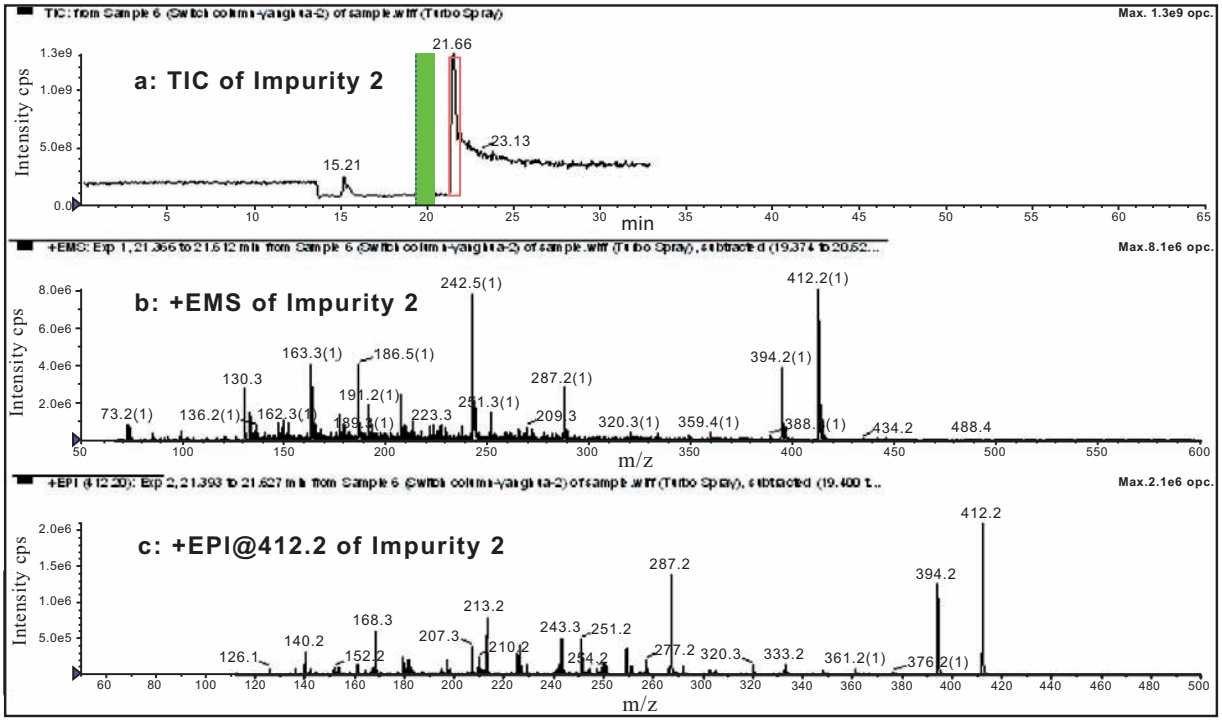

Fig. (8). Mass spectra of Impurity 2: (a) TIC, (b) EMS, and (c) EPI@412.2 [M+H].

