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Analysis of etimicin sulfate by liquid chromatography with pulsed amperometric detection

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Abstract

A new method for determination of etimicin's (ETM) purity and content is developed by liquid chromatography (LC) and pulsed amperometric detection (PAD). A reversed-phase ion-pair LC method with pulsed amperometric detection on a gold electrode after post-added NaOH is described. The mobile phase consisted of an aqueous solution containing 0.033 mol L^{-1} oxalic acid, 0.012 mol L^{-1} heptafluorobutyric acid, and 210 mL L^{-1} acetonitrile with pH 3.40 adjusting by dilute NaOH solution. The total analysis time was not more than 30 min. The effects of the different chromatographic parameters on the separation were also investigated. A number of commercial samples of etimicin sulfate were analyzed using this method.

Keywords: Etimicin; Pulsed amperometric detection; Liquid chromatography

1. Introduction

Etimicin (ETM), which is mainly used as the sulfate, is a new semi-synthetic, water soluble aminoglycoside antibiotic obtained by chemical modification of gentamycin C_{1a} (Fig. 1) [1], It is active against both Gram-positive and Gram-negative bacteria, including strains which are resistant to other aminoglycosides and similar as netilmicin [2–4]. The oto- and nephro-toxicity of etimicin are substantially lower than those of other aminoglycisides antibiotics and even lower than netilmicin [3,4]. Nevertherless, etimicin still has a narrow therapeutic range and it must be careful to monitor the levels in the blood.

Etimicin is the 1-*N*-ethyl derivative of gentamycin C_{1a}. Therefore, gentamycin C_{1a} can be expected to be contained as a possible impurity in the samples. The 3″-*N*-ethyl (ETM-1), and 1,3″-*N*-ethyl (ETM-2) derivatives of gentamycin C_{1a}, and some intermediates such as 3,2′,6′-*N*-ethanoyl-gentamycin C_{1a} (P_1) and 1-*N*-ethyl-3,2′,6′-*N*-ethanoyl-gentamycin C_{1a} (P_2) can be also formed during synthesis of etimicin and they possessed weakly antibacterial activity [1]. As can be seen, neither etimicin nor its related substances contain a significant UV absorbing chromophore. Microbiological assay which is not able to distinguish between the main components and the impurities in the drug, reversed-phase liquid chromatography with pre-column derivatization with *o*-phthalaldehyde (OPA) [5] and with 1-fluoro-2,4-dinitrobenzene [6] in which derivatized etimicin was used as internal standard, has been described. However, no LC method has been described to analyze etimicin sulfate as a drug substance and to determine possible impurities. Evaporative light-scattering detection (ELSD) is prescribed in The Ph. Chinese for the determination of etimicin content [7], which was not able to determine possible impurities in the drug, either. Moreover, a volatile mobile phase is required for ELSD detection and it is low sensitive in detection. A wide variety of methods for the analysis of aminoglycoside antibiotics have been published over the years, including microbiogical assay [8], immunoassays [9,10], capillary electrophoresis [11], thin layer chromatography [12], gas–liquid chromatography [13], and high-performance liquid chromatography with pre-column derivatization with detection of UV [14,15], electrochemical [16] and mass spectrometry [17,18]. Etimicin has a structure of aminoglycoside and the methods mentioned above should also be applicable to

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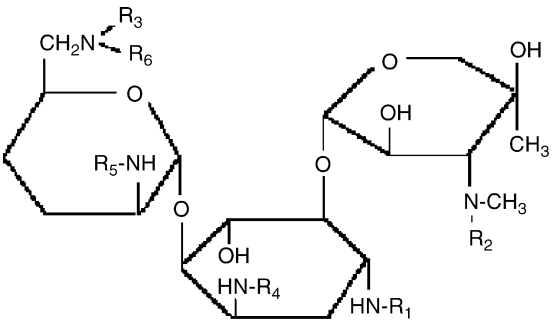

GMC_{1a}	R_1=R_2=R_3=R_4=R_5=R_6=H
ETM	R_1=C_2H_5, R_2=R_3=R_4=R_5=R_6=H
ETM-1	R_1=H, R_2=C_2H_5, R_3=R_4=R_5=R_6=H
ETM-2	R_1=R_2=C_2H_5, R_3=R_4=R_5=R_6=H
P_1	R_1=R_2=R_3=H, R_4=R_5=R_6=CH_3CO
P_2	R_1=C_2H_5, R_2=R_3=H, R_4=R_5=R_6=CH_3CO

Fig. 1. Structure of some etimicin components.

it. Since pre-column derivatization is cumbersome, time consuming and gives some problems with quantitation or results in unstable derivatives, electrochemical detection especially pulsed amperometric detection (PAD) is the better, pulsed amperometric detection has been demonstrated for the sensitive detection of numerous aminoglycoside antibiotics [19–24]. But up to now, the determination of etimicin with PAD has not been reported. To our knowledge, no paper has been published describing the composition of commercial etimicin samples.

