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Short communication

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

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ABSTRACT

A selective, sensitive and simple high performance liquid chromatography tandem mass spectrometric (HPLC–MS/MS) method for determining quetiapine in human plasma was developed and validated. One-step protein precipitation with acetonitrile was used to pretreat plasma samples. Carbamazepine was used as internal standard. An automated liquid handling workstation with 96-well protein precipitate plate was used to facilitate the process. The chromatographic separation was achieved on a Waters Xbridge C_{18} column (3.5 μm, 2.1 mm × 50 mm). Gradient elution was set with a mobile phase of acetonitrile/water (containing 10 mM ammonium acetate and 0.1% formic acid).The flow rate was 0.4 mL/min and total analytical run time was 3 min. The analysis was conducted using a triple quadrupole tandem mass spectrometer with an electrospray ionization source operating in positive ion mode. The multiple reaction monitoring of transition were *m/z* 384.2 → 253.1 for quetiapine and *m/z* 237.0 → 194.0 for carbamazepine, respectively. The linear concentration range for the standard curve of quetiapine was 0.5–400 ng/mL for a 5 μL injection of the pretreated sample (original plasma sample, 50 μL). The intra-day and inter-day accuracy and precision were all less than 15%. The method was successfully used in a bioequivalence study comparing two quetiapine extended-release tablets in Chinese volunteers.

1. Introduction

Quetiapine fumarate (Seroquel®) is an atypical antipsychotic with demonstrated efficacy in schizophrenia and bipolar disorder. The antipsychotic activities of quetiapine are thought to exert from antagonizing both serotonin 5-HT_2 and dopamine D_2 receptor [1]. Quetiapine causes fewer adverse effects compared with other antipsychotic with less extrapyramidal effects, weight gain, abnormal ECG and prolactin levels [2–4]. Long-term use of quetiapine has demonstrated that it is a more tolerated and satisfactory option than the first-generation antipsychotics [5]. Patient compliance is a substantial issue in schizophrenia. The modified-release formulation of quetiapine (quetiapine XR) are developed later to help with this issue. Quetiapine XR can be administered once-daily. Dose escalation is simpler and more rapid, which facilitates its application [6]. Therefore, it is believed that quetiapine with the perceived benefits and less risk characteristic should be the subject of further research along with other newly introduced antipsychotics [7]. The absorption after oral administration is quite well [8]. Maximal plasma concentrations are reached in about 5 h [9]. It also goes through extensive metabolism by the P450 isoenzyme CYP3A4 and by oxidation in the liver. The elimination half-life is about 6–7 h [8].

Several HPLC and LC–MS methods have been applied for the analysis of quetiapine in plasma. A lower limit of quantification (LLOQ > 10 ng/mL) has been reported [10], but such performance may not be sufficient for quantitation of low levels of the drug. Moreover, retention times of over 10 min for quetiapine have been reported [11], which makes the method less applicable to the analysis of massive samples. Meanwhile, some methods consume large volumes (≥500 μL) of plasma [12], increasing difficulties for repeat sampling and reanalysis. In addition, only solid phase extraction (SPE) and liquid–liquid extraction (LLE) have been employed for plasma pretreatment in the case of quetiapine determination [13–17], however, LLE is time consuming requiring an evaporation step and large solvent consumption. SPE is also relatively tedious and expensive. Simpler, faster and more efficient pretreatment schemes with high throughput should be explored to meet the challenges posed by today's clinical analysis, which typically can require analysis of thousands of samples.

In this report we describe a simple one-step protein precipitation process coupled with HPLC–MS/MS analysis, which affords a rapid, sensitive and selective method for quantification of quetiapine in

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human plasma. The method was subsequently used in a bioequivalence study in Chinese healthy volunteers.

2. Experimental

2.1. Materials and reagents

Quetiapine fumarate (purity: 99.9%, lot: 100815-201202) and the internal standard (IS) carbamazepine (purity: 99.7%, lot: 100142-201105) were obtained from the National Institutes for Food and Drug Control (Beijing, China). Acetonitrile was obtained from Thermo Fisher Scientific (USA). Formic acid and ammonium acetate were HPLC grade and obtained from Sigma (USA). Deionized pure water was produced by a Milli-Q integral water purification system (Millipore Corporation, USA). Human plasma with lithium heparin, which was obtained from healthy volunteers, served as blank plasma sample.

2.2. Chromatographic conditions

A Shimadzu HPLC system comprised of a LC-20AD prominence pumps, a DGU-20A3 prominence degasser, a CTO-20AC prominence column oven and a SIL-20AC XR prominence auto-sampler. Chromatographic separation was performed on an XBridge C_{18} column (2.1 mm × 50 mm, 3.5 μm, Waters, USA) maintained at 40 °C. A gradient mobile phase system was used to elute the analyte with a flow rate of 0.4 mL/min. The mobile phase at the start of the run was a mixture of 70% water (containing 10 mM ammonium acetate and 0.1% formic acid) and 30% acetonitrile, increased to 95% acetonitrile at 0.8 min and was kept for 1.2 min. Then the solvent combination was reversed back to 30% acetonitrile at 2.2 min and kept for 0.8 min. The total run time was 3 min. The sample injection volume was 5 μL.