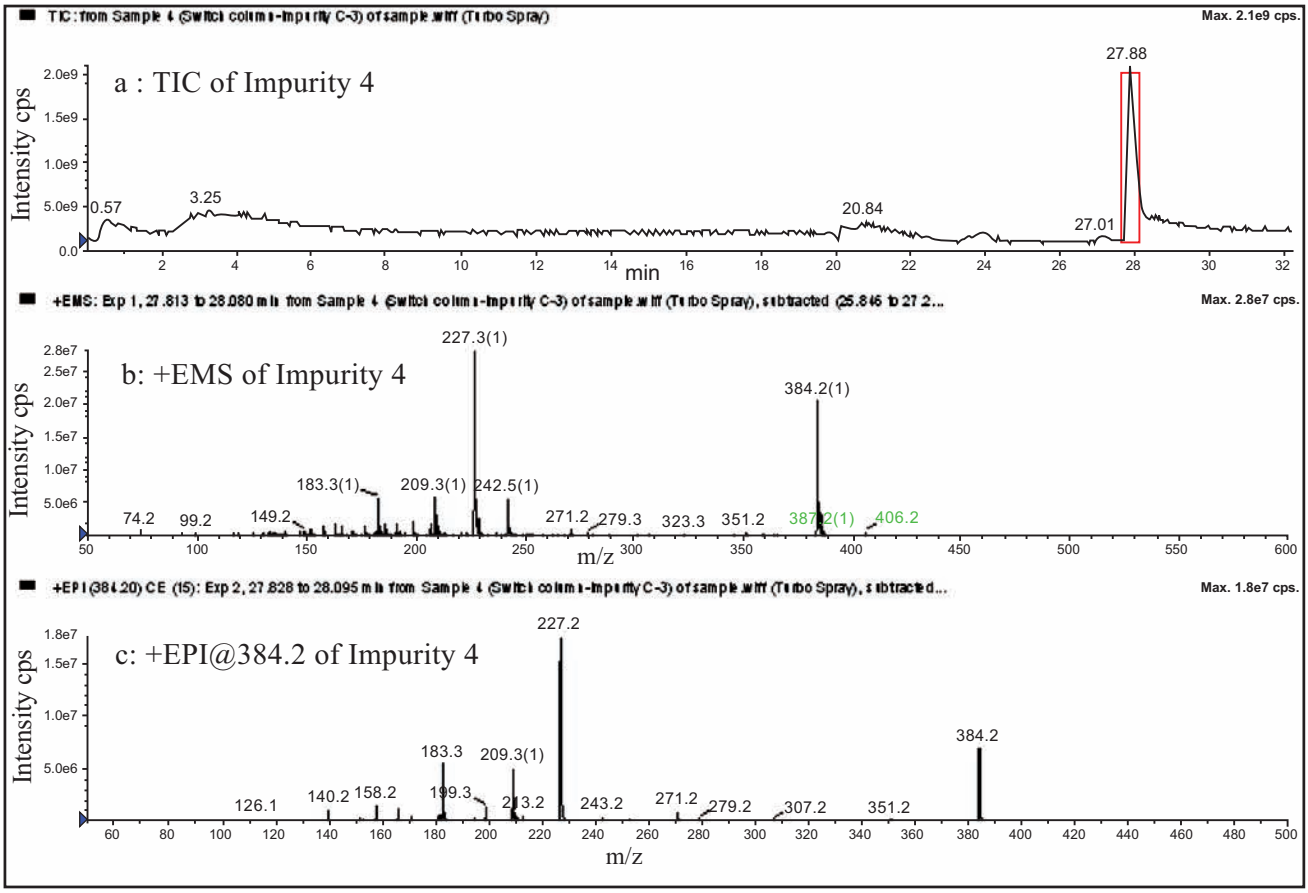

Fig. (9). Mass spectra of Impurity 4: (**a**) TIC, (**b**) EMS, and (**c**) EPI@384.2 [M+H].

