Validated LC–MS-MS Method for the Determination of Quetiapine in Human Plasma: Application to a Pharmacokinetic Study

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A sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS-MS) method for the determination of quetiapine was developed and validated over the linearity range 1–1500 ng/mL with 0.1 mL of plasma using clozapine as the internal standard. Detection was performed on a triple-quadrupole tandem mass spectrometer using positive electrospray ionization and quantification was performed by selected reaction monitoring mode. The MS–MS ion transitions monitored were m/z 384.1 → 253.1 and 327.0 → 270.0 for quetiapine and clozapine, respectively. The between- and within-run precision was less than 7.44% and accuracy was less than 10.2%. The lower limit of quantification was 1 ng/mL. The extraction recoveries of quetiapine were over 90%. The method is proved to be accurate and specific, and was applied to the pharmacokinetic study in healthy Chinese volunteers.

Introduction

Quetiapine (Figure 1), a dibenzothiazepine derivative, is classified as a second-generation antipsychotic drug. It is an antagonist at the serotonin 5HT₁A and 5HT₂ receptors, dopamine D₁ and D₂ receptors, histamine H₁ receptor and adrenergic α₁ and α₂ receptors. Binding at both serotonin and dopamine receptors accounts for quetiapine’s antipsychotic effects (1–5). Quetiapine was approved by the U.S. Food and Drug Administration in September 1997 and is also currently approved in over 70 countries worldwide for the treatment of psychosis associated with schizophrenia (1–5).

The oral absorption of quetiapine in the body is rapid, with a median time to reach maximum observed plasma concentration ranging from 1 to 2 hours. The absorption of quetiapine is unaffected by food and the drug is approximately 83% bound to serum proteins (3–5). Quetiapine exhibits linear pharmacokinetics, so that blood levels change roughly as a proportion of dose taken. Furthermore, its pharmacokinetics does not appear to be affected by ethnic background, gender, body weight or cigarette smoking (1, 2, 6–8). Quetiapine is primarily metabolized in the liver by the cytochrome P450 3A4 isoenzyme and is primarily excreted in the urine (3–5).

Quetiapine undergoes extensive metabolism that results in very low plasma concentrations (ng/mL levels), and the activity is primarily attributed to the parent drug. Mean maximum concentrations (C_max) of 53 to 117 μg/L were observed after administration of the 25 mg dosage form. For these relatively low doses (10 to 25 mg), the plasma concentration of quetiapine declined with a mean apparent terminal elimination half-life (t1/2) ranging from 3.1 to 5.5 hours, but at doses of 250 mg and higher, the mean t1/2 has been found to be approximately 6 hours (3–5). Due to the low plasma concentrations of quetiapine, a more sensitive analytical method is needed for its determination in plasma. Several high-performance liquid chromatography (HPLC) methods have been reported for the determination of quetiapine (9–15). However, none of these methods is sensitive enough for determination of the expected drug levels, and some of them are time-consuming and require complex sample pretreatment or long run times. Gas chromatography–mass spectrometry (GC–MS) methods have also been employed, but quetiapine needs to be derivatized before analysis (16–18). For analysis of many plasma samples, the derivatization step increases the time of sample preparation and the cost of the method.

Liquid chromatography–tandem mass spectrometry (LC–MS-MS) methods provide high sensitivity while avoiding a tedious derivatization step. Recently, a few LC–MS-MS methods have been reported for quantification of quetiapine (19–26). Nirogi et al. developed an LC–MS-M method for the determination of quetiapine, but this method was not designed for the determination of human plasma (24). Zhou et al. reported an LC–MS-MS method for simultaneous determination of clozapine, olanzapine, risperidone and quetiapine in 0.5 mL plasma with a lower limit of quantification (LLOQ) of 20 ng/mL (25). Lin et al. reported an LC–MS-M method for quantification of quetiapine in human plasma and liver microsomes (26). The method was validated over a limit concentration range of 1–500 ng/mL with LLOQ of 1 ng/mL, but the average recovery was approximately 70%. Given that many techniques including LC, GC–MS and LC–MS-MS have been previously reported for the determination of quetiapine, certain limitations in a variety of biological samples still remain. Thus, a more specific and more efficient analytical method with adequate concentration range for pharmacokinetic study and full validation was required to accurately determine the concentration of quetiapine in biological samples. This study presented a small plasma sample (0.1 mL), and higher sample extraction recoveries of quetiapine, quantitative matrix effect data, adequate concentration range of 1.0–1500 ng/mL and a shorter run time resulted from an isocratic elution system used that provided another alternative for the determination of quetiapine in human plasma by the LC–MS-MS method.

