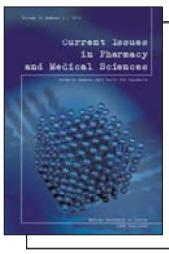

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# The stability of cefoselis sulfate in aqueous solutions in accordance with the ICH guidelines for stability testing

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**ABSTRACT**

The stability of cefoselis sulfate in accordance with the ICH Guidelines for the Stability Testing for Medicinal Substances and Pharmaceutical Products was investigated. The study was conducted at different pH, temperature, oxidative factor and with a varying exposure time. The tests of photostability were also done. Changes in cefoselis sulfate concentration were followed using the HPLC method with UV detection. Cefoselis sulfate was classified depending on the stress conditions applied and data obtained in the study as extremely labile in base pH, very labile in neutral pH, labile in acidic pH and in the presence of an oxidative factor. In the photostability test cefoselis sulfate was classified as photolabile.

**Keywords:** cefoselis sulfate, stability in aqueous solutions, ICH Guidelines, HPLC

## INTRODUCTION

The time of cephalosporins has begun since 1948. The substance isolated from cultures of *Cephalosporium acremonium* by Giuseppe Brotzu was effective against *Salmonella spp.* Cephalosporin C isolated in 1961, was a first antibiotic from the cephalosporis. The first drug cephalothin - was launched by Eli Lilly in 1964. Until this time the cephalosporins made a great contribution in clinical use. There are currently over twenty cephalosporin antibiotics available commercially. Unfortunately, until 1964 antimicrobial resistance became a significant worldwide problem. Antibacterial activities of cephalosporins are coincided with their resistance to the degradation [1]. Cephalosporins are susceptible to degradation in aqueous solutions [2,3] and in solid state [2, 4-10]. The structure causing grater alkaline stability of cephalosporins usually causing greater acid degradation, but on the other hand antibiotics should be stable at acidic pH especially if orally used. Acid-stable cephalosporins are one of the main targets of research of new antibiotics.

Cefoselis sulfate (Fig. 1) is a new, parenteral, fourth generation cephalosporin [11]. It has a broad spectrum of antibacterial activity against Gram positive and Gram negative bacteria including *Pseudomonas aeruginosa* [11]. Cefoselis sulfate contains imidazopyrazolium methyl group at the C3 position determining antibacterial activity against methicyllin-resistant *Staphylococcus aureus* [11]. This group will probably determine greater stability of cefoselis sulfate in acid than base pH and wide plateau region in neutral pH. The aim of this work was to classify cefoselis sulfate according to ICH guidelines for stability testing.

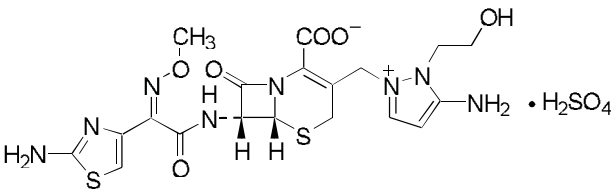


**Fig. 1.** Chemical structure of cefoselis sulfate

## MATERIAL AND METHODS

*Chemicals and apparatus.*Cefoselis sulfate was obtained from Xingcheng Chempharm Co., Ltd. Taizhou, Zhejiang, China. It is white or light yellow crystalline powder containing 99.5% cefoselis sulfate, 0.1% related substances and complies with the Chinese Pharmacopoeia 2005 standard.

All other chemicals and solvents were obtained from Merck KGaA (Germany) and were of analytical grade. High quality pure water was prepared by using the Milli-

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pore purification system (Millipore, Molsheim, France, model Exil SA 67120).

The analytical system consisted of a quaternary pump (L-7100), an autosampler (L-7200), a column oven (L-7360) and diode array detector (L-7455) (all Merck Hitachi products). As the stationary phase a Lichrospher RP-18 column, 5 μm particle size, 250 mm 4 mm (Merck, Darmstadt, Germany) was used. The mobile phase consisted of 5 volumes of acetonitrile and 95 volumes of ammonium acetate, 12 mmol $L^{-1}$, pH of the mobile phase was 7.15. The flow rate of the mobile phase was 1.0 mL $min^{-1}$. The wavelength of the DAD detector was set at 260 nm. Separation was performed at 30°C. The HPLC method has been evaluated and validated for the determination of cefoselis sulfate in stability studies [12]. Photodegradation stability studies were performed using Suntest CPS+ (Atlas®) with filter Solar ID65.