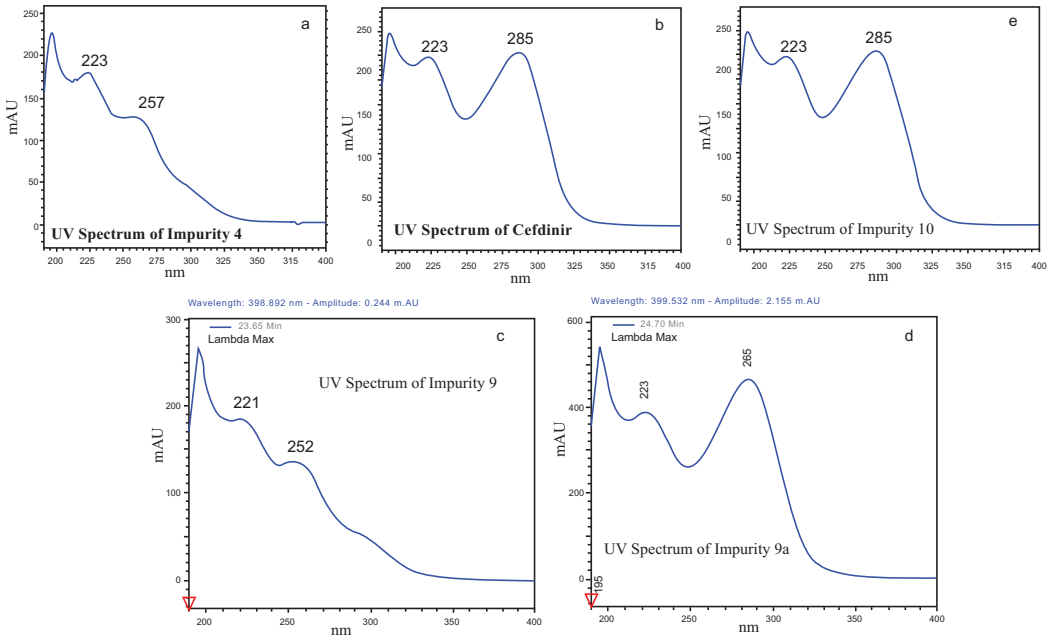

Fig. (10). UV spectra of cefdinir and related impurities (**a**: impurity 4, **b**: cefdinir, **c**: impurity 9, **d**: impurity 9a, **e**: impurity 10).

3.5.4. Impurities 5, 6, 7a, and 8

Impurities 5, 6, 7a, and 8 were found to be typical water bath degradations, as shown in Fig. (**4a**). The EMS and EPI spectra of the four impurities were found to be almost the same. These four impurities were identified together.

The molecular weights of the four impurities were deduced as 413.2 according to the ion peaks at m/z414.2 and m/z436.2. These were proposed to be [M+H] and [M+Na] peaks, respectively, as shown in Fig. (**11**). This was 18 Da more than the molecular weight of cefdinir, suggesting the introduction of one H_2O. Accordingly, these four impurities were deduced to be β-lactam hydrolysis ring-opened products. Two types of methyl cleavage, m/z396.2-m/z 381.2 = 15 Da and m/z352.2-m/z337.2 = 15 Da, were present in the EPI spectra, indicating that a methyl group had formed in those impurities. In the hydrolysis of cefdinir, when the carboxyl group at position 2 and the vinyl at position 3 underwent a nucleophilic reaction, a new γ-lactone ring was generated. This caused the formation of a methyl group. From this it could be inferred that the four impurities were cefdinir β-lactam ring opened γ-lactones. Their stereo-chemical structures were further studied based on the references [23, 24].

Yoshihiko separated and determined the stereo-chemical structures of cefdinir β-lactam ring opened γ-lactones using 10 mM butyl-ammonium hydroxide solution (pH 7.0, containing 0.04 M EDTA disodium)-acetonitrile (17: 3) as a mobile phase [23,24]. Because the mobile phase systems in references and *Chinese Pharmacopoeia* 2010 edition all belonged to the ion pair reagent system, it was inferred that, under the two mobile phases, the elution behavior of those four isomers did not change.

In order to validate this assumption, a column-switching study was designed and performed. The Water bath degradation solution (T-5) was separated by system V firstly, and switched to system VI immediately when the target impurity was eluted out by the system V, then the target impurity was re-analyzed continuously by system VI. Calculate the retention time of the four isomers in system VI separately by minus the switch time point from the retention time in system V and system VI. It was shown that the retention time of impurities 5,6,7a,8 in system VI were 15.6min, 18.3min,18.9min, and 20.3min respectively, as shown in Fig. (**12**). Therefore, the 4 kinds of isomers had the same elution behavior in the two different chromatographic systems.