This paper describes a simple, selective and highly sensitive method by LC coupled with electrospray ionization (ESI) triple-quadrupole MS for the determination of quetiapine in human plasma. The developed method for the plasma samples and the validated LC–MS-MS analytical method were further applied to a pharmacokinetic study in a group of healthy Chinese male volunteers after an oral administration of a...
200-mg quetiapine tablet. The related pharmacokinetic parameters were obtained.

**Experimental**

**Chemicals and reagents**
Quetiapine and clozapine (internal standard, IS; Figure 1) were purchased from Synpac-Kingdom Pharmaceutical Co., Ltd. (Taiwan). Methanol and acetonitrile, LC grade, was obtained from E. Merck (Darmstadt, Germany). Formic acid, sodium hydroxide and diethyl ether were of analysis grades and purchased from Merck. Double distilled water was prepared by a Millipore Milli-Q purification system (Millipore, Bedford, MA).

**LC conditions**
The LC system consisted of a Waters 515 pump and an autosampler (Waters 717 plus Autosampler, Waters Instruments, MA). The analytical column was a Phenomenex Luna C18 column (2.0 mm x 150 mm, i.d., 5 μm), and the column was maintained at room temperature. The mobile phase was composed of 85% CH₃CN (aq) with 1 mM NH₄OAc and 0.1 mM HCOOH. The flow rate was 0.3 mL/min and the injection volume was 5 μL.

**Mass spectrometer conditions**
A Quattro Ultima triple-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with ESI was used, and the detection was performed in positive ionization mode. Capillary voltage and cone voltage were set at 3200 and 60 V, respectively. The temperature of ion source was 80°C with ultra high-purity nitrogen as cone gas and nebulizer gas (150 L/h). Desolvation gas was heated to 320°C and set at a flow rate of 560 L/h. With argon as collision gas, selected reaction monitoring (SRM) was applied to detect quetiapine and clozapine by monitoring the ion transition of m/z 384.1 → 253.1 and 327.0 → 270.0, respectively. The collision energy for quetiapine and clozapine was set at 23 eV and 38 eV. Data acquisition was performed using MassLynx 3.5 software (Micromass Ltd., Manchester, UK).

**Preparation of calibration standards and quality control samples**
A stock solution of 0.5 mg/mL for quetiapine was prepared in 50% methanol and serially diluted with 50% methanol to give a series of standard working solutions with concentrations of 0.02, 0.06, 1, 2, 6, 10, 12, 18, 24, 30 and 100 ng/μL. A stock solution of 0.5 mg/mL for clozapine was prepared in 50% methanol and then further diluted with 50% methanol to yield a working solution of 0.5 ng/μL.

Plasma calibration standards of quetiapine (1, 50, 100, 300, 600, 900, 1200 and 1500 ng/mL) were prepared by spiking the appropriate amount of the standard solutions in blank plasma from healthy, non-smoking volunteers. Quality control (QC) samples were prepared from a pool of different sources of blank plasma at the concentrations of 1, 3, 500, 1300 and 1500 ng/mL and were used in pre-study validation.

**Sample preparation**
To an aliquot of 100 μL plasma sample, 50 μL of 0.5 ng/mL clozapine solution was added as an IS. Fifty μL of 0.1N NaOH was added and vortexed for 1 min. The mixture was then vortexed for 3 min to ensure adequate mixing followed by an addition of 3 mL diethyl ether. After 5 min of centrifugation, the organic layer was transferred into another tube. The samples were then evaporated to dryness under N₂ stream and reconstituted in 1 mL of 50% MeOH. Finally, 5 μL of aliquots were injected onto LC–MS-MS.

**Method validation**
The following parameters were determined for the validation of the analytical method developed for quetiapine in human plasma: matrix effect, selectivity, linearity, precision, accuracy, LLOQ, recovery and stability (27). During pre-study validation, six validation runs were conducted on six separate days. Each validation run consisted of a set of the calibration standards at eight concentrations over the concentration range.

Six different sources of blank plasma were used to assess the matrix effect. The absolute and relative matrix effects were previously defined by Matuszewski et al. (28). The absolute matrix effect was evaluated by comparing the peak areas of analytes added to extracted blank plasma to those of extracted water. The relative standard deviation (RSD) of the mean peak area of analytes in the extracted blank plasma indicated the relative matrix effects.

Selectivity was evaluated by comparing chromatograms of six blank plasma samples from six different sources to make sure there were no significant interfering peaks at retention time at LLOQ of the analytes.

