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Short communication

Development and validation of a sensitive U-HPLC–MS/MS method with electrospray ionization for quantitation of ranolazine in human plasma: Application to a clinical pharmacokinetic study

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ABSTRACT

A simple, sensitive and high-throughput ultra high-performance liquid chromatography electrospray ionization mass spectrometry (U-HPLC–ESI-MS/MS) method has been developed and validated for the determination of ranolazine in human plasma. Propafenone was employed as the internal standard (I.S.). The analytes were chromatographically separated on a BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm) with a mobile phase consisting of acetonitrile and aqueous ammonium acetate solution (0.06% formic acid, 7.5 mmol L^{-1} ammonium acetate, 40:60, v/v). Detection of the analytes was achieved using positive ion electrospray ionization via multiple reactions monitoring mode. The mass transitions were m/z 428.3 → 279.3 for ranolazine and m/z 342.4 → 115.9 for propafenone. The assay was linear over the concentration range 1–3000 ng mL^{-1}, with correlation coefficients ≥0.997. The intra- and inter-day coefficients of variation were less than 8.9% in terms of relative standard deviation and accuracy ranged from 93.0 to 108.9% at all quality control levels. The validated method was a simple sample preparation procedure and short run-time (<2.0 min) method, which was successfully applied to a phase I pharmacokinetic study of ranolazine in Chinese healthy volunteers.

1. Introduction

Ranolazine (Fig. 1), (±)-N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy) propyl]-1-piperazine acetamide is a novel compound that is approved by the US FDA in January 2006 for the treatment of chronic angina pectoris in combination with amlodipine, β-adrenoceptor antagonists or nitrates, in patients who have not achieved an adequate response with other anti-anginals [1,2]. Ranolazine is the first approved agents from a new class of anti-anginal drug in almost 25 years, unlike existing anti-ischemic agents, studies with ranolazine had shown it to be hemodynamically neutral, with little effect on blood pressure and heart rate [3].

Since ranolazine lacks strong characteristic UV absorption, a HPLC–UV detection method does not provide suitable sensitivity and selectivity for the determination of ranolazine in biological samples [4,5]. Recently, two LC–MS methods with selected ion monitoring (SIM) have been published [6,7]; both of them allowed the quantitation of ranolazine in rat plasma with the lower limit of quantitation (LLOQ) above 20 ng mL^{-1} and relatively long HPLC/MS analysis time (6 or 7 min per sample) [8]. Although LC–MS/MS methods have been reported for the analysis of ranolazine in plasma, but the lower limit of quantitation can not meet the requirements for our pharmacokinetic studies and also sample pre-treatment procedure with solid-phase extraction (SPE) procedure or liquid–liquid extraction was time-consuming [9–11]. Recently, the ultra-performance LC (U-HPLC) has been introduced and quickly adopted in quantitative analysis of biological matrix. The van Deemter equation indicates that, as the particle size decreases to less than 2.5 μm, there is a significant improvement in efficiency that will not reduce with increased LC flow rates. Compared with conventional HPLC columns, U-HPLC, by utilizing 2.5 μm particle, greatly increased the separation throughput and efficiency, resulting in LC peaks as narrow as or less than 2 s [12].

According to the report, after a single oral dose of 500 mg ranolazine tablet in 48 h, the mean plasma concentration of ranolazine was about 2.5 ng mL^{-1}. In addition, plasma levels of ranolazine can vary widely between individuals. It was essential to establish an assay capable of quantifying ranolazine at concentrations down to 1.0 ng mL^{-1} for evaluation and interpretation of

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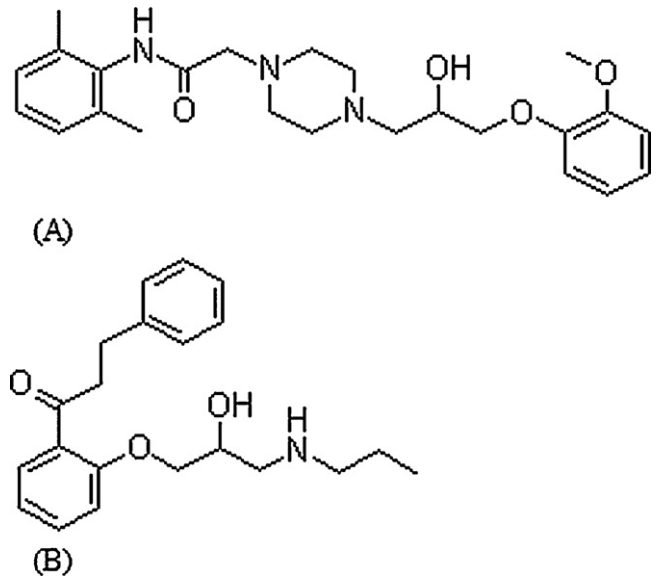

Fig. 1. Chemical structures of ranolazine [(A) MW: 427.54, (±)-N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-1-piperazine acetamide] and IS [(B) propafenone, MW: 341.45, 1-[2-[2-hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylpropan].

pharmacokinetic data. In our study, a simple, rapid (RT = 2.0 min), low LLOQ (1.0 ng mL^{-1}) and small injection volume (3 μL), sensitive U-HPLC–ESI-MS/MS method was developed. Following validation, this method was successfully applied to phase I pharmacokinetic studies of ranolazine performed to assess and compare the PK properties of ranolazine after administration of single oral doses (500, 1000 and 1500 mg) in healthy Chinese volunteers.