2.3. Mass spectrometric conditions

An API 5500 tandem triple quadrupole mass spectrometer was used for analysis (Applied Biosystems, USA). The mass spectrometer was operated in positive electrospray ionization (ESI) mode. Quantification was conducted in MRM mode using the transitions *m/z* 384.2 → 253.1 and *m/z* 237.0 → 194.0 for quetiapine and carbamazepine, respectively. The optimized mass spectrometric conditions were as follows: entrance potential (EP), 10 V; ion spray voltage, 5500 V; curtain gas (CUR) and nitrogen, 20 L/min, nebulizer gas (GS1), 50 L/min; auxiliary gas (gas2), 50 L/min; collision activation dissociation (CAD), medium; turbo heater temperature (TEM), 500 °C; declustering potential, 100 V; collision energy (CE), 32 eV for quetiapine and 20 eV for carbamazepine respectively. Ultra high purity nitrogen gas was used as the collision gas. All parameters of HPLC and MS were controlled by Analyst software (version 1.5.2). Watson LIMS™ software (version 7.2, Thermo Fisher Scientific, Inc, PA, USA) was used for the linear regression and concentration data processing.

2.4. Preparation of stock and working solutions, standards and quality control samples

Quetiapine (10.0 mg) was dissolved with 30-mL methanol, then transferred to a 50-mL volumetric flask, and diluted with 20-mL water to volume. The final concentration of quetiapine stock solution was 200 μg/mL. Then the stock solution of quetiapine was diluted with acetonitrile:water (1:9, *v/v*) to obtain working solution of quetiapine (10 μg/mL). Carbamazepine (IS) stock solution was prepared in a similar manner to reach the final concentration of 200 μg/mL. Then the stock solution of carbamazepine was diluted with acetonitrile to obtain working solution of IS (25 ng/mL). Stock and working solutions were all stored in the refrigerator at 4 °C.

Standard samples were prepared by serial dilution of stock solution with plasma to get the desired concentrations of 0.5, 1, 5, 12.5, 50, 100, 200 and 400 ng/mL for quetiapine. The quality control (QC) plasma samples (1, 50, 300 ng/mL quetiapine) were diluted in a similar manner.

2.5. Sample preparation

Protein precipitation was performed using a TOMTEC™ Quadra 4 liquid handling workstation and a Waters Ostro™ protein precipitation 96-well plate. Aliquots (50 μL) of each plasma sample were added into the wells of the Ostro plate followed by 450 μL of carbamazepine working solution (25 ng/mL). Then the 96-well plate was vortexed at 1400 rpm for 4 min. After that, the plate was placed on a rack of TOMTEC workstation and −7 in.Hg of vacuum was applied for 10 min to collect the eluent. A 50 μL aliquot of each eluent was further diluted with 450 μL of acetonitrile:water (1:9, *v/v*). A 5-μL aliquot of each sample was injected into the HPLC–MS/MS system for the analysis.

2.6. Application of the method to a bioequivalence study

The HPLC–MS/MS method for quantification of quetiapine was fully validated based on FDA recommendations. Then it was applied to a human bioequivalence study comparing two extended-release tablets of quetiapine fumarate at a single dose of 200 mg. This was a randomized, 2-way crossover, single-dose, 2-period study with a wash-out period of at least 7 days. Fasting and fed bioequivalence studies were conducted in 2 different groups of healthy male Chinese volunteers. Blood samples were obtained at time zero (pre-dosing), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, and 48 h. Fifteen blood samples were obtained over 48 h from each volunteer after oral drug administration for each period. At each time point for each subject, 5 mL of blood was collected. The blood sample was centrifuged at 1811*g* at 4 °C for 10 min. Plasma samples were separated and stored in the deep freezer at −70 °C until they were used for analysis. The ethics committee of Beijing Anding Hospital (Beijing, China) approved the study protocol. Clinical research was conducted in Beijing Anding Hospital. The test preparation consisted of a quetiapine 200 mg extended-release tablet manufactured by a domestic company. An extended release tablet containing 200 mg of quetiapine, manufactured by AstraZeneca UK Limited, was used as a reference preparation.

An incurred sample reanalysis (ISR) to check method reproducibility was conducted in 36 volunteers who were randomly selected. In each period for each selected volunteer, one sample was taken around the time of C_{max} and one was taken in the elimination phase. The difference between the initial and reanalyzed concentrations should be within 20% of their mean for at least 67% of the repeat.

3. Results

3.1. HPLC–MS/MS analysis

The HPLC–MS/MS in MRM mode under the optimized conditions provided a very sensitive, effective, and selective method for quantification of quetiapine. Direct infusion was used to determine the best mass spectrometry conditions for measurement of quetiapine and carbamazepine. The mass spectra for the compounds showed parent molecular ion peaks at *m/z* of 384.2 for quetiapine and 237.0 for carbamazepine. The product ion scan showed that the *m/z* 253.1 and 194.0 were the most abundant product ions for quetiapine (Fig. 1A) and carbamazepine (Fig. 1B), respectively. Parameters such as flow rate of auxiliary gas and desolvation gas, spray and capillary voltage were optimized to achieve the highest intensity for the protonated molecules of quetiapine and carbamazepine.

LC columns, mobile phase compositions, gradient elution, and flow rates were tested and compared to achieve good chromatographic separation and efficient ionization. The addition of the two modifiers (10 mM ammonium acetate and 0.1% formic acid) was a key factor in