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Original Article

Determination of torasemide in human plasma and its bioequivalence study by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry ☆

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ABSTRACT

A sensitive and selective method using high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (HPLC–ESI–MS) to determine the concentration of torasemide in human plasma samples was developed and validated. Tolbutamide was chosen as the internal standard (IS). The chromatography was performed on a Gl Sciences Inertsil ODS-3 column (100 mm × 2.1 mm i.d., 5.0 μm) within 5 min, using methanol with 10 mM ammonium formate (60:40, v/v) as mobile phase at a flow rate of 0.2 mL/min. The targeted compound was detected in negative ionization at *m/z* 347.00 for torasemide and 269.00 for IS. The linearity range of this method was found to be within the concentration range of 1–2500 ng/mL (r=0.9984) for torasemide in human plasma. The accuracy of this measurement was between 94.05% and 103.86%. The extracted recovery efficiency was from 84.20% to 86.47% at three concentration levels. This method was also successfully applied in pharmacokinetics and bioequivalence studies in Chinese volunteers.

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1. Introduction

Torasemide (1-isopropyl-3-([4-(3-methyl-phenylamino) pyridine]-3-sulfonyl) urea, CAS: 56211–40–6, Fig. 1A) is a new high-efficiency loop diuretic, acting the same as other pyridine sulfonylurea medications in blocking the Na^+–K^+–$2Cl^-$ carrier to promote excretion of water on the thick ascending limb of the loop of Henle [1]. It has been successfully used to treat oedematous states associated with chronic congestive heart failure [2], renal disease [3] and hepatic cirrhosis [4], and low-dose torasemide has also been used to control arterial hypertension [5]. Research proves that torasemide is safer and better tolerated than furosemide in chronic heart failure patients and associated with a trend in reducing all-cause mortality [6]. Lower incidences of abnormal serum potassium levels and hypotension were also observed in patients receiving torasemide compared with those receiving other diuretics [7].

Torasemide is well absorbed and yields a bioavailability of 80%–90%. It is highly bound to protein (99%) [1]. The time of peak reaches at 1 h, and the elimination half-life varies from 3 to 4 h. Torasemide is metabolized by the hepatic cytochrome P450 system, followed by both renal and non-renal excretion of metabolites. The total amount of torasemide and metabolites in urine is 83%, including 25% of torasemide, 11% of M1, 3% of M3, and 44% of M5. Both M1 and M3 are active and probably contribute to the diuretic action of torasemide [8]. Due to its high activity, a low therapeutic dose is required.

A rapid, sensitive and reliable determination of torasemide in human plasma is essential to evaluating its pharmacokinetics in clinical trials and bioequivalence researches. Several methods have been reported including gas chromatography–tandem mass spectrography (GC–MS) [9], capillary zone electrophoresis (CZE) [10], high-performance liquid chromatography–tandem ultraviolet detection (HPLC–UV) [11–19] and high-performance liquid chromatography–tandem electrochemical detection (HPLC–ED) [20–22] to determine the concentration of torasemide as a single analyte [9,12,18–21] or in combination with its important metabolites [10,11,13–16] or with other diuretic drugs [23,24] in plasma [11–19] or other biological samples [9,13,20–24]. Barroso et al. [9] applied GC–MS to determine the concentration of torasemide in human urine, which needs complicated preparation for derivatization and is not suitable for bioequivalence studies of large

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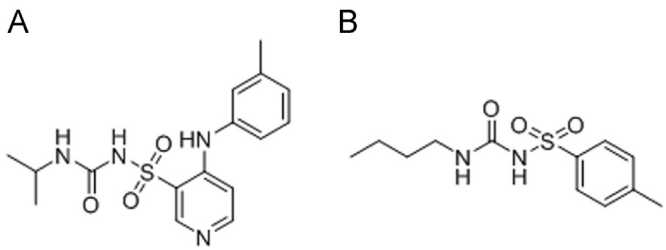

Fig. 1. Structure of (A) torasemide and (B) tolbutamide (IS).

quantity of samples. Additionally, several studies used HPLC with low sensitivity detectors [11–21]. These methods cannot detect the low concentration points such as the last several points in elimination phase, which will lead to inaccurate pharmacokinetic parameters. In this case, high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS) can be a better option because it combines advantages of HPLC and MS, which allows a rapid, sensitive and selective quantification of drugs in complex biological samples. However, there are only few reports regarding the determination of torasemide concentration in human plasma using HPLC–MS.

In this study, a new method using HPLC–ESI–MS was developed to determine the concentration of torasemide in human plasma rapidly, sensitively and selectively. This method was successfully applied in pharmacokinetics and bioequivalence studies in healthy Chinese volunteers.

