Shanghai University of Engineering Science[1], Shanghai; National Institutes for Food and Drug Control[2], People's Republic of China

Influence of impurities on the specific optical rotation of cefozopran

Shu-Yu Liu[1,2], Ya-Ping Li[2], Chang-Qin Hu[2]

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Chang-Qin Hu, National Institutes for Food and Drug Control, No. 2 Tiantan Xili, Chingwen district, Bejing 100050, People's Republic of China
hucq@nicpbp.org.cn

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The impurities of cefozopran hydrochloride are analyzed using high-performance liquid chromatography (HPLC) with UV absorbance and optical rotation (OR) detection. The results show that the impurities can affect the specific optical rotation of the cefozopran product. Due to the different composition of impurities, the Chinese cefozopran hydrochloride product has a specific optical rotation different from the Japanese product. The relationship between impurity limits and specific optical rotation of cefozopran hydrochloride is revealed. The results provide a scientific rationale for setting the limit of specific optical rotation of cefozopran hydrochloride.

1. Introduction

Cefozopran hydrochloride is a cephem anti-microbial agent developed by Takeda Chemical Industries, Ltd. It became clinically available in the late 1990 s and is often used for antibacterial prophylaxis in abdominal surgery and for the treatment of postoperative intra-abdominal infections (Kobayashi et al. 1997). In Japan, cefozopran hydrochloride is the first-line antimicrobial agent for febrile neutropenia (Masaoka 2004). The 14th edition of the Japanese Pharmacopoeia regulated that the specific optical rotation of cefozopran hydrochloride should fall between -73° and -78° (JP 2001). However, the specific optical rotation of cefozopran hydrochloride manufactured in China often sparingly falls within this range and some manufacturer's product may even have specific optical rotation as high as about -45°. In this work, we study the relationship between the specific optical rotation of cefozopran hydrochloride and its impurity profile to determine the cause for the difference in the specific optical rotation between Chinese imitation products and Japanese products. The results provide a scientific rationale for setting the limit of specific optical rotation of cefozopran hydrochloride.

2. Investigations, results and discussion

2.1. HPLC–UV–OR analysis

Figures 1–3 show the HPLC–UV–OR chromatograms of the cefozopran degradation products. The main degradation products detected in the HPLC-UV chromatograms (Figs. 1a, 2a and 3a) are labeled in the order of increasing retention time as impurity 1, impurity 2, . . ., impurity 12, respectively. It can be seen that impurities 1 and 3 were formed only in acidic degradation, impurities 10 and 11 were formed only in basic degradation, and impurities 2 and 8 were formed only in oxidative degradation. Impurities 7 and 9 were detected under all degradation conditions, indicating that they can be easily generated. The optical activities of the impurities are shown in the HPLC-OR chromatograms (Figs. 1b, 2b and 3b). Note that the OR peaks offset slightly from the UV peaks because the detectors were connected in series. Nine of 12 main impurities showed optical rotation. Specifically, 5 impurities (impurities 1, 8, 9, 10, 11) had positive optical rotation, 3 impurities (impurities 3, 6, 7) had negative optical rotation, and impurity 2 was racemic. Therefore, the presence of impurities can affect the specific optical rotation of the cefozopran product.

The optical correction factor are calculated by the following equation:

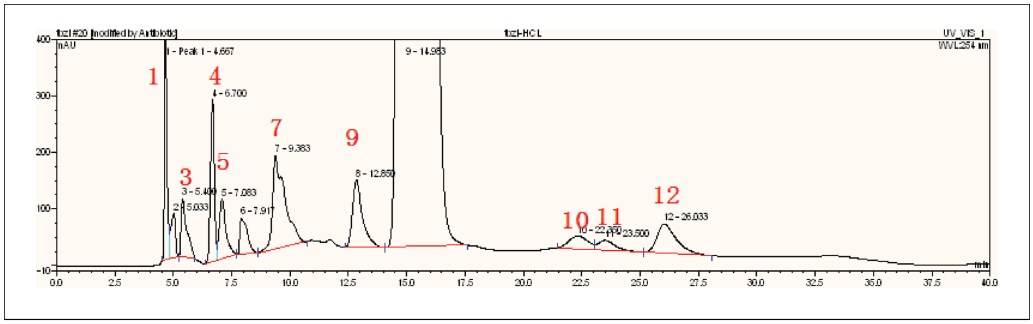
$$f_{OR} = \frac{\mathrm{Ai(OR)}/Ai(UV)}{A_R(OR)/A_R(UV)} \quad (1)$$