In this way, the stereo-chemical structures of impurities 5, 6, 7a, and 8 were characterized with the help of the references, as shown in Figs. (**1d**, **1e**, **1f**, and **1g**) [23, 24].

3.5.5. Impurity 9

The ions at m/z396.2 and m/z 418.2 in the EMS spectrum were hypothesized to be the [M+H] and [M+Na] ion peaks, as shown in Fig. (**13**). The molecular weight of impurity 9 was deduced to be 395.2, which was equivalent to the molecular weight of cefdinir. There was a significant difference between the UV spectrum of impurity 9 and that of cefdinir (Fig. **10c** vs. **10b**). The UV spectrum of impurity 9 was similar to that of impurity 4. Like impurity 4, impurity 9 had a new γ -lactone ring. This ring altered the UV-conjugated groups. Impurity 9 was deduced to be cefdinir lactone, as shown in Fig. (**1h**).

3.5.6. Impurity 9a

The ions at m/z396.2 and m/z 418.2 in the EMS spectrum were considered to be the [M+H] and [M+Na] ion peaks, as shown in Fig. (**14**). The molecular weight of impurity 9a was deduced to be 395.2. This was equivalent to the molecular weight of cefdinir. The typical A fragmental ions such as the peaks at m/z 227.2(A2), m/z 152.2 (A1), and m/z 170.2 (A1) were detected. However, the typical B fragmental ions at m/z 243.2 and m/z 271.2 were not detected. It was suggested that part of the nuclear structure of impurity 9a differs from that of cefdinir.