*Solutions.* All degradation studies in solutions were done at cefoselis sulfate concentration of 0.4 mg $mL^{-1}$. Hydrolytic reactions were carried out in water (12 h at 90°C), in hydrochloric acid (0.1 M for 8 h at 90°C and 0.01 M for 8 h at 40°C) and in solution of sodium hydroxide (0.1 M for 8 h at 90°C, 0.01 M for 8 h at 40°C and 0.01 M for 2 h at 21°C). Oxidative studies were conducted at room temperature in 3% $H_2O_2$ for 6 h. Photodegradation studies were made in water solution, in a photostability chamber, at room temperature. The samples were exposed to $1.2\times10^6$ Lux h and $6.0\times10^6$ Lux h. Suitable controls were kept under dark conditions.

The solutions were stored in heat chambers in glass vials except samples for photodegradation, which were stored in quartz cuvette. Samples were taken at intervals shown above, cooled to room temperature and neutralised. 50 μL of each obtained solution was analyzed immediately, using the reverse-phase HPLC method [12]. Quantitative tests were conducted. The results obtained are part of the analytical profile of cefoselis sulfate and may be used for comparing the stability of cephalosporins.

## RESULTS AND DISCUSSION

The stability tests of cefoselis sulfate were performed according to the ICH Guidelines for the Stability Testing for Medicinal Substances and Pharmaceutical Products. Analytical procedures were drawn up for complex stability testing, which involved hydrolysis reactions under acidic, alkaline and neutral conditions as well as oxidative and photostability reactions. The study was conducted at different pH, temperature, oxidative factor and with a varying exposure time. The tests of photostability were also done.

*Stability studies in hydrochloric acid.* At the beginning of stability studies of cefoselis sulfate in hydrochloric acid 0.1 M HCl was used. The study was conducted at 90°C for 8 h. Under these conditions, complete decomposition of substrate was observed; therefore, the concentration of hydrochloric acid was reduced to 0.01 M HCl and the temperature was decreased to 40°C. The time of exposure was not changed (Fig. 2). Under this condition, the degradation level was 40 %. Cefoselis sulfate was classified as very labile (Tab. 1).

*Stability studies in sodium hydroxide.* At the beginning of stability studies of cefoselis sulfate in sodium hydroxide 0.1 M NaOH was used. The study was conducted at 90°C for 8 h. Under these conditions, complete decomposition of substrate was observed. Similar results in sodium hydroxide 0.01 M NaOH at 40°C for 8 h were observed, therefore the concentration of NaOH, temperature and time of exposition were reduced. In 0.01 M NaOH and 21°C for 2 h the degradation level was 85% (Fig. 3). Cefoselis sulfate was classified as extremely labile (Tab. 2).