where A_i(OR) and A_i(UV) are the OR and UV peak area of the impurity and A_R(OR) and A_R(UV) are the OR and UV peak area of cefozopran, respectively. The optical correction factors of the detected impurities are listed in Table 1. The impurity 3 had the most negative optical correction factor and thus the greatest impact on the specific optical rotation of cefozopran. The OR peak area of impurity 3 would be equal to that of cefozopran when the UV peak area of impurity 3 is as small as 1/364.8 of that of cefozopran.

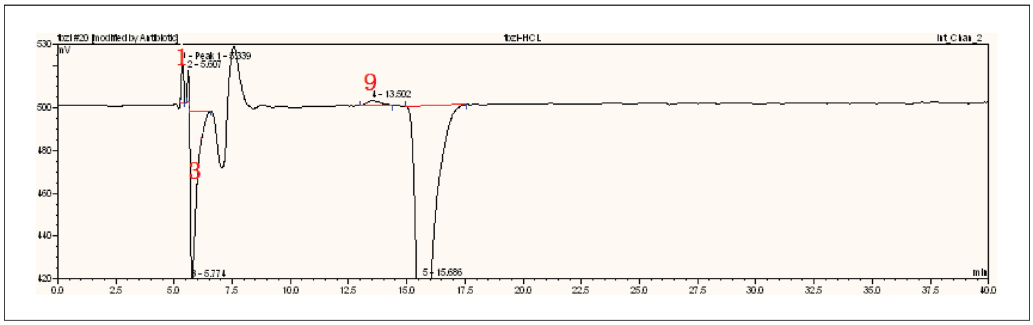
2.2. The impurity profile of the Chinese product and the Japanese product

Figure 4 shows the HPLC-UV chromatograms of the Chinese cefozopran product (batch number 060516) and the Japanese cefozopran product (batch number HK 798). It can be seen that the composition of impurities are significantly different between the Chinese and the Japanese product. The impurities 1 and 2 in the Chinese product were not detected in the Japanese product, and the content of impurity 7 in the Japanese product was much higher than in the Chinese product. Impurity 11 was detected in the Chinese product but was absent in the Japanese product. In addition, new impurities 13 and 14 were found in the Japanese product.

Figure 5 shows the HPLC-OR chromatograms of the Chinese and Japanese cefozopran product. In the Japanese product only impurities 7 and 9 were detected, and other impurities had neg-



(a)



(b)

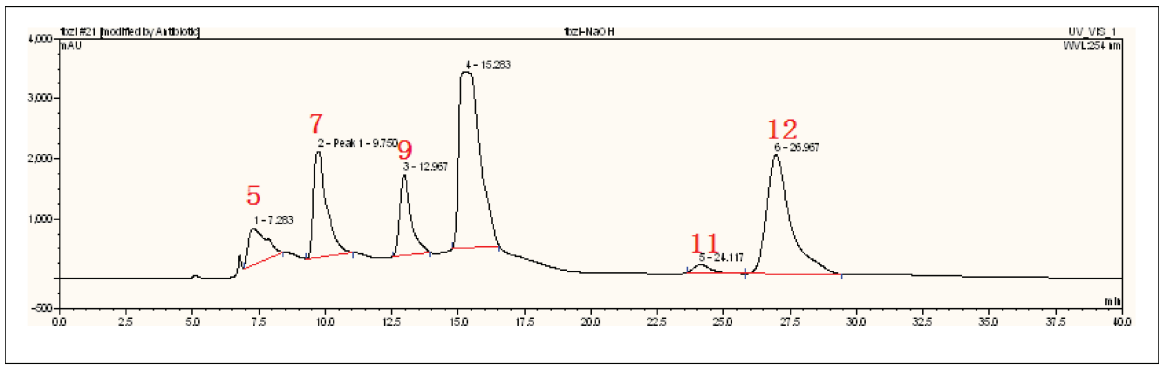
Fig. 1: (a) HPLC-UV chromatogram of acid-induced degradation products. (b) HPLC-OR chromatogram of acid-induced degradation products

ligible influence on the optical rotation. Impurity 9 had positive optical rotation and impurity 7 had negative optical rotation, therefore they offset each other and weakened the overall impact on the optical rotation of Japanese cefozopran product. In the Chinese product, only impurity 9 was observed, which had opposite optical rotation to cefozopran and thus increased the specific optical rotation of the Chinese cefozopran product.