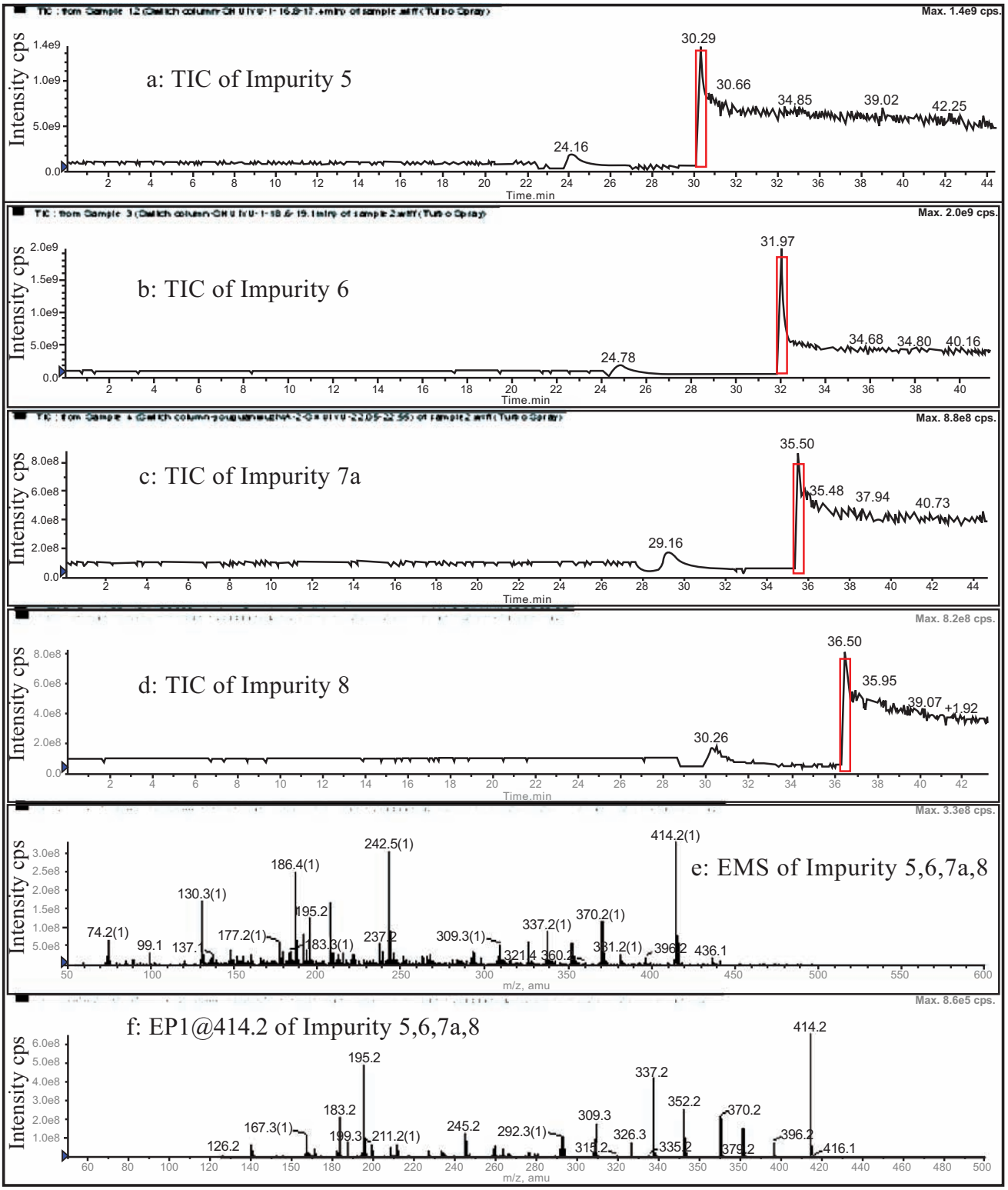

Fig. (11). Mass spectra of impurities 5, 6, 7a, 8 (a–d: TIC of impurity 5, 6, 7a, 8; e: EMS of 5, 6, 7a, 8; f: EPI@414.2 of impurities 5, 6, 7a, 8).