Like other aminoglycoside antibiotics [19–24], etimicin is polybasic cations at low pH, which are high polar species, and it can interact with the ion-pair agents to form ion-pair compounds which are less polar than the parent aminoglycosides and are amenable to separation by the preferred techniques of reversed-phase HPLC. In this work an ion-pair chromatography combined with PAD is described. The mobile phases that were investigated and further optimized. The quality of separation on different stationary phases was compared. Finally, the method has been applied to analyze some commercial samples of etimicin.

2. Experiment

2.1. Reagents, reference substances, and samples

Distilled deionizes water of 18.2 MΩ cm was used throughout. Heptafluorobutyric acid (Alfa Aesar, Heysham, Lancs). Standard etimicin and gentamycin C_{1a} were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The 3-*N*″-ethyl and 1,3″-*N*-ethyl derivatives of gentamycin C_{1a} (ETM-1, ETM-2), 3,2′,6′-*N*-ethanoyl-gentamycin C_{1a} (P_1) and 1-*N*-ethyl-3,2′,6′-*N*-ethanoyl-gentamycin C_{1a} (P_2) were obtained from Jiangsu Institute of Microbiology (Wuxi, China). Three commercial samples of etimicin were provided by Hangzhou Aida Pharmaceutical Co. Ltd. (Hangzhou, China), Hailan Aike Pharmaceutical Co. Ltd. (Hailan, China), Changzhou Fangyuan Pharmaceutical Co. Ltd. (Changzhou, China), respectively. Oxalic acid and sodium hydroxide were of analytical grade. Methanol and acetonitrile were of HPLC-grade.

2.2. Apparatus

Chromatographic analyses were carried out using a P680 HPLC Pump (Dionex, Sunnyvale, CA, USA), with a fixed loop of 20 μL. Sodium hydroxide was added post-column using a pneumatic device, PC10 Pneumatic controller from Dionex (Sunnyvale, CA, USA). The ZORBAX Rx-C_8 column (250 mm × 4.6 mm I.D.) from Agilent (Palo Alto, CA, USA). German Century SIL C_{18} column (AQ 5 μm, 150 mm × 4.6 mm I.D.) from Dianlian Jiangshen Separating Science and Technology Company (Dianlian, China). Acclaim™ 120 C_{18} column (5 μm120 Å, 150 mm × 4.6 mm I.D.) from Dionex (Sunnyvale, CA, USA). The temperature of the column was maintained at 35 °C. ED50A Electrochemical Detector from Dionex (Sunnyvale, CA, USA) was equipped with a gold working electrode with a diameter of 3 mm, an Ag/AgCl reference and stainless steel counter electrode. The cell of the pulsed amperometric detector was placed in the air keeping the temperature at 35 °C. All the instrument control and data collection were performed by a Dionex Chromeleon 6.5.

2.3. Chromatography

The mobile Phase is consisted of an aqueous solution containing 0.033 mol L^{-1} of oxalic acid, 0.012 mol L^{-1} heptafluorobutyric acid, 210 mL L^{-1} acetonitrile, adjust the apparent pH to 3.4 with dilute sodium hydroxide solution. Filter the mobile phase through a 0.45 μm filter and sonicated before use. The flow rate was 1.0 mL min^{-1}. All substances to be analyzed were dissolved in the mobile phase. To allow pulsed amperometric detection, 0.52 mol L^{-1} NaOH was added post-column (0.5 mL min^{-1}) through a mixing-tee from a nitrogen pressurized reservoir (30 psi) and mixed in a packed reaction coil (Dionex, 375 μL), linking to the electrochemical cell. The flow rate for the addition of the base is not critical, but it should be reproducible between runs and must be pulse-free. It was necessary to raise the pH of the mobile phase to approximately 13 to improve the sensitivity of the detection [25]. The 0.52 mol L^{-1} NaOH solution was made starting from a 2.62 mol L^{-1} aqueous solution, which was pipetted into N_2 degassed water to avoid carbonates that foul the electrodes. For this reason, it is advisable to pipette the NaOH solution from the center of the bottle and to use only two-thirds of the bottle.

The time and voltage parameters for the pulsed amperometric detector were set as follows: E_1, E_2, and E_3 were, respectively +0.12, +0.70, and −0.60 V with the assigned pulse durations t_1: 0–0.40 s, t_2: 0.41–0.60 s, and t_3: 0.61–1.00 s, integration of the signal was done between 0.20 and 0.40 s.