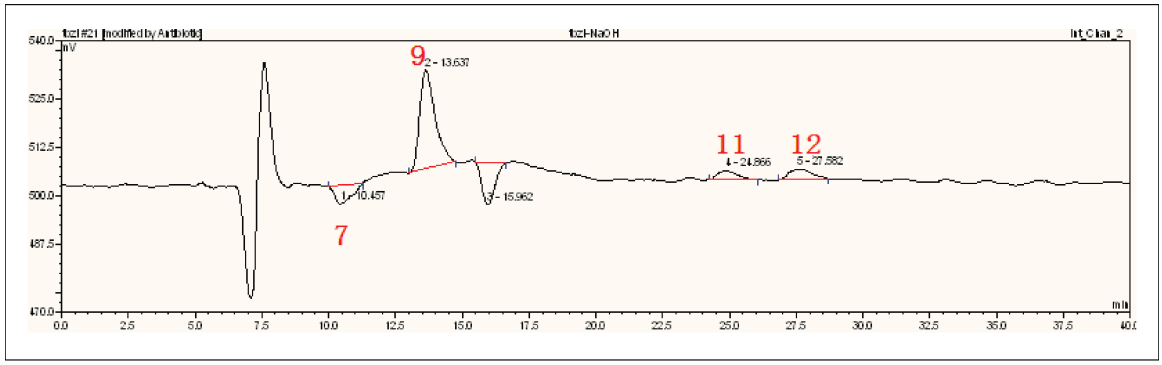
In addition, the impurity 2 (RRT = 0.34) in the Chinese product had negative optical rotation, and the impurity 2 was detected in the Japanese product to have positive optical rotation although it was not observed in the HPLC-UV chromatogram of the Japanese product. The impurity 2 (RRT = 0.34) is a racemic oxidative degradation product (see Fig. 3b), thus the content of the specific enantiomer of impurity 2 may differently affect the optical rotation of the cefozopran product.

2.3. Effect of impurity profile on the specific optical rotation of cefozopran

The specific optical rotation of a substance is directly influenced by its purity. As shown in Table 2, the purity of cefozopran batch 060605 was determined to be 93.6% by qNMR and mass bal-



(a)



(b)

Fig. 2: (a) HPLC-UV chromatogram of base-induced degradation products. (b) HPLC-OR chromatogram of base-induced degradation products