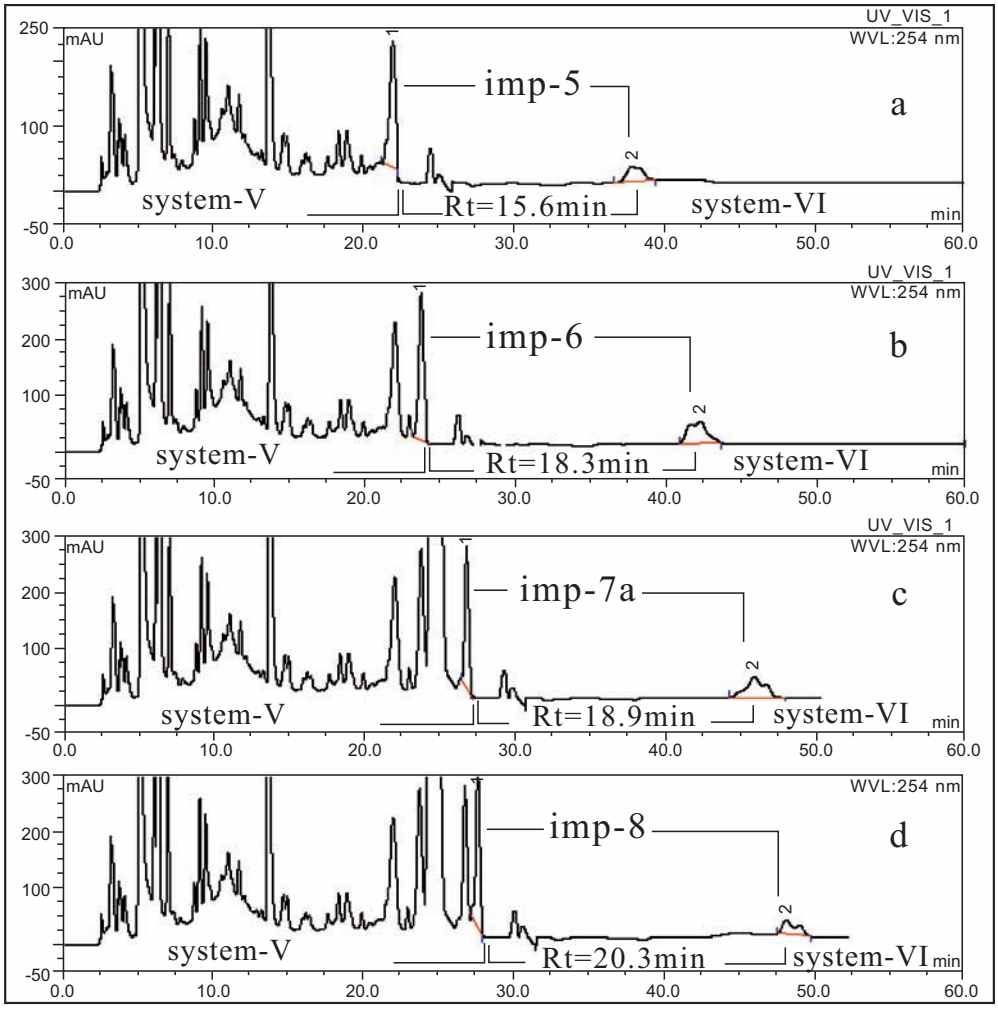

Fig. (12). The typical chromatograms of impurities 5,6,7a,8 in two different chromatographic systems by column-switching technology (a:impurity 5, b:impurity 6, c: impurity 7a, d: impurity 8).

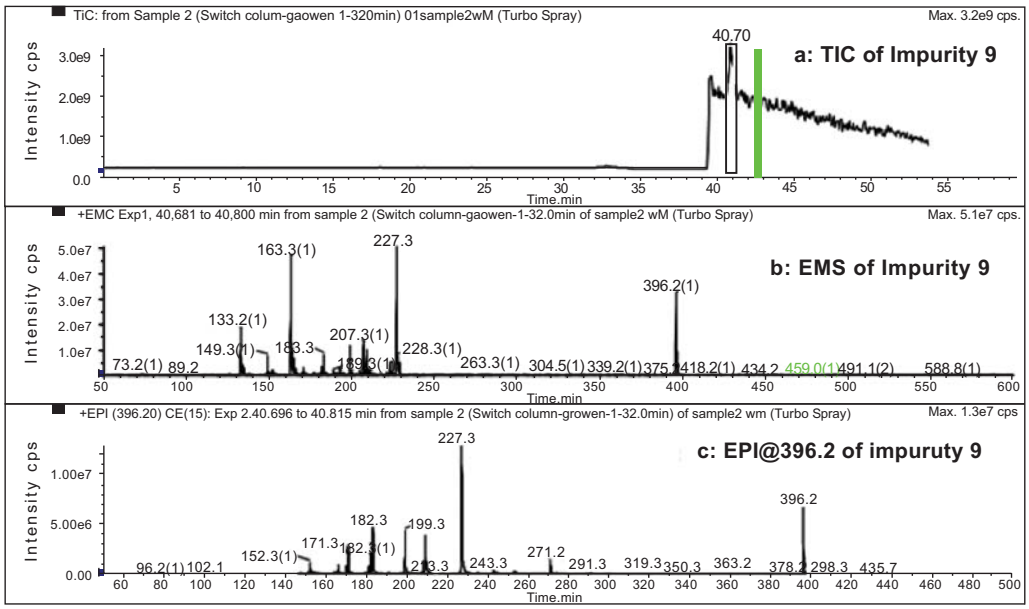

Fig. (13). Mass spectra of impurity 9 (a: TIC of impurity 9; b: EMS of impurity 9; c: EPI@396.2 of impurity 9)