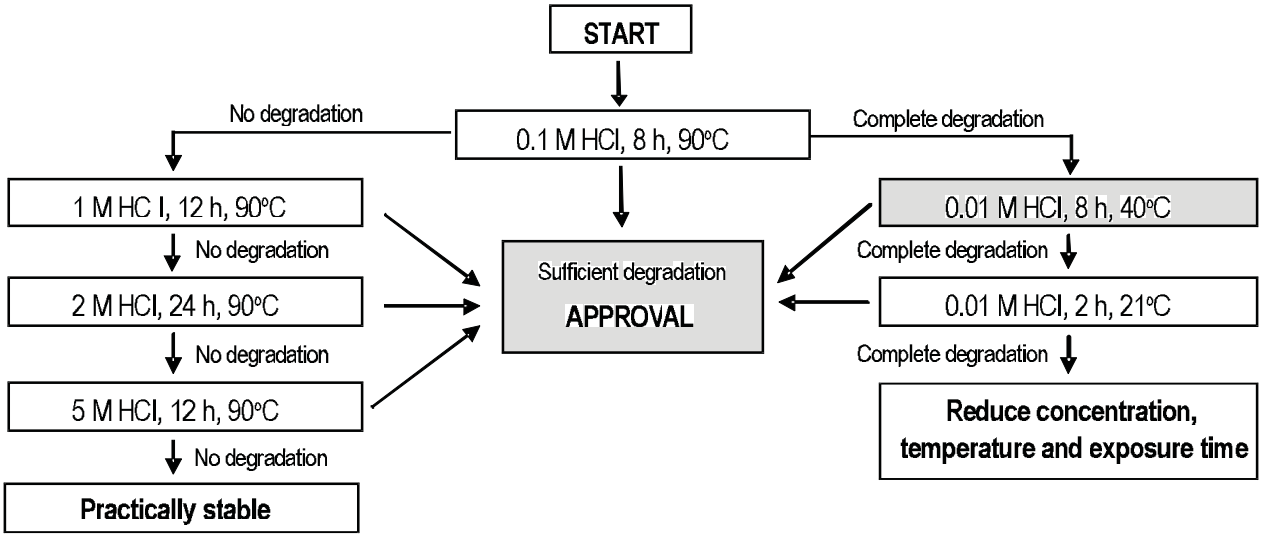


**Fig. 2.** Stability studies of cefoselis sulfate in hydrochloric acid solution

**Table 1.** Classification of cefoselis sulfate into stability classes in hydrochloric acid

| Drug category | HCl concentration | Time of exposure | Temperature | Degradation level |
|---|---|---|---|---|
| Extremely labile | 0.01 M | 2 h | 21°C | Sufficient |
| Very labile | 0.01 M | 8 h | 40°C | Sufficient |
| Labile | 0.1 M | 8 h | 90°C | Sufficient |
| Stable | 1 M | 12 h | 90°C | Sufficient |
| Very stable | 2 M | 1 day | 90°C | Sufficient |
| Practically stable | 5 M | 2 days | 90°C | No degradation |

**Table 2.** Classification of cefoselis sulfate into stability classes in sodium hydroxide

| Drug category | NaOH concentration | Time of exposure | Temperature | Degradation level |
|---|---|---|---|---|
| Extremely labile | 0.01 M | 2 h | 21°C | Sufficient |
| Very labile | 0.01 M | 8 h | 40°C | Sufficient |
| Labile | 0.1 M | 8 h | 90°C | Sufficient |
| Stable | 1 M | 12 h | 90°C | Sufficient |
| Very stable | 2 M | 1 day | 90°C | Sufficient |
| Practically stable | 5 M | 2 days | 90°C | No degradation |