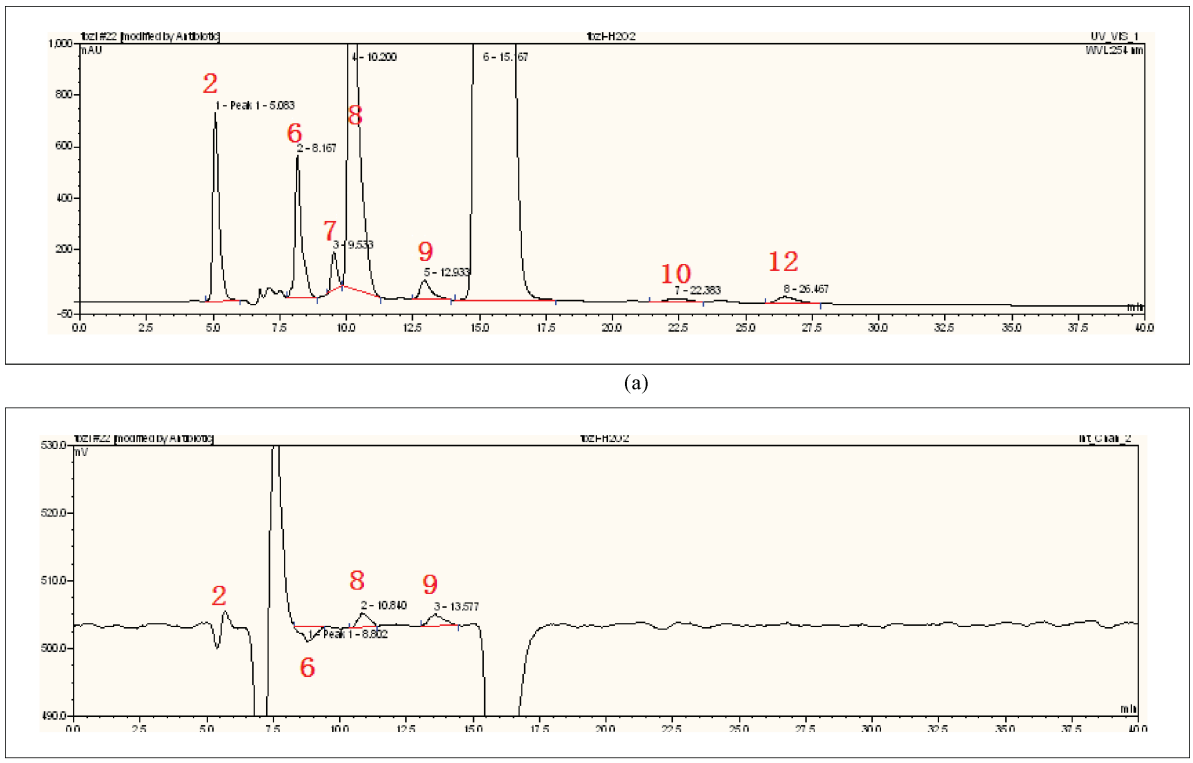


Fig. 3: (a) HPLC-UV chromatogram of oxidative degradation products. (b) HPLC-OR chromatogram of oxidative degradation products

ance. The purity of cefozopran batch 060516 was determined to be 93.3% by HPLC using cefozopran batch 060605 as the reference. The specific optical rotation of cefozopran batch 060605 and 060516 were −75.3441° and −74.5609° respectively, which are in the middle of the limit (between −73° and −78°) prescribed in the Minimum Requirement for Antibiotic Products of Japan. Therefore, it can be concluded that the presence of about 1% of impurities does not significantly influence the specific optical rotation of cefozopran hydrochloride.

A series of cefozopran hydrochloride (batch 060605) solutions were prepared (0.2–10 mg/mL) to quantify the relationship between optical rotation and OR peak area. As shown in Fig. 6,

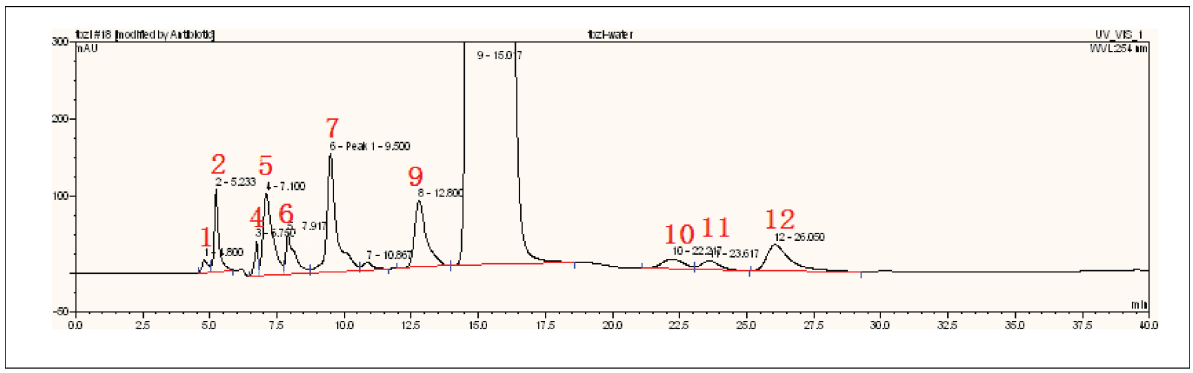
Table 1: Specific optical rotation of cefozopran impurities

