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Characterization of the impurities and isomers in cefetamet pivoxil hydrochloride by liquid chromatography/time-of-flight mass spectrometry and ion trap mass spectrometry

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ABSTRACT

Ten impurities and isomers in cefetamet pivoxil hydrochloride drug substance made in China were separated and identified by HPLC-MS^n (TOF and TRAP). Their fragmentation patterns and structural assignment were studied based on the HPLC-MS^n data. Among the ten impurities and isomers, impurity VII was isolated by preparative HPLC, and its structure was confirmed by ^1H NMR data.

1. Introduction

Cefetamet pivoxil hydrochloride is one of the third generation cephalosporin antibiotics. It presents high activity against both gram-positive and gram-negative micro-organisms and resistance to β-lactamases, which makes it a widely used antibiotics. Cefetamet pivoxil hydrochloride is unstable and some degradation products are produced readily during the production of cefetamet pivoxil hydrochloride and storage. The clinical effect of cefetamet pivoxil hydrochloride will be impacted because some impurities have less antibacterial activities and more toxicities than cefetamet pivoxil hydrochloride. Therefore, it is necessary to characterize and control these impurities in order to meet the requirements of FDA. Fig. 1 shows the structure of cefetamet pivoxil hydrochloride.

To identify impurities, they should be collected by preparative liquid chromatography and then analyzed by NMR spectroscopy, and so on. It is evident that such a procedure is labor intensive and requires a large amount of degradation material. Moreover, this indirect technique can induce degradation of the compound of interest. Consequently, it is difficult to know whether the identified compound was truly an impurity or simply an artifact. This is especially true for β-lactams which have limited stability in organic solvents like methanol or acetonitrile. A direct coupling method is thus more suitable.

The on-line combination of liquid chromatography and mass spectrometry (HPLC-MS^n) has developed quickly as an identification tool. HPLC-MS^n combines separation capability of chromatography with qualitative advantages of mass spectrometry. A large amount of structural information can be obtained from LC–MS–MS and LC–MS–MS–MS analysis, which shows great superiority to identify the impurities. Structural identifications and analysis of the impurities in cephalosporin antibiotics have been reported [1–12], however, no report can be found regarding the impurities study in cefetamet pivoxil hydrochloride. The aim of this study was to separate and characterize unknown impurities in cefetamet pivoxil hydrochloride by HPLC-MS^n (TOF and TRAP), to isolate stable impurity VII by preparative HPLC, and confirm its structure by ^1H NMR.

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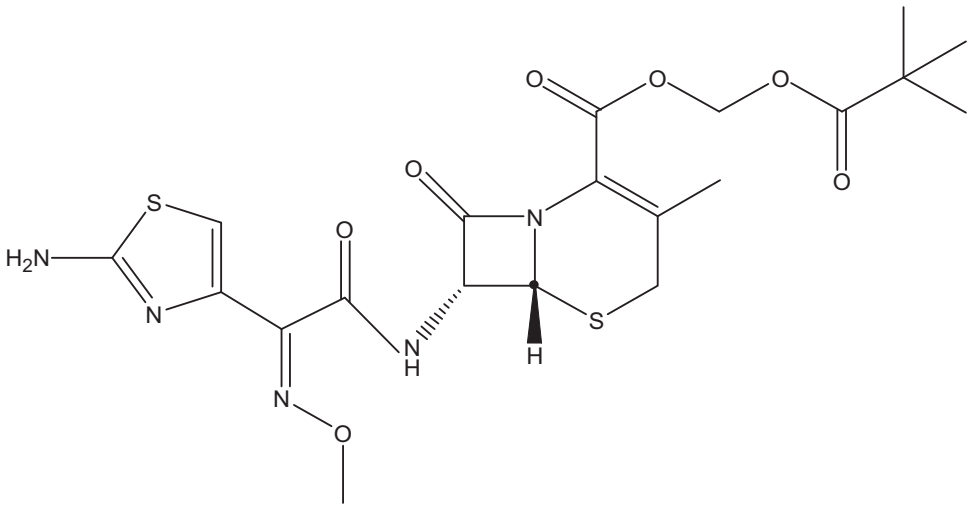

Fig. 1. The structure of cefetamet pivoxil hydrochloride.