![Figure 1. The chemical structures: (A) quetiapine; (B) clozapine.](https://academic.oup.com/chromsci/article-abstract/50/3/277/352861/278)
The linearity was confirmed by plotting the peak area ratio of quetiapine (Y) to the internal standard versus quetiapine concentration (X). The unknown sample concentrations were calculated from the equation \( y = mx + b \). The calibration curve was obtained by weighted (1/x) least-squares regression analysis.

The within-run and between-run precision and accuracy were determined by analyzing a set of QC samples (n = 6) at each of the five levels, 1, 3, 500, 1300 and 1500 ng/mL. The precision of the assay for quetiapine was evaluated by determining the RSD and accuracy was evaluated by the relative percent error (RE) and was evaluated as \( \frac{\text{mean calculated concentration} - \text{concentration spiked}}{\text{concentration spiked}} \times 100\% \). The acceptable values for RSD and RE were within 15% for validation QC samples.

LLOQ defined in the presented study is the lowest plasma concentration on the calibration curve that can be measured by precision and accuracy. The acceptable values for RSD and RE were below 20% for LLOQ.

The absolute recovery for quetiapine in human plasma was determined by comparing the responses of plasma samples spiked before extraction with the corresponding standard solution without extraction. Triplicate human plasma samples of three concentrations (3, 500 and 1300 ng/mL) were prepared and injected into LC–MS-MS.

Freeze and thaw stability for quetiapine in plasma samples was studied in 3 cycles with two concentrations (3.0 and 1300 ng/mL) in 3 replicates.

**Application of the method**

The LC–MS-MS procedure was developed to determine quetiapine concentrations in human plasma 0–48 h after an oral administration of 200 mg quetiapine tablet. This pharmacokinetic study was approved by the Ethics Committee. The volunteers fasted for 12 h (overnight) with subsequent drug administration and then continued to fast for a further 4-h period after dosing. All volunteers gave written informed consent.

### Table 1

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Quetiapine</th>
<th>Clozapine</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>91.7 (3.3)</td>
<td>96.2 (2.4)</td>
</tr>
<tr>
<td>1300</td>
<td>90.2 (4.3)</td>
<td>94.5 (2.8)</td>
</tr>
</tbody>
</table>

*The results are expressed as absolute matrix effect percentage (relative matrix effect percentage). Six different sources of blank plasma were used in the experiments.

Fifty milliliters of 0.5 ng mL\(^{-1}\) clozapine were spiked with the analytes of interest at 3.0 and 1300 ng mL\(^{-1}\).
consent before the study. Eight milliliters of blood were removed by venepuncture before dosing and at 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 h thereafter. Blood samples were collected into heparinized tubes. After each blood sampling, plasma was separated by centrifugation at 1080 × g for 5 min and stored at –70°C until analysis.

Results and Discussion

Method development

Liquid–liquid extraction was necessary and important because this technique not only purifies but also concentrates the samples. Diethylether was used because of its high extraction efficiency and low interference.

The positive product ion mass spectra of quetiapine and the IS are shown in Figure 2. The most intensive product ion was observed at m/z 253.1 for quetiapine and m/z 270.0 for the IS. Therefore, the precursor-to-product ion transitions m/z 384.1 → 253.1 for quetiapine and m/z 327.0 → 270.0 for the IS in the selected reaction monitoring mode were used for the quantitation of quetiapine and IS.

Matrix effect

The absolute and relative matrix effects are shown in Table I. The results indicated that little ion suppression occurred for quetiapine and clozapine (IS). The relative matrix effects were all less than 4.3%, which indicated that the impact from extracted plasma matrix was consistent and limited.

Selectivity

Figure 3 shows the chromatograms of quetiapine and clozapine (as IS) eluted under an isocratic mobile phase system. The results demonstrated that the retention times for quetiapine and clozapine were 1.8 and 2.3 min, respectively. The retention time for quetiapine and sample run time was much shorter than in the previous study done by Lin et al. where a longer run time was observed using a gradient elution program (26). No significant interfering peaks were observed due to endogenous compounds or reagent. These chromatograms indicated that the liquid–liquid extraction method from plasma produced a clean matrix free from the interference of endogenous substances.

Linearity

The linearity was determined by plotting the peak-area ratio (y) of quetiapine to IS versus the nominal concentration (x) of quetiapine in plasma. Representative regression equation for the calibration curve was y = 0.03485x + 0.00257 (r = 0.999) over the concentration range of 1–1500 ng/mL for quetiapine in human plasma.