Impurity	Relative Retention Time (RRT)	Degradation path	Optical correction factor	Specific optical rotation	Specific optical rotation of cefozopran with single impurity	
					Impurity content ≤0.5%	Impurity content ≤1%
1	0.31	Acid	22.6	1708.2°	≤ −66.6°	≤ −57.7°
2	0.34	Oxygen	N/A [a]	N/A [a]	N/A [a]	N/A [a]
3	0.36	Acid	–364.8	−27540.9°	≤ −212.8°	≤ −350.2°
4	0.45	Acid	N/A [a]	N/A [a]	N/A [a]	N/A [a]
5	0.47	Acid, alkali	N/A [a]	N/A [a]	N/A [a]	N/A [a]
6	0.53	Oxygen	–3.5	–265.0°	≤ −76.4°	≤ −77.4°
7	0.63	Alkali	–1.5	–115.0°	≤ −75.7°	≤ −75.9°
8	0.67	Oxygen	0.55	41.8°	≤ −74.9°	≤ −74.3°
9	0.85	Acid, alkali, oxygen	12.9	976.1°	≤ −70.2°	≤ −65.0°
10	1.48	Acid,oxygen	N/A [a]	N/A [a]	N/A [a]	N/A [a]
11	1.58	Alkali	7.8	592.7°	≤ −72.2°	≤ −68.8°
12	1.76	Alkali	0.55	41.8°	≤ −74.9°	≤ −74.3°

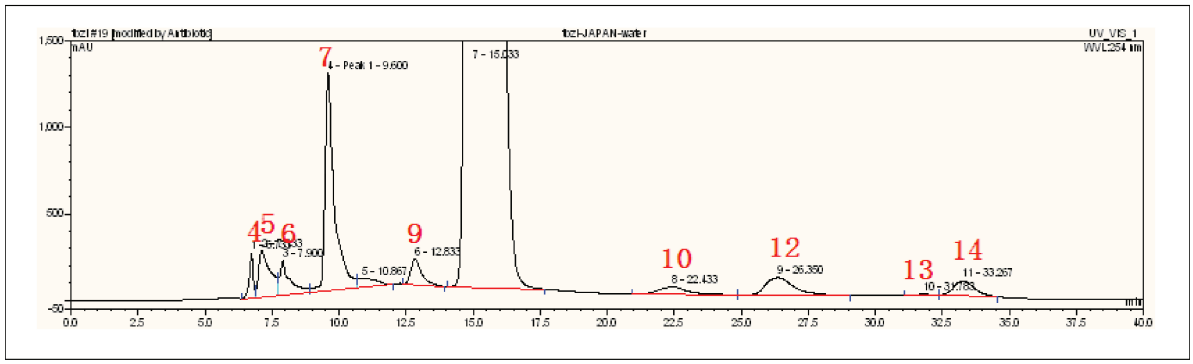
Relative retention time is calculated by dividing the retention time of the impurity by the retention time of cefozopran.
[a] N/A indicates no optical rotation.

Table 2: Purity of cefozopran hydrochloride (batch number 060605)

	Mass balance method				qNMR method		
	Total impurity	Water	Volatile material	Residue	1	2	3
Content	1.44%	4.29%	0.37%	0.035%	94.8%	93.2%	91.7%
Content of cefozopran hydrochloride		93.9%				93.2%	
Average content				93.6%			



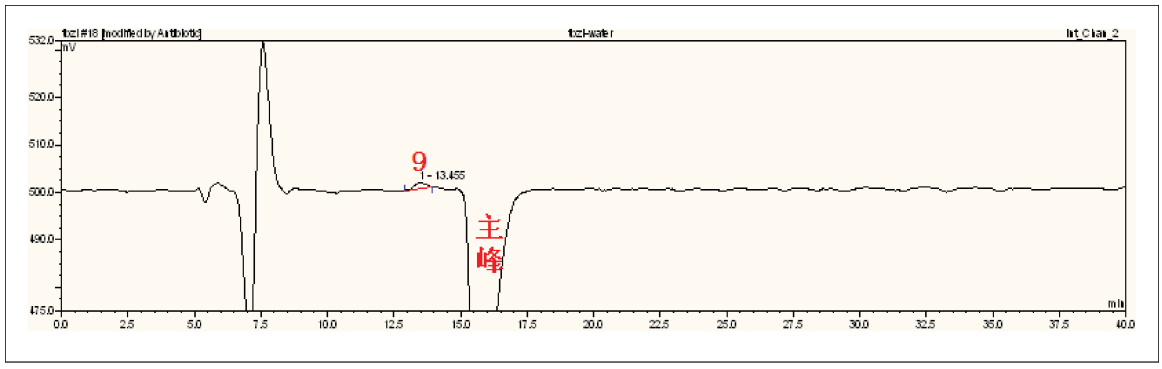
(a)