2. Materials and methods

2.1. Chemicals and reagents

Ranolazine pharmaceutical product (purity >99.3%) and Ranolazine 500 mg sustained-release tablets were provided by Fujian Tianquan Pharmaceutical Holdings Co., Ltd. (Longyan, People's Republic of China). Ranolazine, the reference standard (purity >99.3%), and the internal standard, propafenone hydrochloride (purity >99.9%), was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, People's Republic of China). HPLC-grade acetonitrile were purchased from Merck KGaA (Darmstadt, Germany). Formic acid and ammonium acetate, also of HPLC grade, were obtained from Tedia Company, Inc. (Fairfield, Ohio). Distilled water was deionized by using a Milli-Q Gradient system A10 (Millipore, Bedford, MA, USA) and was used throughout the study.

2.2. Instrumentation

The U-HPLC–MS/MS system (Waters Corporation, Milford, Massachusetts) consisting of an acquity ultra high performance LC and electrospray ionization tandem mass spectrometer (U-HPLC–ESI-MS/MS; Quattro Premier XE, Waters Corporation) Chromatographic analysis of ranolazine and propafenone (I.S.) were performed on a Waters Acquity U-HPLC^{TM} BEH C_{18} column (50 mm × 2.1 mm, i.d., 1.7 μm particle size). All data were acquired employing MassLynx V4.1 Quantitative Analysis version analyst data processing software.

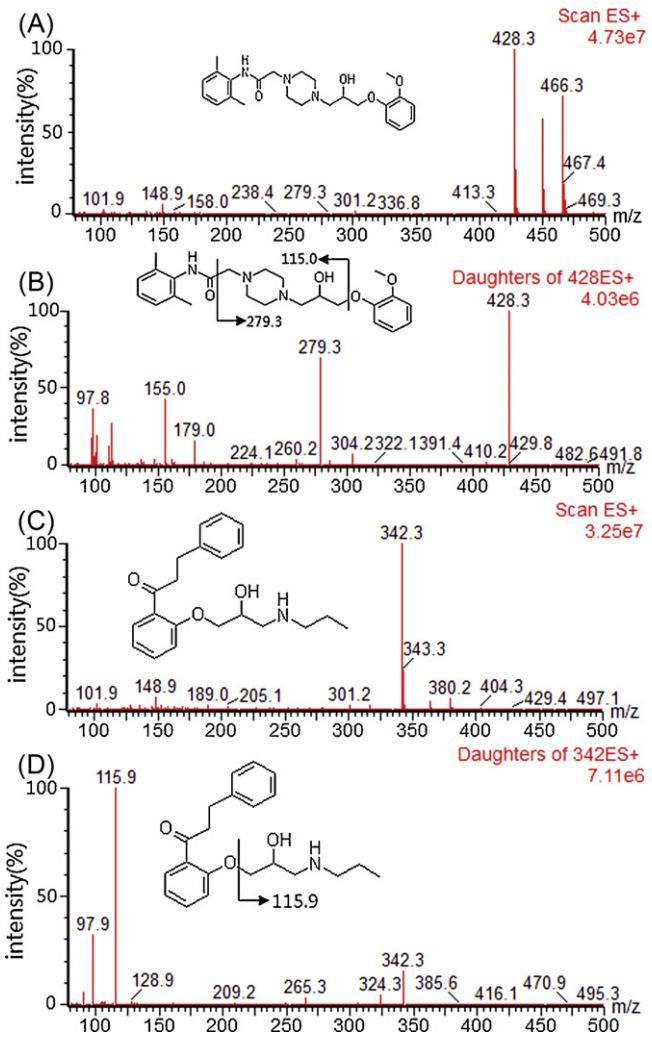

Fig. 2. The precursor and product ion scan mass spectra of ranolazine and the internal standard, propafenone. (A) Precursor ion scan mass spectra of ranolazine and (B) product ion scan mass spectra of ranolazine. (C) Precursor ion scan mass spectra of propafenone and (D) product ion scan mass spectra of propafenone.

2.3. U-HPLC–ESI-MS/MS conditions

The mobile phase composition was a mixture of acetonitrile–water containing (0.06% formic acid, 7.5 mmol L^{-1} ammonium acetate) in a ratio 40:60 (v:v). Measurements were made at a flow rate 0.35 mL/min at 40 °C column temperature. Mass spectrometric detection was performed on a U-HPLC–MS/MS system (Waters Corporation, Milford, Massachusetts) using multiple reaction monitoring (MRM). A turbo electrospray interface in positive ionization mode was used. The main working parameters of the mass spectrometer are summarized as follows: capillary voltage was 0.7 kV, the ionization sources and the desolvation temperature were 120 °C and 400 °C, respectively; desolvation gas flow rate was 750 L/h; the Ar gas was used for collision activated dissociation in Q2; the optimized collision cone voltage for the analyte and I.S. were 38 and 35 eV, and the optimized collision energies chosen for the analyte and I.S. were 24 and 22 eV, respectively. Fig. 2 shows the product ion mass spectra of $[M+H]^+$ of the analyte and I.S.

2.4. Sample preparation

Frozen human plasma samples were thawed at ambient temperature, 100 μL of the plasma sample was added to 200 μL