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Hydrophilic interaction chromatography combined with tandem mass spectrometry method for the quantification of tobramycin in human plasma and its application in a pharmacokinetic study

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ABSTRACT

A fast, sensitive and specific hydrophilic interaction chromatography combined with tandem mass spectrometry (HILIC-MS/MS) method was developed for the determination of tobramycin in human plasma. With sisomycin as internal standard, the analysis was carried out on a hilic column (150 mm × 2.1 mm, 3.5 μm) using a mobile phase consisting of acetonitrile: 5 mM ammonium acetate and 0.1% formic acid (60:40, v/v). The detection was performed by tandem spectrometry via electrospray ionization (ESI). Linear calibration curves were obtained in the concentration range of 10.51–1051 ng/mL for tobramycin, with a lower limit of quantification of 10.51 ng/mL. The intra- and inter-day precision (RSD) values were below 15% and accuracy (RE) was 1.3–5.7% at all QC levels. The method was applicable to the clinical study of the pharmacokinetics of tobramycin in healthy volunteers.

1. Introduction

Tobramycin is a broad spectrum aminoglycoside antibiotic and widely used for the treatment of infections caused by different bacteria. It is particularly useful for the treatment of *P. aeruginosa* in patients with cystic fibrosis. It has a narrow therapeutic range and monitoring of the drug is required to reduce serious side effects such as nephro and ototoxicity [1–4]. Dosage alterations based on the results of drug monitoring have been found to improve efficacy and minimize toxicity [5].

Analysis of tobramycin is quite challenging due to its physicochemical properties. The lack of a UV chromophore makes direct UV detection unfeasible. Therefore, traditionally, analysis of aminoglycosides has been performed using derivatization with ultraviolet, fluorescent or electrochemical detection [6–14]. However, derivatization methods require complicated sample preparation procedures. Moreover, the sensitivity of these techniques is relatively low. These may not meet the requirement of desired throughput speed and sensitivity in biosample analysis. An alternative for the detection of aminoglycosides is mass spectrometry with high selectivity and specificity. Thus, LC–MS [15] and LC–MS/MS [16,17] were used in the determination of tobramycin in biosamples with lower limit of quantification (LLOQ) of 200 ng/mL [15], 50 ng/mL [16], 150 ng/mL [17] and 100 ng/mL [18]. However, aminoglycosides are extremely hydrophilic compounds. Due to their highly polar characteristics, the use of simple chromatographic methods for separation are not applicable: aminoglycosides are positively charged at the pH range employed in reversed-phase HPLC and are not retained on conventional C_{18} bonded silica columns without an ion pairing reagent [16,18]. Ion-pair chromatography [17] has been described to prolong the retention of aminoglycosides. But electrospray ionization (ESI) MS detection of ion pairs is not ideal because the sensitivity of mass spectrometry will be reduced (due to suppression of ionization). An alternative for separation of hydrophilic compounds is hydrophilic interaction chromatography (HILIC). HILIC is a type of liquid chromatography that allows high-resolution separation of highly polar compounds. Only one HILIC-MS/MS method was used in the determination of tobramycin in serum [19]. However, this method needs a time-consuming and expensive sample – extraction. Moreover, this method has a long analysis time (10 min) and a high LLOQ (100 ng/mL).

This paper describes a fast, selective and highly sensitive approach, which enables the determination of tobramycin at 10 ng/mL in plasma with good accuracy using hydrophilic

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interaction chromatography combined with tandem mass spectrometry (HILIC-MS/MS) method. The total analysis time was 3.5 min.

2. Experimental

2.1. Reagents and chemicals

Tobramycin reference standard (99.2% of purity) and sisomycin (99.3% of purity) (Fig. 1) were obtained from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). Acetonitrile, ammonium acetate (HPLC grade) and formic acid (HPLC grade) were purchased from Dikma (11 Orchard Road, Suite 106, Lake Forest, CA 92630, USA). All other chemicals were of analytical grade and purchased from Guangzhou chemical reagent factory (Guangzhou, China). Water was purified by redistillation and filtered through 0.22 μm membrane filter before use (Grade 1 water).

2.2. Apparatus and operation conditions

2.2.1. Liquid chromatography

The chromatography was performed on Agilent 1200 system with autosampler and column oven enabling temperature control of analytical column. The Inertsil HILIC column (150 mm × 2.1 mm, 3.5 μm) was employed. The column temperature was maintained at room temperature. The mobile phase consisted of acetonitrile: 5 mM ammonium acetate and 0.1% formic acid (60:40, v/v) at an isocratic flow rate of 0.30 mL/min. The injection volume was 5 μL.