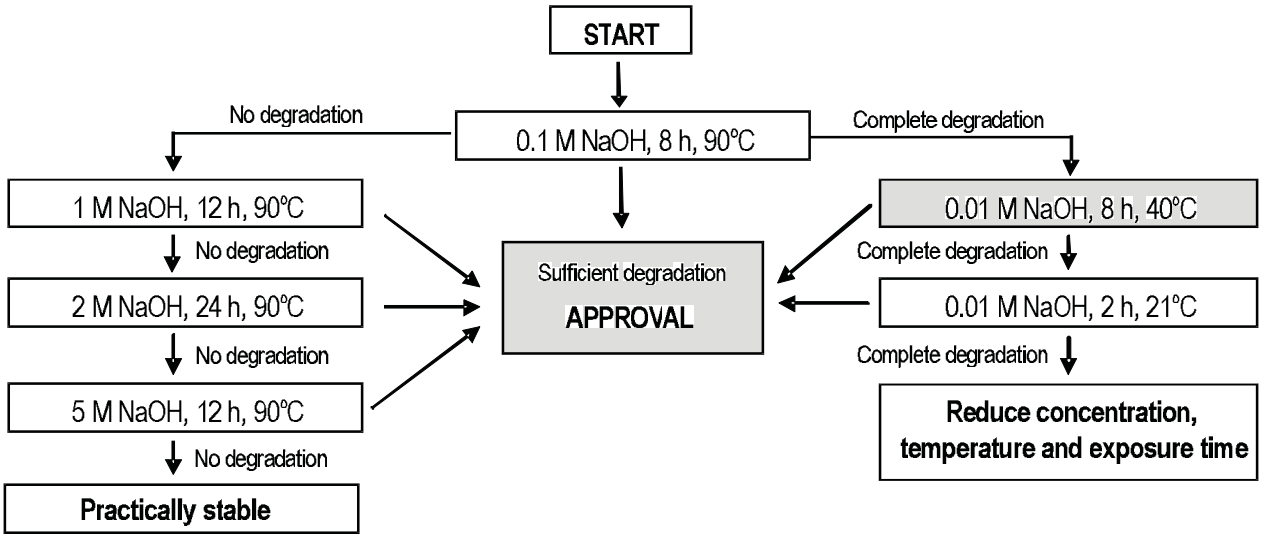


**Fig. 3.** Stability studies of cefoselis sulfate in sodium hydroxide solution

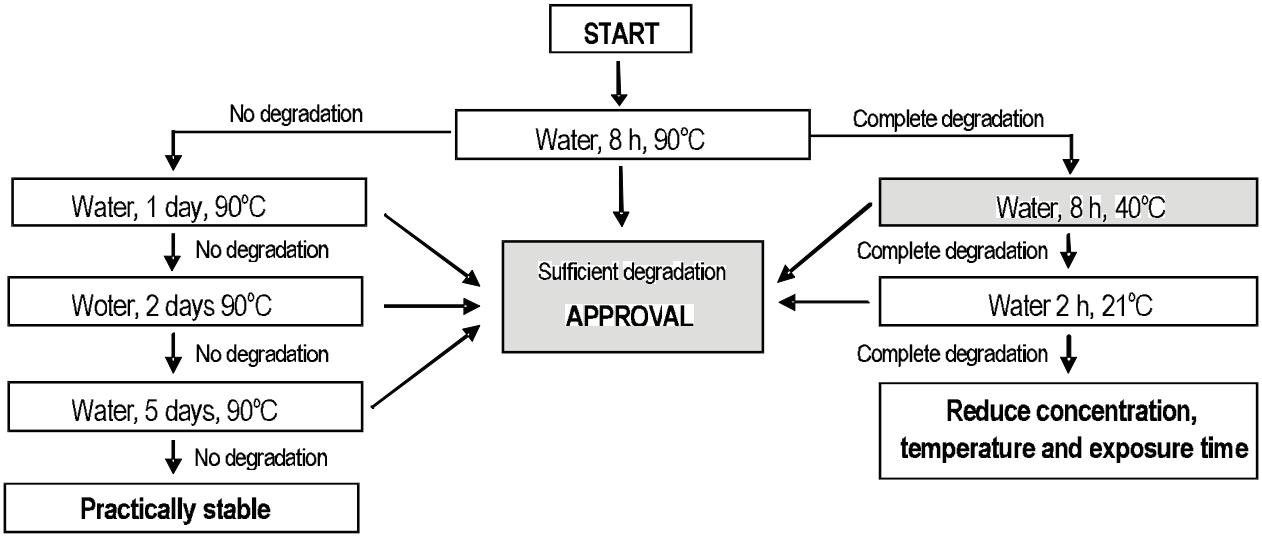


**Fig. 4.** Stability studies of cefoselis sulfate in water

*Stability studies in water.* Stability studies of cefoselis sulfate in water were carried out as shown in (Fig. 4) and started with incubation for 12 h at 90°C. The degradation level was 65%. Cefoselis sulfate was classified as labile (Tab. 3).

*Stability studies in the presence of an oxidative factor.* In the first stage of stability studies in the presence of an oxidative factor cefoselis sulfate was incubated in 3% $H_2O_2$ at 21°C for 6 h (Fig. 5). The degradation level was 17%. Cefoselis sulfate was classified as labile (Tab. 4).

*Photostability studies.* Photostability tests were performed as shown in Fig. 6. Samples were exposed to $1.2\times10^6$ Lux h and the degradation level was 47%. Cefoselis sulfate was classified as photolabile (Tab. 5).

