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Determination of ranolazine in human plasma by LC–MS/MS and its application in bioequivalence study

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ABSTRACT

A simple, sensitive and specific liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for quantification of ranolazine in human plasma. The analytical method consists in the precipitation of plasma sample with methanol, followed by the determination of ranolazine by an LC–MS/MS. The analyte was separated on a Peerless Cyano column (33 mm × 4.6 mm, 3 μm) an isocratic mobile phase of methanol–water containing formic acid (1.0%, v/v) (65:35, v/v) at a flow rate of 1.0 ml/min. Protonated ions formed by a turbo ionspray in positive mode were used to detect analyte and internal standard (IS). The MS/MS detection was made by monitoring the fragmentation of m/z 428.20 → 279.50 for ranolazine and m/z 448.30 → 285.20 for internal standard on a triple quadrupole mass spectrometer. The method was validated over the concentration range of 5–2000 ng/ml for ranolazine in human plasma with correlation coefficient of 0.9937 (S.D.: ±0.00367, range: 0.9895–0.9963). The accuracy and precision values obtained from six different sets of quality control samples analyzed in separate occasions ranged from 94.53 to 117.86 and 0.14% to 4.56%, respectively. Mean extraction recovery was 82.36–94.25% for three quality control (QC) samples and 88.37% for IS. Plasma samples were stable for three freeze–thaw cycles, or 24 h ambient storage, or 1 and 3 months storage at −20 °C. Processed samples (ready for injection) were stable up to 72 h at autosampler (4 °C). The developed method was successfully applied for analyzing ranolazine in plasma samples for a bioequivalence study with 12 healthy volunteers.

1. Introduction

Ranolazine, (±)-N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-1-piperazine acetamide (Fig. 1) is an interesting anti-anginal and anti-ischemic agent [1]. In January 2006, the U.S. Food and Drug Administration (FDA) approved ranolazine for use in chronic stable angina following completion of a study mandated the FDA's special protocol assessment process, with the reported data in Journal of the American College of Cardiology (JACC) [2]. Ranolazine is the first approved agents from a new class of anti-anginal drug in almost 25 years. Ranolazine is believed to have its effects via altering the trans-cellular late sodium current. It acts by altering the intracellular sodium level that ranolazine affects the sodium-dependent calcium channels during myocardial ischemia [3]. Thus, ranolazine indirectly prevents the calcium overload that causes cardiac ischemia [4].

Ranolazine also modulates the metabolism of ischemia myocardial cells and improves the efficiency of oxygen use, by increasing myocardial glucose oxidation and decreasing fatty acid oxidation [5,6]. Ranolazine is extensively metabolized in the liver by the cytochrome P450 (CYP) 3A and 2D6 enzymes, with 5–10% being excreted unchanged by the kidneys [7]. Three major metabolites of ranolazine are produced by dearylation, O-demethylation and N-dealkylation, which are all at levels greater than 10% of the parent drug [7,8].

Since ranolazine lacks strong characteristic UV absorption, a HPLC-UV detection method is not sensitive and selective for the determination of ranolazine in biological samples [9]. Herron et al. developed a LC–MS method with solid-phase extraction (SPE) procedure for quantification of ranolazine and its metabolites in human plasma, but the method was not sensitive enough for pharmacokinetic studies and did not provide a detailed description of the method [10]. Recently, two LC–MS methods with selected ion monitoring (SIM) have been published for the quantitation of ranolazine in rat plasma, but the lower limit of quantitation (LLOQ) was above 20 ng/ml [11,12]. Hence the main objective of this work was to develop a simple one-step precipitation, sensitive, accurate and reliable mass spectrometry (LC–MS/MS) method for the quantification of ranolazine in human plasma.

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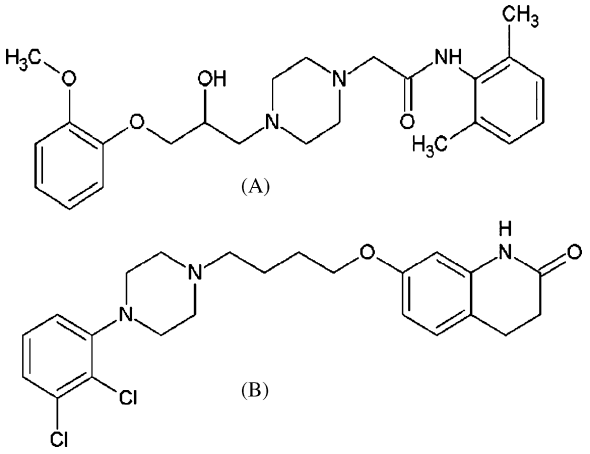

Fig. 1. Chemical structure of: (A) ranolazine and (B) aripiprazole (IS).

2. Experimental

2.1. Materials and reagents

Ranolazine dihydrochloride (>99%) was obtained from Ravenbhel Healthcare Pvt. Ltd. (Jammu, India). Aripiprazole (Fig. 1) (>99%) used as internal standard (IS) was supplied by Sun Pharmaceutical Industries Ltd. (Mumbai, India). Formic acid (98%) (analytical-reagent grade) and methanols (HPLC grade) were purchased from Merck Pvt. Ltd. (Mumbai, India). HPLC grade water (resistivity of 18 M cm) generated from Milli Q water purification system was used throughout the analysis. The blank human plasma with EDTA-K_3 anticoagulant was collected from Clinical Pharmacological Unit (CPU) of Bioequivalence Study Centre, Jadavpur University, Kolkata, India.

