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Determination of the active metabolite of moguisteine in human plasma and urine by LC–ESI-MS method and its application in pharmacokinetic study☆

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ABSTRACT

In this study, a sensitive and reproducible electro-spray ionization liquid chromatography–mass spectrometry (LC–ESI-MS) method was established to determine the concentration of M1, the main active metabolite of moguisteine in human plasma and urine. The analysis was performed on a Diamonsil® $C_{18}(2)$ column (150 mm × 4.6 mm, 5 μm) with the mobile phase consisting of 0.1% formic acid–acetonitrile (57:43, v/v, pH = 3.0) at a flow rate of 0.8 mL min^{-1}. The pseudo-molecular ions $[M+H]^+$ (*m/z* 312.2 for M1 and 446.3 for glipizide) were selected as the target ions for quantification in the selected ion monitoring (SIM) mode. Plasma samples were analyzed after being processed by acidification with formic acid and protein precipitation with acetonitrile. Urine samples were appropriately diluted with blank urine for analysis. Calibration curve was ranged from 0.02 to 8 μg mL^{-1}. The extraction recovery in plasma was over 90%. Both the inter- and intra-day precision values were less than 7.5%, and the accuracy was in the range from −6.0% to 6.0%. This is the first reported LC–ESI-MS method for analyzing M1 in human plasma and urine. The method was successfully applied to the pharmacokinetic study after oral administration of single-dose and multiple-dose of moguisteine tablets in healthy Chinese subjects.

1. Introduction

Moguisteine, (*R*,*S*)-2-(2-methoxyphenoxy)-methyl-3-ethoxycarbonyl-acetyl-1,3-thiazolidine) (Fig. 1), is a novel peripheral non-narcotic antitussive agent by having inhibitory effect on the airway [1,2]. The antitussive function is similar to that of codeine, but no interaction with central opioid receptors is observed [3]. Moguisteine is effective against experimentally induced cough in a dose related manner. It was also well tolerated after oral administration of healthy subjects [4–6]. The ATP-sensitive K^+ channels in tracheobronchial tract may play an important role in the antitussive effect of moguisteine inhaled [7,8].

Moguisteine is a prodrug. Previous studies showed that moguisteine underwent a prompt and complete presystemic hydrolysis to produce its principal active metabolite, the free carboxylic acid, named as M1. M1 is partly metabolited to M2, the sulfoxidation derivative. The pharmacological activity of M2 is unknown, and there is no M2 standard from the market. Thus, M1 as the target compound is determined for studying the pharmacokinetics of moguisteine. So far, there is no study on the metabolism of moguisteine. The potential interaction of moguisteine with other drugs is still expected to be researched in future.

An HPLC method for the analysis of M1 in plasma and urine samples has been reported previously [1], however, its disadvantages with the complex sample preparation and the long injection time were presented. In the present study, we aim to develop a simple and sensitive LC–ESI-MS method for the determination of M1 in plasma and urine samples. It was also successfully applied to evaluate the pharmacokinetic characteristics of moguisteine after single-dose and multi-dose of moguisteine tablets in healthy Chinese subjects, which provides guidance for clinical application.

2. Materials and methods

2.1. Chemicals and instrumentation

Moguisteine tablet (batch 091224) and M1 standard (purity 99.5%) were obtained from Shanchuan Pharmaceutical Co., Ltd. (Shandong, China). Glipizide standard (purity 99.1%), used as the internal standard (I.S.), was obtained from Yunmen Pharmaceutical Co., Ltd. (Shandong, China). Methanol and acetonitrile of HPLC grade were from J.T. Baker. Formic acid, HPLC grade, was from Acros

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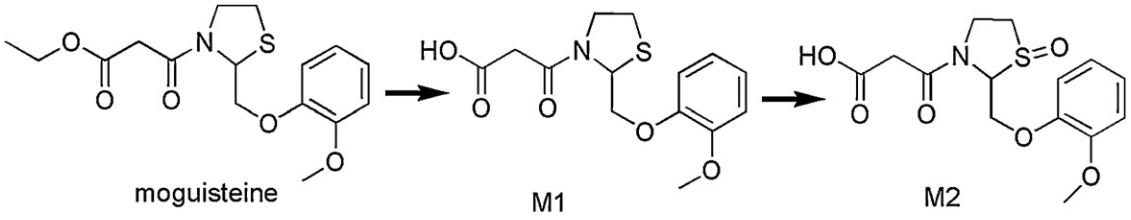

Fig. 1. Metabolic pathway of moguisteine in man.

Organics. Blank human plasma was provided by Shandong Blood Center of China.

Agilent 1100 series LC system (equipped with a quadruple pump, a vacuum degasser, a thermostated column compartment and an autosampler) and an Agilent 1946D single quadrupole mass spectrometric detector equipped with ESI source were used for mass detection (Agilent Technologies, USA). Data analysis was accomplished by using Agilent ChemStation software (Version A.10.01).

