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Short Communication

Determination of bevantolol in human plasma using liquid chromatography–electrospray ionization tandem mass spectrometry and its application to a bioequivalence study

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ABSTRACT

A liquid chromatography–electrospray ionization tandem mass spectrometry method was established and validated for the determination of bevantolol in human plasma using propranolol as the internal standard. The optimal chromatographic behavior of bevantolol and propranolol was achieved on a Welch Ultimate XB-C_{18} column (5 μm, 150 mm × 2.1 mm, Maryland, USA) with a mobile phase of acetonitrile–water (40:60, v/v) containing 10 mM ammonium acetate and 0.1% formic acid. The mass spectrometer was operated in selected reaction monitoring mode using the transition *m/z* 346.1 > 165.1 for bevantolol and *m/z* 260.3 > 116.1 for propranolol. Sample preparation was carried out through protein precipitation with acetonitrile. The calibration curves were linear over the range of 5.00–1000 ng/ml. The intra- and inter-day precisions were less than 6.7% and 6.6%, respectively. This method was successfully applied to the bioequivalence study of two kinds of bevantolol hydrochloride tablets in 24 Chinese male volunteers in fasting and postprandial experiment.

1. Introduction

Bevantolol, 1-[(3,4 dimethyoxyphenethyl)-amino]-3-(m-tolyloxy)-2-propanol, is a cardioselective beta-adrenoreceptor antagonist with a lack of intrinsic beta sympathomimetic activity and with weak membrane-stabilizing and local anesthetic properties [1]. Bevantolol is an effective agent in the management of mild to moderate hypertension and stable angina pectoris. Interestingly, bevantolol has been shown to cause a lowering effect on peripheral vascular resistance [2].

To date, some assays for the determination of bevantolol in plasma have been reported, including GC [3] and HPLC–UV [4–7]. GC was not ideal for high-throughput analysis. Some of these published HPLC methods showed long HPLC run times (over 8 min [7], 40 min [5,6]) or high lower limits of quantification (LLOQ) (20 ng/ml [6], 40 ng/ml [5,7]), or involved liquid–liquid extraction (LLE) [4] and solid phase extraction (SPE) [5,7] prior to analysis, which is time-consuming and not economical. In addition, large-volume plasma samples (1 ml [5,6], 0.5 ml [7]) were used in some of these methods.

This paper presents, for the first time, the development and validation of a rapid, sensitive, high throughput and simple LC–MS/MS method for the determination of bevantolol in human plasma.

2. Experimental

2.1. Chemical and reagents

Bevantolol hydrochloride and propranolol hydrochloride reference standards with purities of 99.9% and 100% were purchased from National Institutes for Food and Drug Control (Beijing, PR China). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). A Milli-Q® reagent-grade water system (Millipore, MA, USA) was used to produce ultrapure water. Other chemicals and reagents were of analytical grade. Drug-free human plasma was provided by the Blood Center of Tongji

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Hospital of Tongji Medical College (Wuhan, PR China) and was stored at −80 °C.

The test preparation (bevantolol hydrochloride tablets, 50 mg, Batch No. 11092701) was provided by Shanghai Haini Pharmaceutical Co. Ltd, Yangtze River Pharmaceutical Group (Shanghai, PR China). The reference preparation (bevantolol hydrochloride tablets, 50 mg, Batch No. 0022) was purchased from Nippon Kayaku Co. Ltd.

2.2. Chromatographic conditions

The separation work was carried out on an Agilent 1200 series HPLC system (Agilent Technologies, Wilmington, DE, USA) equipped with a G1311A quat pump, G1329A autosampler, G1316A column oven and G1322A degasser. Chromatographic analysis was conducted using an isocratic elution on a Welch Ultimate XB-C18 column (5 μm, 150 mm × 2.1 mm, Maryland, USA) protected by a Phenomenex ODS guard column (5 μm, 4.0 mm × 3.0 mm, Torrance, CA, USA) with a mobile phase of acetonitrile–water (40:60, v/v) containing 10 mM ammonium acetate and 0.1% formic acid at a flow rate of 0.3 ml/min. The sample injection volume was 5 μl, and the column temperature was maintained at 30 °C. The total LC analysis time per injection was 3.5 min.

2.3. Mass spectrometric conditions

An API 4000 MS/MS system (Applied Biosystems, Foster City, CA, USA) with a TurboIonspray source operated in positive electrospray ionization (ESI ±) mode with selected reaction monitoring (SRM) for LC–MS/MS analyses. The precursor to product ion transitions were m/z 346.1 ≥ 165.1 for bevantolol and m/z 260.3 ≥ 116.1 for the IS with dwell times of 200 ms. The optimal ESI-MS/MS parameters were as follows: the ion spray voltage and source temperature were 5500 V and 550 °C; the gas rates for nebulizing gas (GAS1), turbo gas (GAS2), curtain gas (CUR) and collision gas (CAD) were set to 50, 50, 25 and 4 psi, respectively; the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were set to 40, 29, 10 and 10 V, respectively, for bevantolol and 70, 25, 10 and 10 V, respectively, for the IS.