2.2. LC–MS/MS

The liquid chromatographic (LC) system consists of LC-20AD pump, SIL-20AC autosampler and CTO-10ASvp column oven (Shimadzu, Kyoto, Japan). The LC–MS/MS system consisting of turbo spray and atmospheric pressure ionization source (API-2000) with triple quadrupole tandem mass spectrometer (AB Sciex Instruments, Foster, CA) was used for quantitative determination of ranolazine in plasma. Data integration was performed with Analyst 1.4.1 software version (AB Sciex Instruments, Foster, CA).

LC separation was performed on a Peerless Cyano column (33 mm × 4.6 mm, 3 μm) from Chromatopak, Mumbai, India. The mobile phase consists of a mixture of methanol–water containing formic acid (1.0%, v/v) (65:35, v/v). The LC system was operated isocratically at 1 ml/min. The column eluent was split and approximately 400 μl was introduced in the mass spectrometer.

A triple quadrupole mass spectrometer (MS–MS) was used with API source and channel electron multiplier (CEM) detector in positive ion detection mode. The collision energy (CE) and other parameters for the analyte and IS were optimized by infusing each compound solution with a concentration of 500 ng/ml in water. A high voltage of 5.5 kV was applied to the spray needle. The source temperature was set at 550 °C, using nitrogen (5.0 grade) at 7 l/min as auxiliary gas and zero grade air as nebulizer gas at a pressure of 80 psi. The setting of curtain gas and collision gas flow at instrument was 15 and 12 (arbitrary scale), respectively. Multiple reaction-monitoring (MRM) mode was used for scanning throughout this study. The transitions selected were m/z 428.20 → 279.50 and m/z 448.30 → 285.20 for ranolazine and IS, respectively, with a dwell time of 200 ms per transition.

2.3. Preparation of standard and quality control samples

The stock solutions of analyte and IS were prepared by dissolving the accurately weighted standard compound in water to give final concentration of 1 mg/ml. The working solutions of 50, 250, 1000, 2500, 5000, 10,000 and 20,000 ng/ml of the analyte and 2500 ng/ml of IS were prepared from the stock solution. These working solutions of analyte and IS were used to prepare the calibration and quality control (QC) samples. A seven-point standard curve was prepared by spiking the 0.1 ml of working solution of analyte and IS into the 0.8 ml blank plasma to obtain final concentrations of 5, 25, 100, 250, 500, 1000 and 2000 ng/ml for the analyte and 250 ng/ml for IS. All stock solutions and working standard solutions were stored in polypropylene vials at −20 °C freezer.

The linear regression of the peak area ratio of analyte/IS vs. concentration using a weighted 1/concentration^2 was used to obtain calibration curve. The regression equation of the calibration curve was then used to calculate the plasma concentration. The back calculated values of the concentrations were statistically evaluated.

QC samples were made using the stock solution. Four levels of QC samples in plasma were 5.0 (lower limit of quantitation), 15 ng/ml (low), 1000 ng/ml (medium), and 1600 ng/ml (high) for the analyte. QC samples were prepared in a 50-ml pool, then aliquoted into pre-labeled 2 ml polypropylene vials and stored at −20 °C.

2.4. Sample preparation

The samples were prepared by taking 0.9 ml of plasma sample in polypropylene tube. Then 0.1 ml of IS working solution (2500 ng/ml) was added to each tube and all the samples were vortex mixed for 30 s. Then 1.0 ml of methanol was added and mixed for 15 min by cyclo mixer. All the samples were centrifuged for 15 min at 5000 rpm. 1.0 ml supernatant clear solution was separated and filtered through 0.2 μm membrane filter. The resulting samples were transferred into a 1.0 ml glass vial, which was loaded into autosampler cabinet, and 25 μl aliquot was injected into the LC–MS/MS system.

2.5. Method validation

The method was validated for selectivity, linearity, precision, accuracy, recovery and stability according to the principles of the Food and Drug Administration (FDA) industry guidance [13]. Three validation batches were processed on three separate days. Each batch included two set of calibration standards and six replicates of LLOQ, low-, medium-, and high-concentrations of QC samples.

Accuracy and precision were determined by analyzing six replicates of three QC samples (low-, medium-, and high-concentration). Accuracy (DEV) was determined as percent difference between the mean observed concentration and the nominal concentration [14]:

$$\mathrm{DEV}\,(\%) = \frac{\text{nominal} - \text{observed}}{\text{nominal}} \times 100$$

The precision of the assay was assessed by the between-run and within-run precision. The between-run precision (BRP) was defined as

$$\mathrm{BRP}\,(\%) = \frac{\sqrt{(\mathrm{MS_{bet}} - \mathrm{MS_{wit}})/n}}{\mathrm{GM}} \times 100$$

The within-run precision (WRP) was calculated as

$$\mathrm{WRP}\,(\%) = \frac{\sqrt{\mathrm{MS_{wit}}}}{\mathrm{GM}} \times 100$$