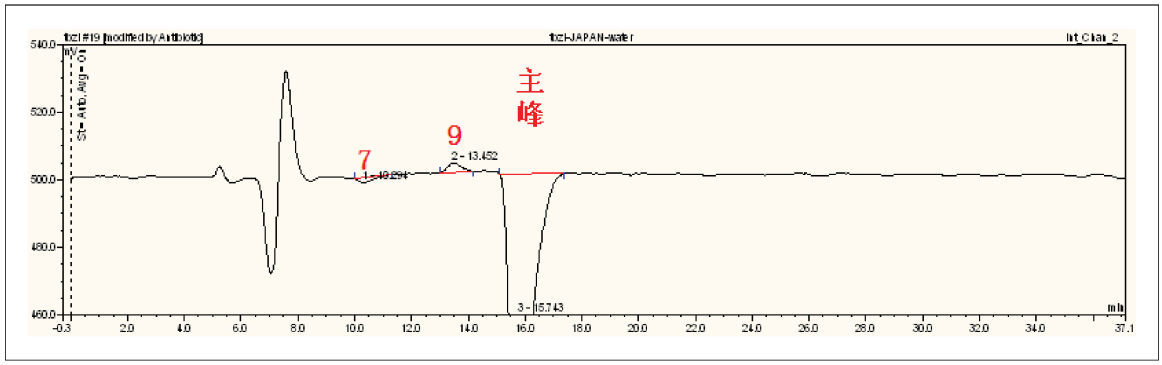
(b)

Fig. 4: HPLC-UV chromatograms of cefozopran. (a) Chinese product (batch number 060516), (b) Japanese product (batch number HK 798)

at the concentration range of 0.2–10 mg/mL, the optical rotation and the OR peak area of cefozopran hydrochloride can be described by the linear equation $y = 102.12x - 0.1515$, where the coefficient $R = 0.9966$. According to this equation, the specific optical rotation of cefozopran batch 060516 and batch HK798 are calculated to be 74.2 and 74.9, respectively. Therefore, the theoretical specific optical rotation of cefozopran hydrochloride is in the middle of the limit (-73° to -78°) set by the Minimum Requirement for Antibiotic Products of Japan.



(a)



(b)

Fig. 5: HPLC-OR chromatograms of cefozopran. (a) Chinese product (batch number 060516), (b) Japanese product (batch number HK 798)

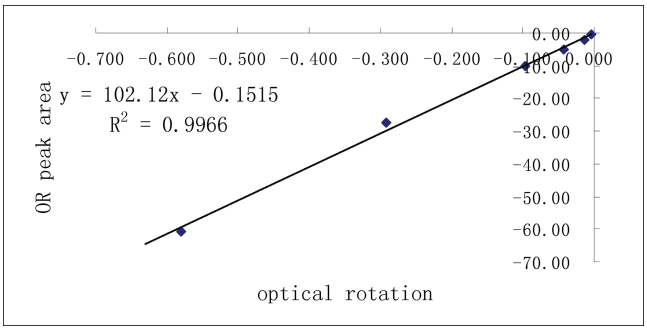


Fig. 6: Relationship between optical rotation and OR peak area for cefozopran hydrochloride

2.4. Relationship between impurity limits and specific optical rotation of cefozopran

Assume that the theoretical specific optical rotation of cefozopran hydrochloride is −75.5°, which is in the middle of the limit (−73° to −78°) set by the Minimum Requirement for Antibiotic Products of Japan. According to the results of Table 1, impurity 3 (RRT = 0.36) has the greatest impact on the upper limit of specific optical rotation, and impurity 1 (RRT = 0.31) has the greatest impact on the lower limit of specific optical rotation. The ICH guidelines require that any individual impurity be no more than 0.5% and the largest single impurity be no more than 1%. According to the requirement, the limit of specific optical rotation of cefozopran is calculated as follows. The upper limit of specific optical rotation reaches −212.8° in the presence of 0.5% impurity 3 and −350.2° in the presence of 1.0% impurity 3. The lower limit of specific optical rotation reaches −66.6° in the presence of 0.5% impurity 1 and −57.7° in the presence of 1.0% impurity 1. Furthermore, the total impurities are usually no more than 2.0% for cephalosporins. The upper limit of specific optical rotation of cefozopran is not more than −214.0° when only three negative impurities (0.5%) are considered. The lower limit of specific optical rotation of cefozopran is not more than −57.6° when the largest four positive impurities (0.5%) are considered. Therefore, the specific optical rotation of cefozopran prepared through different processes may vary from −57.6° to −214.0° due to different impurity profile. It is necessary to set the limit of specific optical rotation of cefozopran on the basis of the practical production process and impurity profile.