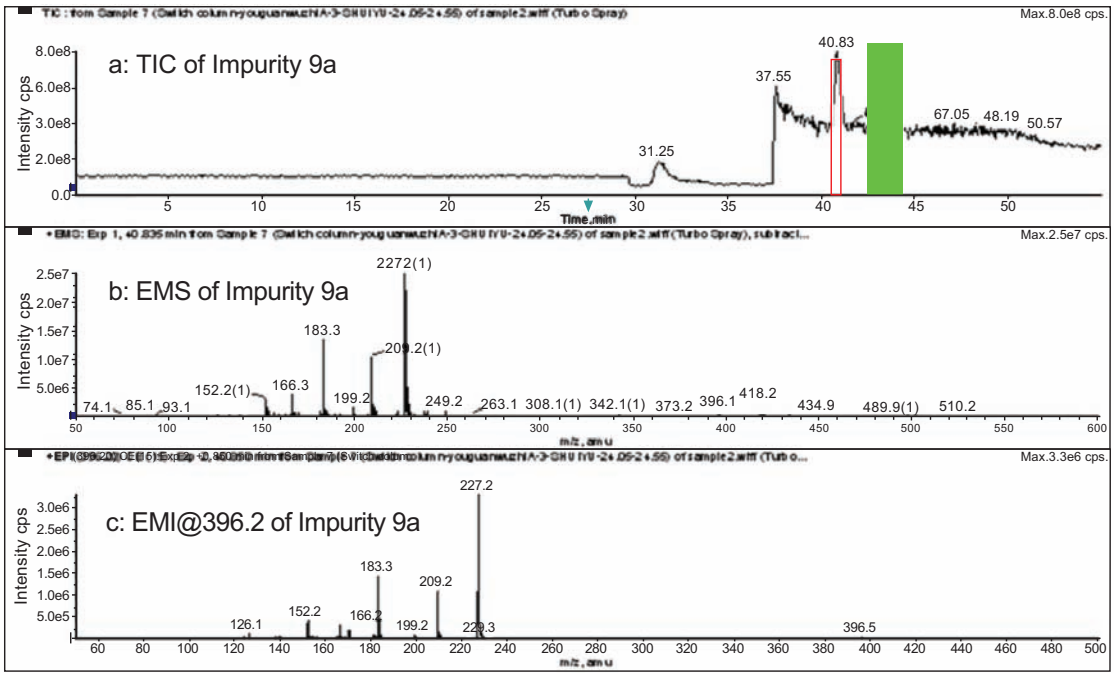

Fig. (14). Mass spectra of impurity 9a (a: TIC of impurity 9a; b: EMS of impurity 9a; c: EPI@396.2 of impurity 9a).

The UV spectrum of impurity 9a was consistent with that of cefdinir (Fig. (**10d**) vs. Fig. (**10b**)), suggesting that the UV conjugated group of impurity 9 was the same as that of cefdinir. It was reported that the configuration of carbon atom at position 6 changed when cefdinir was placed under acid conditions [15, 16]. Impurity 9a was deduced to be the isomer of cefdinir at position 6, as shown in Fig. (**1i**).

3.5.7. Impurity 10

The ions at m/z396.2 and m/z 418.2 in the EMS spectrum of impurity 10 were characterized as the [M+H] and [M+Na] ion peaks separately,as shown in Fig. (**15**). The molecular weight of impurity 10 was deduced to be 395.2. This was consistent with the molecular weight of cefdinir. The EPI spectrum @396.2[M+H] of impurity 10 was also similar to