2.2. LC–ESI-MS conditions

The separation was performed on a Diamonsil® $C_{18}(2)$ column (150 mm × 4.6 mm, 5 μm), and eluted by the mobile phase of 0.1% formic acid–acetonitrile (57:43, v/v) at a flow rate of 0.8 mL min^{-1}. The column temperature was maintained at 25 °C by using a thermostated column compartment.

The MS conditions were as follows: positive ion mode; capillary voltage, 4000 V; source temperature, 300 °C; drying gas flow, 12 L min^{-1}; nebulizer gas, 50 psi; fragment electric voltage, 70 V for M1 and 100 V for glipizide. The pseudo-molecular ions $[M+H]^+$ (m/z 312.2 for M1 and 446.3 for glipizide) were selected as the target ions for quantification in the selected ion monitoring (SIM) mode.

2.3. Standard solutions and sample preparation

M1 and glipizide (I.S.) standards were accurately weighted and dissolved in methanol for obtaining 1.0 mg mL^{-1} of stock solution. Stock solution was further diluted with methanol for 100, 10 and 1 μg mL^{-1} working solutions of M1, and 10.0 μg mL^{-1} of I.S. All were stored at 4 °C, and equilibrated to room temperature before use (approximately 15 min).

Two hundred microliters of plasma sample was mixed with 20 μL of glipizide (I.S.) working solution, 20 μL of 10% formic acid and 500 μL of acetonitrile (protein precipitation reagent), vortex-mixed for 2 min, and then centrifuged at 10,800 rpm for 10 min. The supernatant was transferred to the vial for analysis. 10 μL of urine sample was appropriately diluted to 1 mL with blank urine, added with 50 μL of I.S. working solution, 50 μL of 10% formic acid, vortex-mixed for 1 min, and centrifuged at 10,800 rpm for 5 min, and then injected for analysis.

2.4. Method validation

The specificity of the method was evaluated by comparing chromatograms of the standard solutions of M1 and I.S., blank plasma, blank urine, blank plasma or blank urine spiked with M1 and I.S., plasma or urine from a subject after oral administration of moguisteine tablets. At least six blank plasma or urine samples from different individuals were examined for the specificity of the method. Calibration curves were made by analyzing spiked calibration samples at 0.02, 0.1, 0.5, 1, 2, 4 and 8 μg mL^{-1} on each analysis day. The linearity of calibration curves was assessed by linear regression with a weighting factor of the reciprocal of the concentration squared ($1/x^2$).

The matrix effects were evaluated by extracting fifteen different lots of blank plasma or urine and then spiking with M1 at three concentration levels (0.05 μg mL^{-1}, 1 μg mL^{-1} and 7 μg mL^{-1}) and I.S. in five replicates at each concentration level, respectively. The corresponding peak areas were compared with those of standard solutions, and peak area ratio was used to evaluate the matrix effect. The matrix suppression was assessed by post-column infusion of an analyte with a syringe pump post column into the MS detector. The extracted blank matrix was injected by an autosampler onto the analytical column. The MS monitored the signal level from post-column infused analyte solution when injecting blank plasma or urine sample.

The extraction recoveries of M1 were evaluated by comparing the areas of M1 extracted from plasma or urine with those of standard solutions at equivalent concentrations, and five replicates at each concentration level were disposed with the established extraction procedure. The extraction recovery of I.S. was evaluated in a similar way at the working concentration. The lowest limit of quantification (LLOQ) was evaluated by analyzing five replicates of spiked samples at the concentration of 0.02 μg mL^{-1} with acceptable precision.

Accuracy and precision were assessed by analyzing spiked samples at three concentration levels (0.05 μg mL^{-1}, 1 μg mL^{-1} and 7 μg mL^{-1}) for three consecutive days, and five replicates at each concentration level were applied. The mean value of RE for the accuracy should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%. The relative standard deviation (RSD) for the precision determined at each concentration level should not exceed 15% except at LLOQ, where it should not exceed 20%.

The stability of M1 in plasma or urine was evaluated by analyzing samples with five replicates at each concentration level, after storage at −20 °C for 1, 7 or 28 days respectively, or after one or two freeze–thaw cycles (−20 °C to 25 °C).

2.5. Pharmacokinetic studies

The study was approved by the Ethics Committee of the College of Medicine, Shandong University and conformed to the principles of the Declaration of Helsinki. Subjects signed informed consent before any screening item being performed.

2.5.1. Single-dose study

Thirty healthy Chinese subjects, half male and half female, aged of 20–28 years, were randomly assigned to three groups and receive a single oral dose of 200 mg, 400 mg and 600 mg moguisteine tablets, respectively, with 200 mL of warm water after an overnight fast (10 h). Blood samples were collected before and 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 h after administration. Samples were immediately centrifuged at 5000 rpm for 5 min, separated and transported into two EP tubes, labeled and stored at −20 °C for pending analysis. Urine samples of subjects with 400-mg dose were collected at time zero (blank), 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–10, 10–12, 12–24 h after administration. The volume of urine was recorded and stored at −20 °C until assayed.