2. Experimental

2.1. Chemicals and reagents

Cefetamet pivoxil hydrochloride drug substance (batch number: 070504) was provided by Sichuan Direction Pharmaceutical Co. Ltd. (Chengdu, China). Cefetamet acid reference substance was provided by Zhejiang Zhenyuan Pharmaceutical Co. Ltd. (Shaoxing, China). Methanol (Merck, HPLC grade, Darmstadt, Germany) and acetonitrile (Merck, HPLC grade, Darmstadt, Germany) were used to prepare mobile phase.

2.2. High performance liquid chromatography

An Agilent 1260 series liquid chromatography system (Agilent, Santa Clara, CA, USA) equipped with a binary pump and a UV detector was connected to an Agilent G1313A autosampler. Chromatographic separation was carried out at 30 °C using a Thermo Acclaim™ 120 C_{18} (250 mm × 4.6 mm, 5 μm) (Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of 10 mmol L^{-1} ammoniumacetate–methanol–acetonitrile (327:133:504).

The flow rate was 0.8 mL min^{-1}. Injection volume was 10 μL. The LC chromatogram of cefetamet pivoxil hydrochloride drug substance is shown in Fig. 2.

2.3. Mass spectrometry

LC–MS experiment was carried out on Agilent 6538Q TOF high resolution mass spectrometer and AB SCIEX 4000 Q TRAPTM composite triple quadrupole/linear ion trap tandem mass spectrometer (Applied Biosystems, Forster, CA, USA). The column effluent was split using a zero-dead-volume "T" connector, with approximately half of the flow being fed to the mass spectrometer. The mass spectrometer was equipped with an ESI source. The ionization mode was positive. The interface and parameters of TOF mass spectrometer were as follows: a capillary voltage of 3.0 kV, a cone voltage of 35 V, a source temperature of 120 °C, a dissolvation temperature of 300 °C, a cone gas flow of 50 L h^{-1} and a dissolvation gas flow of 400 L h^{-1}. The interface and parameters of TRAP mass spectrometer were as follows: nebulizer pressure [40 psi], dry gas pressure [40 psi], curtain gas pressure [10 psi], dry gas temperature (550 °C), spray capillary voltage (5000 V), CAD: high, DP: 70 V, EP: 10 V, CE: 35 V. TOF mass spectra results of 10 impurities in cefetamet pivoxil

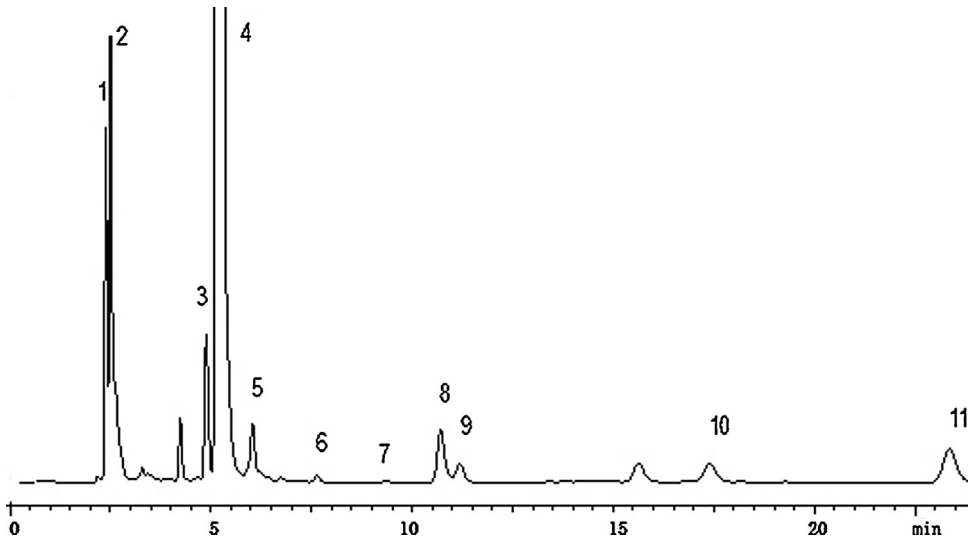

Fig. 2. Chromatogram of cefetamet pivoxil hydrochloride drug substance. (1) Impurity I, (2) impurity II, (3) impurity III, (4) cefetamet pivoxil, (5) impurity IV, (6) impurity V, (7) impurity VI, (8) impurity VII, (9) impurity VIII, (10) impurity IX, (11) impurity X.