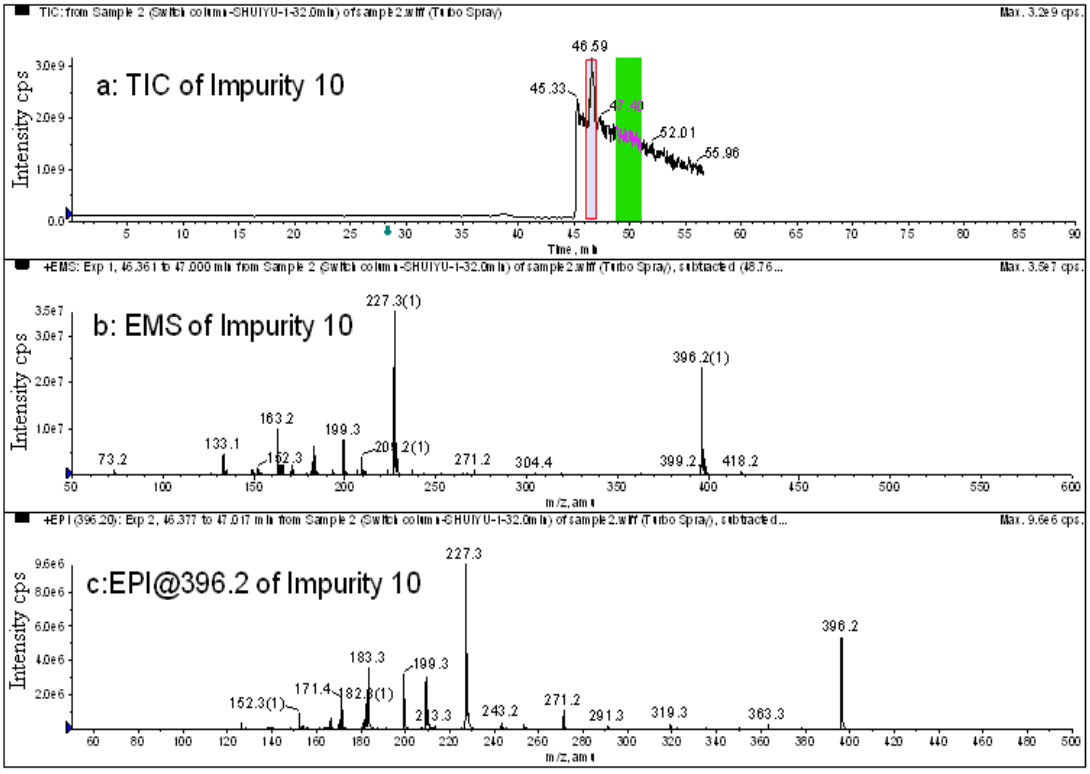

Fig. (15). Mass spectra of impurity 10 (a: TIC of Impurity 10; b: EMS of impurity 10; c: EPI@396.2 of impurity 10).

that of cefdinir, including the typical ion peaks at m/z227, m/z170, and m/z152, which were derived from fragmentation pathway A, and ion peaks at m/z271, m/z243, which were produced by fragmentation pathway B. The UV spectra of impurity 10 and cefdinir were basically consistent with each other (Fig. **10b** vs. **10e**). It was suggested that there was no change in the nuclear structure of impurity 10 and that the structural change was located in the ammonia cefotaxime substituent at position 7. Impurity 10 was characterized as anti-cefdinir, identical to impurity G, as indicated by the relative retention time in USP 34 (Fig. **1j**).

3.5.8. Other Impurities

There were still five other impurities in cefdinir bulk material, impurities 3, 11, 12, 13, and 14. Due to their low concentrations, no mass data could be obtained effectively. These impurities required further study.

4. CONCLUSION

In this study, several related substances found in cefdinir bulk material were analyzed using the chromatographic system described in *Chinese Pharmacopeia* 2010 Edition. In total, 13 impurities were detected. Based on the degradation mechanism of cephalosporins, stress tests were designed and performed. Eight main impurities were found to be degradation products and five impurities were found to have originated from the synthesis process. Because the chromatographic system was not suitable for LC/MS analysis, the target impurities were enriched and desalinated. Then these impurities were identified using column-switching LC/MS technology, the general mass fragmentation pathway of cefdinir, and UV spectra. In this way, this study not only proved that the cefdinir analysis method recommended in *Chinese Pharmacopoeia* had the same separation capability as USP34 and identified the retention behaviors of impurities listed in *Chinese Pharmacopoeia*, but also constituted an effective tool for the rapid identification of impurities in chromatographic systems containing non-volatile salts.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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