3. Experimental

3.1. Instruments

The LC system consisted of a Dionex liquid chromatography instrument equipped with a P680 pump, an ASI-100 automated sample injector, a TCC-100 thermostatted column compartment, a PDA-100 photodiode array detector and a JASCO OR 2090 optical rotation detector. The optical rotation of the samples was measured on an AUTOPOL IV series automatic polarimeter. ^{1}H NMR measurements were made on a Varian 500 spectrometer operating at 500 MHz. A Trace GC Ultra system was used to measure residual solvents.

3.2. Materials

Cefozopran hydrochloride manufactured by Sichuan Industrial Institute of Antibiotic Co., Ltd., China (batch no. 060516 and 060605) and Takeda Chemical Industries, Ltd., Japan (batch no. HK 798) were used. The internal standard 5-methyl resorcinol (purity 97%) was purchased from Sigma, USA. Heavy water was purchased from Cambridge Isotope Laboratories Inc., UK. All HPLC reagents were of chromatographic purity. Acetonitrile was purchased from Fisher, USA, and ammonium acetate and acetic acid were purchased from Sigma-Aldrich (Schnelldorf, Germany).

3.3. Methods

3.3.1. Preparation of cefozopran degradation products

The cefozopran solution (5 mg mL^{-1}) was prepared by dissolving cefozopran hydrochloride (batch no. 060516, 5 mg) in water (1 mL). The cefozopran solution (1 mL) was mixed with hydrochloric acid (37%, 0.05 mL) and allowed to stand at 25 °C for 12 h to give the solution ready for HPLC injection containing acid-induced degradation products. Similarly, the cefozopran solution (1 mL) was mixed with sodium hydroxide (4 mg mL^{-1}, 0.25 mL) or hydrogen peroxide (3%, 0.05 mL) and allowed to stand at 25 °C for 12 h to form base-induced degradation products and oxidative degradation products, respectively. The solutions of the degradation products were then submitted to HPLC analysis.

3.3.2. HPLC method

HPLC analysis was performed on an Alltima C18 chromatographic column (150 × 4.6 mm). The mobile phase consisted of 5/95 acetonitrile/ammonium acetate solution (0.0166 mol L^{-1}, pH adjusted to 3.0 by acetic acid). The flow rate was set to 0.3 mL min^{-1} and the column temperature was kept at 25 °C. Sample injection was 10 μL and UV detection was observed at 254 nm. Because the optical rotation detector is connected in series to the UV detector, the optical rotation peaks offset slightly from corresponding the UV peaks at a time delay of about 0.7 min.

3.3.3. Sample purity determination

The specific optical rotation of the cefozopran samples was determined according to the method defined in Japanese pharmacopoeia 14th edition. The purity of the cefozopran samples was determined by both qNMR and mass balance.

The qNMR experiments were carried out at the following optimized parameters: 90° pulse for 11.000 μs, 29026 data points, 499.609 MHz irradiation frequency, 64 s relaxation delay, 32 scans and gain of 10. Phase and baseline corrections were done manually. The qNMR samples contained 0.04 mol L^{-1} cefozopran hydrochloride and 0.04 mol L^{-1} 5-methyl resorcinol in D_2O. The peaks for the methyl protons in cefozopran and 5-methyl resorcinol were used to quantify the amount of cefozopran.

The mass balance method quantifies all impurities (including moisture and ash) and subtracts the sum of these impurities from 100%. The content of the analyte can be calculated as follows (volatile material means residual solvents):

$$\text{Content\%} = (\text{1-impurity\%}) \times (\text{1-water\%-volatile material\%-sulphated ash\%}) \times 100\% \quad (2)$$

The impurities in cefozopran were determined by HPLC. The water content in cefozopran was determined by the Karl Fischer method using methanol as the solvent. Residual solvents in cefozopran were determined by GC and sulphated ash was determined by routine methods.

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