LLOQ

LLOQ for the determination of quetiapine in human plasma was found to be 1 ng/mL with an accuracy of −4.6% and with within- and between-run precisions of 6.1 and 7.4%, respectively.
Table II.
Precision and Accuracy for the Determination of Quetiapine in Human Plasma*

<table>
<thead>
<tr>
<th>Added concentration</th>
<th>Found concentration</th>
<th>RSD</th>
<th>RE</th>
<th>Found concentration</th>
<th>RSD</th>
<th>RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.984 ± 0.058</td>
<td>6.1</td>
<td>−4.6</td>
<td>0.984 ± 0.073</td>
<td>7.4</td>
<td>−1.6</td>
</tr>
<tr>
<td>3</td>
<td>2.96 ± 0.13</td>
<td>4.5</td>
<td>−1.2</td>
<td>2.94 ± 0.09</td>
<td>2.9</td>
<td>−2</td>
</tr>
<tr>
<td>500</td>
<td>551.0 ± 8.7</td>
<td>1.6</td>
<td>10.2</td>
<td>529.0 ± 11.0</td>
<td>2.1</td>
<td>5.7</td>
</tr>
<tr>
<td>1300</td>
<td>1360.0 ± 49.9</td>
<td>3.7</td>
<td>4.6</td>
<td>1308.0 ± 44.1</td>
<td>3.4</td>
<td>0.6</td>
</tr>
<tr>
<td>1500</td>
<td>1570.0 ± 31.1</td>
<td>2</td>
<td>4.7</td>
<td>1506.0 ± 43.8</td>
<td>2.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SD (n = 6); concentrations are expressed as ng/mL; RSD and RE are expressed as %.

Table III.
Pharmacokinetic Parameters of Quetiapine Calculated from the Plasma Concentrations*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–t∞ (ng h/mL−1)</td>
<td>2330 ± 1036</td>
</tr>
<tr>
<td>Cmax (ng/mL−1)</td>
<td>699 ± 279</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>4.89 ± 0.90</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.94 ± 0.70</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>4.4 ± 0.3</td>
</tr>
</tbody>
</table>

*AUC0–t∞: the area under the plasma drug concentration-time curve from time zero to time infinity; Cmax, the peak drug concentration; MRT, the mean residence time; Tmax, the time to peak drug concentration; T1/2, the terminal half-life.

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method. The mean quetiapine plasma concentrations of 23 volunteers are shown in Figure 4. The related pharmacokinetic parameters are listed in Table III. It was obvious that inter-individual variation existed after the administration of the same amount of quetiapine. The difference between maximum and minimum values of C max was approximately four-fold (1295 and 274 ng/mL), and the time to reach the peak plasma concentration (t max) also showed a difference ranging from 0.5 to 3 h. The significant inter-individual variation was reflected in the extraordinarily large standard deviation.

Conclusions
A sensitive, accurate and selective assay has been developed for the determination of quetiapine in human plasma with a linear range from 1.0 to 1500 ng/mL and a chromatographic run time less than 3 min. The method only needed a small amount of plasma sample and a simple liquid–liquid extraction for sample pretreatment with high extraction recovery and sensitivity up to an ng level (1 ng/mL). The usefulness of this method in the routine analysis was successfully demonstrated by the analysis of a large number of samples from pharmacokinetic studies. This study also provided an alternative option for the quantification of quetiapine in human plasma.

References

respectively. This LLOQ was sufficient for pharmacokinetic studies of quetiapine in humans.

**Accuracy and precision**
As shown in Table II, for each QC level of quetiapine, the within- and between-run precision was less than 6.1 and 7.4%, respectively. The accuracy on the other hand, was within 4.7% for the within-run and 5.7% for the between-run. These indicated acceptable accuracy and precision of the LC–MS-MS method for the determination of quetiapine in human plasma.

**Absolute recovery**
The results showed that the extraction recoveries of quetiapine were 94.1 ± 2.4%, 92.5 ± 2.5% and 92.5 ± 2.9% in the concentrations of 3, 500 and 1300 ng/mL, respectively. The recoveries were much higher compared to that in the previous study by Lin et al., in which 62.9–72.9% recoveries were reported (26).

**Stability**
Freeze and thaw stability for quetiapine in plasma samples was studied in 3 cycles with two concentrations (3 and 1300 ng/mL) in three replicates. The differences of concentration between the initial concentration of the 3 cycles were within 5.5%, indicating that quetiapine in plasma samples was stable after 3 cycles of freeze and thaw.

**In vivo pharmacokinetic study**
After a single oral administration of 250 mg quetiapine to 23 healthy Chinese male volunteers, plasma concentrations of quetiapine were determined by the described LC–MS-MS method.