2.4. Preparation of standard solutions, calibration curves and quality control (QC) samples

A stock solution of bevantolol was prepared at 100 μg/ml (as the free base) in methanol/water (50:50, v/v) and then diluted with the same solvent to serial working solutions with the desired concentrations (50, 200, 500, 2000, 4000, 7000, 10,000 ng/ml for calibration standards and 100, 3000, 8000, 24,000 ng/ml for QC standards). A stock solution of propranolol was prepared at 92 μg/ml (as the free base) in methanol/water (50:50, v/v) and the working solution was diluted to 9200 ng/ml with the same solvent. All solutions were stored at 4 °C when not used.

Plasma calibration standards of bevantolol (5.00, 20.0, 50.0, 200, 400, 700 and 1000 ng/ml) were prepared by adding 20 μl of the serial working standard solutions to 200 μl drug-free plasma. Quality control samples for lower limit of quantification QC (LLOQ), low QC (LQC), medium QC (MQC), high QC (HQC) and dilution QC (DQC) were prepared at 5.00, 10.0, 300, 800 and 2400 ng/ml in the same manner.

2.5. Plasma sample preparation

Twenty microliters of the IS working solution (9200 ng/ml) and 20 μl of methanol/water (50:50, v/v) were added to a 200 μl aliquot of human plasma and vortex-mixed. Protein precipitation was carried out by the addition of 600 μl acetonitrile. After vortex mixing for 1 min and centrifuging at 13,000 rpm for 10 min, the supernatant was diluted with water in a 1:3 (v/v) ratio; then, 200 μl of the mixture was transferred into autosampler vials, and 5 μl was injected into the LC–MS/MS system.

2.6. Method validation

The method was validated according to the EMA 2012 Guideline on bioanalytical method validation [8].

2.6.1. Selectivity

Selectivity was evaluated by comparing the chromatograms of six lots of blank plasma with the corresponding spiked plasma to investigate potential interferences near the retention times of the analyte and IS.

2.6.2. Calibration curves

Seven calibration standards for bevantolol were prepared at 5.00, 20.0, 50.0, 200, 400, 700 and 1000 ng/ml. The calibration curves (analyte peak area/IS peak area versus analyte concentration) were obtained based upon a least square linear regression fit ($y = ax + b$) with a weighting factor of $1/x^2$. The calibration curves had to have correlation coefficient (r) of 0.99 or better.

2.6.3. Accuracy, precision and lower limit of quantification (LLOQ)

The intra-batch and inter-batch accuracy and precision were assessed by the analysis of three separate batches of human plasma samples. Each batch included one set of calibration standards and five replicates of QC samples at LLOQ, LQC, MQC and HQC levels.

2.6.4. Matrix effect, recovery, dilution integrity and carry-over

The matrix effect defined as matrix factor (MF) was obtained as the ratio of the peak area of bevantolol and IS dissolved in the supernatant of the processed blank plasma from six subjects to that of standard solutions at equivalent concentration (LQC and HQC levels). The RSD of the six IS-normalized MF calculated by dividing the MF of bevantolol by the MF of IS should not be greater than 15%. The recovery was performed at LQC, MQC and HQC levels ($n = 5$) by comparing the response of extracted QC samples with the response of standards in the matrix at the same concentration. Dilution integrity was assessed by analyzing five replicates of a four-fold dilution of the DQC sample (2400 ng/mL) with blank plasma prior to extraction. Carry-over of bevantolol and IS was evaluated by analyzing extracted samples of blank plasma immediately after an upper limit of quantification (ULOQ) sample ($n = 6$). Carry-over in the blank sample should not be greater than 20% of LLOQ and 5% of IS.

2.6.5. Stability

Stability tests including three freeze-thaw cycles (−80 °C in plasma for 24 h), storage for 45 days at −80 °C, the treated plasma sample in autosampler vials at 4 °C for 24 h and the untreated plasma at room temperature for 24 h were evaluated using five replicates of QC samples at LQC, MQC and HQC levels. Samples were considered to be stable if their assay values were within ±15% of the nominal values.

The stability of stock solutions and working solutions of bevantolol and IS (with an appropriate dilution) were evaluated by comparing the peak area of the stock solutions and working solutions kept at 4 °C for 45 days with that of freshly prepared solutions.

2.7. Bioequivalence study

The method was applied to determine the bevantolol concentration in a randomized, two-period, crossover and single oral dose