2. Materials and methods

2.1. Solvent and chemicals

Torasemide test tablets (Batch No: 441104) were supplied by Jiangsu D&R Pharmaceutical Corporation (Taizhou, China). Torasemide reference tablets (Batch No: 14052320) were purchased from SZYY Group Pharmaceutical Limited (Taizhou, China). Torasemide reference standard (Batch No: 100605–200401) was obtained from National Institute for Food and Drug Control (Beijing, China). Tolbutamide (internal standard, IS, Fig. 1B) standard (Batch No: 40716, 99.9% purity) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Glibenclamide standard (Batch No: 121633–201017) was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol of chromatographic grade was purchased from Merck, Germany. Ammonium formate, ethyl acetate and hydrochloric acid were of analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). All other chemicals were of analytical grade. Double distilled water was prepared by passing through Milli-Q water System (Merck Millipore, Massachusetts, U.S.).

2.2. Instrumentation and operation condition

2.2.1. Liquid chromatography

Liquid chromatography was performed using a Shimadzu LC-10AD HPLC system consisting of an autosampler (SIL-HTc). Chromatography was carried out with Gl Sciences Inertsil ODS-3 column (100 mm × 2.1 mm i.d., 5.0 μm) using methanol: 10 mM ammonium formate (60:40, v/v) as mobile phase. The temperatures of column and autosampler were maintained at 40 °C and 15 °C, respectively. The analysis was completed in 5 min at a flow rate of 0.2 mL/min. Data acquisition and processing was accomplished using Shimadzu LC–MS solution software for LCMS-2010A system.

2.2.2. Mass spectrometry

The ESI source was set at negative ionization mode. The $[M-H]^-$, *m/z* 347.00 for torasemide and $[M-H]^-$, *m/z* 269.00 for tolbutamide were selected as detecting ions, respectively. The quantification was performed via peak area. MS operating conditions were optimized as follows: drying gas 1.5 L/min, curved desolvation line (CDL) temperature 250 °C, block temperature 200 °C and probe voltage 1.65 kV.

2.3. Preparation of standard solution

Standard stock solutions of torasemide (1 mg/mL) and tolbutamide (1 mg/mL) were prepared in methanol and stored at 4 °C before use. Standard working solutions of torasemide for calibration curve and quality control (QC) were prepared by diluting standard stock solution with mobile phase to 4, 20, 40, 200, 2000 ng/mL and 10 μg/mL. IS working solution was prepared by diluting standard stock solution with mobile phase to 10 μg/mL.

2.4. Sample preparation

Sample preparation was performed by liquid–liquid extraction. Two hundred microliters of plasma samples were transferred to 10 mL centrifuge tubes and extracted by 2.0 mL of ethyl acetate after addition of 10 μL of IS working solution (10 μg/mL) and 20 μL of 10% hydrochloric acid solution. The mixture was vortex-mixed for 3 min, and then centrifuged at 3500 rpm for 10 min. 1.5 mL of organic layer was transferred to another clean glass tube and evaporated under a steady stream of nitrogen to dryness in a water bath at 40 °C. The residue was re-dissolved in 200 μL of mobile phase and vortexed for 30 s. Following centrifugation (16,000 rpm, 10 min), aliquots of 5 μL of the supernatant were injected into the HPLC–ESI–MS system.

2.5. Calibration curve and QC samples

Calibration curves were prepared by spiking blank plasma with appropriate amount of torasemide working solution mentioned above to yield concentrations of 1, 3, 10, 50, 200, 800, 1500, 2000 and 2500 ng/mL. The following steps were the same as those described in Section 2.4. The blank plasma samples (with or without IS) were also analyzed to confirm absence of interferences, but the results for blank samples were not used as parts of the calibration curves.

QC samples, which were analyzed at the same time with test samples, were prepared at concentrations of 3 (low level), 800 (middle level) and 2000 ng/mL (high level). The total amount of QC samples was no less than 5% of the test samples in the same batch.

2.6. Method validation

The method was validated according to the bioanalytical method validation guidance currently accepted by US Food and Drug Administration (USFDA) [25].

2.6.1. Specificity

The specificity of this method was investigated by preparing and analyzing 6 blank plasma samples from different sources of individual humans. It was assessed by comparing the chromatograms obtained from blank plasma with those from the sample spiked with torasemide at lower limit of quantitation (LLOQ). Each blank sample was also tested for the visible interference.

2.6.2. Linearity, LLOQ and limit of determination (LOD)

Linearity was completed in 5 replicates at concentration levels

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