2.2.2. Mass spectrometry

Detection was performed on a Sciex API 4000 Qtrap MS system equipped with a Turbo Ionspray interface. Mass spectral setting to operate in positive-ion mode (ESI+) were: ion source voltage: 5000 V; ion source temperature: 400 °C; collision gas (N_2): medium; curtain gas: 20 psi; nebulizer gas: 40 psi; auxiliary gas: 60 psi. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 468.2 → m/z 163.0 for tobramycin and m/z 448.2 → m/z 160.0 for sisomycin, respectively. The product ion spectra of tobramycin and sisomycin are shown in Fig. 2. Data acquisition and processing were performed with the Analyst software 1.4.2.

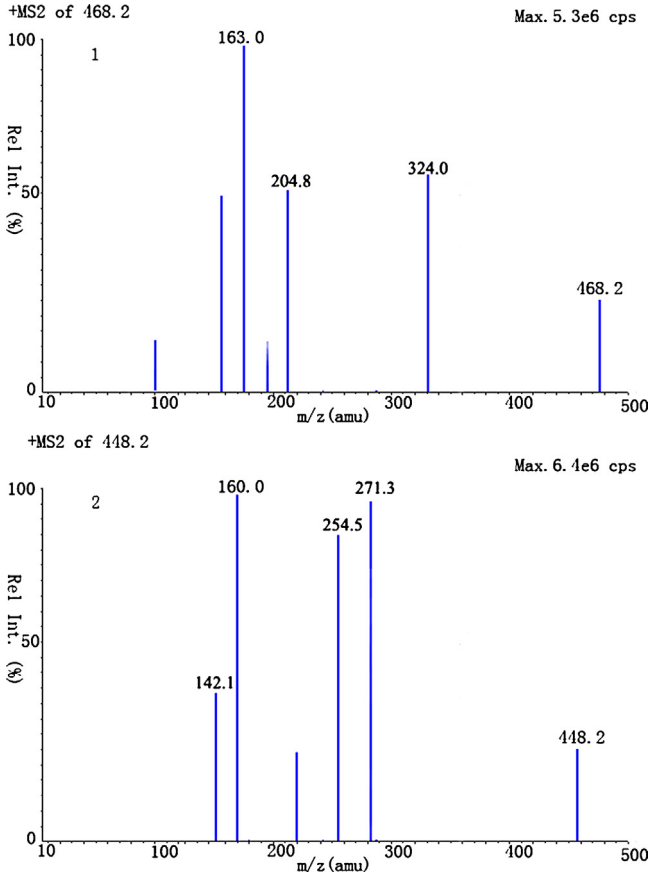

Fig. 2. Product ion spectra of tobramycin (1) and sisomycin (2).

2.3. Preparation of standards and quality control samples

Standard stock solutions of tobramycin and sisomycin were both prepared in water at the concentration of 105.1 μg/mL and 112.1 μg/mL. Then the stock solutions were serially diluted with methanol: water (1:1) to provide working standard solutions of desired concentrations. All the solutions were stored at 4 °C.

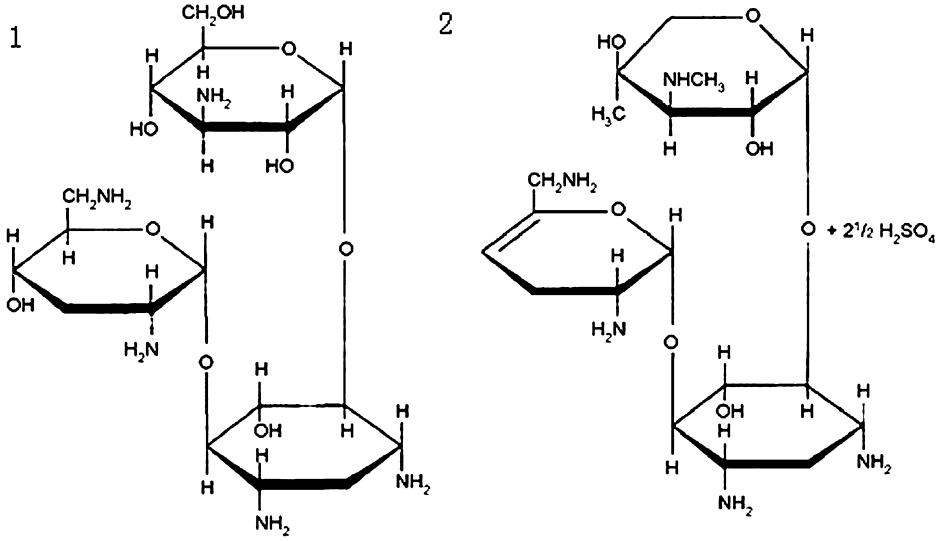

Fig. 1. Chemical structures of tobramycin (1) and sisomycin (I.S.) (2).