**Table 3.** Classification of cefoselis sulfate into stability classes in water

| Drug category | Time of exposure | Temperature | Degradation level |
|---|---|---|---|
| Extremely labile | 2 h | 21°C | Sufficient |
| Very labile | 8 h | 40°C | Sufficient |
| Labile | 12 h | 90°C | Sufficient |
| Stable | 1 day | 90°C | Sufficient |
| Very stable | 2 days | 90°C | Sufficient |
| Practically stable | 5 days | 90°C | No degradation |

Table 4. Classification of cefoselis sulfate into stability classes in the presence of oxidative factor

| Drug category | $H_2O_2$ concentration | Time of exposure | Temperature | Degradation level |
|---|---|---|---|---|
| Extremely labile | 1% | 30 min | 21°C | Sufficient |
| Very labile | 1% | 3 h | 21°C | Sufficient |
| Labile | 3% | 6 h | 21°C | Sufficient |
| Stable | 3% | 1 day | 21°C | Sufficient |
| Very stable | 10% | 1 day | 21°C | Sufficient |
| Practically stable | 30% | 2 days | 21°C | No degradation |

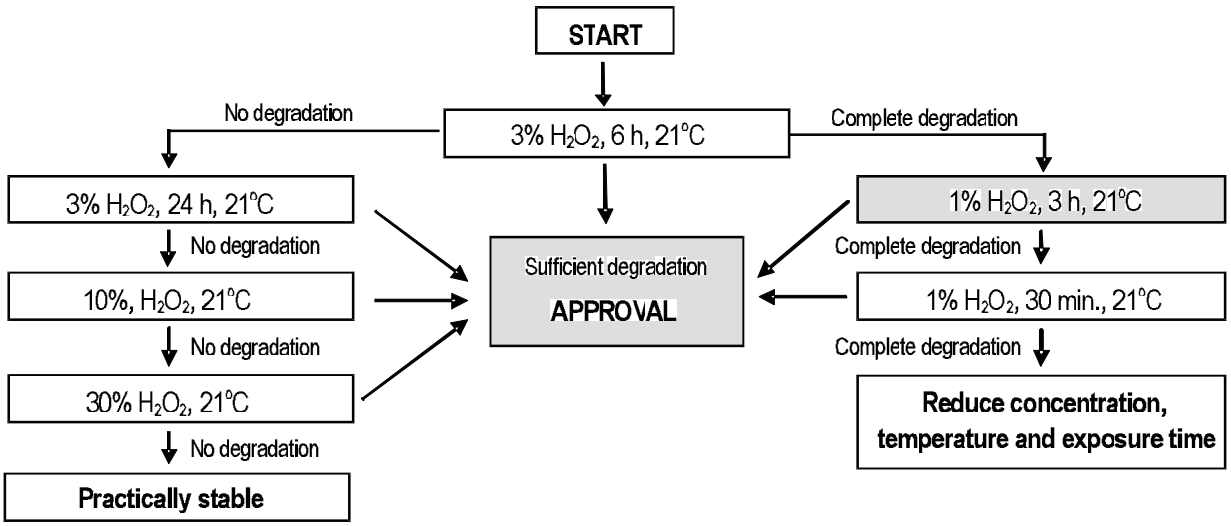


**Fig. 5.** Stability studies of cefoselis sulfate in the presence of an oxidative factor

Table 5. Classification of cefoselis sulfate into stability classes for photostability studies

| Drug category | Total exposure | Temperature | Degradation level |
|---|---|---|---|
| Photolabile | $1.2\times10^6$ Lux h | 21°C | Complete or sufficient |
| Photostable | $6.0\times10^6$ Lux h | 21°C | No degradation |

## CONCLUSION

On the basis of the results of our study, cefoselis sulfate is classified as follows: in acidic conditions – very labile (0.01 M HCl, 8h, 40°C, 40% degradation), in alkaline conditions – extremely labile (0.01 M NaOH, 2h, 21°C, 85% degradation), in neutral conditions – labile (water, 12h, 90°C, 65% degradation), in the presence of an oxidative factor – labile (3% $H_2O_2$, 6h, 21°C, 17% degradation). In photostability studies cefoselis sulfate was photolabile.

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