ORIGINAL ARTICLE

# Development and Validation of a Rapid LC/MS-MS Method for Measuring Plasma Quetiapine and Norquetiapine in Psychiatric Patients

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**Background:** Quetiapine (QTP) is an atypical antipsychotic drug that is widely used to treat psychiatric patients. N-desalkylquetiapine (NQTP) is the major and active metabolite.

**Objective:** The present study aimed to develop and validate a method for measuring QTP and NQTP concentrations in human plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

**Materials and Methods:** Plasma samples were processed using liquid-liquid extraction, and the analytes were separated on a C18 column. The analysis was conducted using a triple quadrupole tandem mass spectrometer with a mobile gradient phase consisting of 0.1% formic acid in 10 mM ammonium formate and 100% acetonitrile. The total run time was 6 min.

**Results:** The calibration curves for QTP and NQTP were in a linear range of 2.0 to 600.0 ng/ml. The within-run and between-run precision for QTP was 10.29% and 11.16%, whereas those for NQTP were 8.21% and 12.87%, respectively. The within-run and between-run accuracy for QTP was <111.74%, <100.34%, <110.53%, and <104.11% for NQTP, respectively. The lower limit of detection for QTP and NQTP was 2.0 ng/ml.

**Conclusion:** An LC-MS/MS method for measuring QTP and NQTP levels in human plasma was validated and successfully applied to measure these analytes in psychiatric patients.

**Keywords:** Quetiapine; Norquetiapine; Antipsychotics; LC-MS/MS; Plasma concentrations; Liquid-liquid extraction

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Quetiapine (QTP) is an atypical antipsychotic medication known as a second-generation antipsychotic that results in a lower risk of extrapyramidal side effects. QTP has been approved by the United States Food and Drug Administration since 1997 for treating patients with manifestations of psychotic disorders, including schizophrenia and bipolar disorder[1-4]. Approximately 89% of QTP is metabolized to the reactive metabolite N-desalkylquetiapine (norquetiapine, NQTP) primarily by CYP3A isoforms, including CYP3A4 and CYP3A[1, 5]. QTP acts on serotonin receptors (5-HT1A, 5-HT2A, and 5-HT2C), dopamine receptors (D1 and D2), histamine receptors (H1), and adrenergic receptors (α1 and α2) in the brain[6]. NQTP has a high affinity for the norepinephrine transporter (NET) and the serotonin receptors (5-HT2C and 5-HT1A)[7]. The efficacy of QTP treatment in psychiatric patients was recently evaluated; therefore, it is important to measure QTP and NQTP levels to improve QTP efficacy and lower adverse events in psychiatric patients.

Due to the extensive metabolism of QTP, which results in very low plasma concentrations, the therapeutic

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range of QTP is 70 to 170 ng/mL. However, current HPLC methods are limited when measuring QTP blood levels at low concentrations[8]. Therefore, a sensitive and specific method is needed to establish the pharmacokinetics of QTP and NQTP in humans, such as LC-MS/MS. Although several LC-MS/MS assays have been reported, these methods have some limitations, including low sensitivity, which is insufficient for measuring plasma sample analytes in clinical practice[9-12]. Previous studies have described complex, low-sensitivity, and expensive extraction methods, such as protein precipitation and solid-phase extraction[13-16]. Moreover, the long retention time of 20 min for the analytes is inconvenient for analyzing large numbers of samples in clinical practice[13,17]. Moreover, the methods for measuring NQTP levels in human plasma are limited by low sensitivity[12,18].

In the present study, we developed and validated a sensitive, simple, and cost-effective LC-MS/MS method for the simultaneous measurement of QTP and NQTP levels. This assay was successfully applied to determine QTP and NQTP levels in the plasma of dementia patients treated with QTP. This method can be used to provide routine clinical laboratory services, thereby enhancing outcomes in dementia and other psychiatric conditions.

## Objective

The present study aimed to develop and validate a method for measuring QTP and NQTP concentrations in human plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

## Materials and Methods

### Materials

Quetiapine fumarate (99.7%), norquetiapine (97.7%), and the internal standard (IS, risperidone) (99.7%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) and methanol (MeOH) were HPLC grade (RCI Labscan limited, Bangkok, Thailand). Analytical grade ammonium formate was obtained from Sigma-Aldrich (St. Louis, MO, USA). Formic acid and ethyl acetate were from Carlo Erba Reagent SAS (Val de Reuil, France). Blank human plasma was obtained from the blood blank at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, and stored at -20°C until use. The ethics committee approved this study for Human Research, Khon Kaen University (HE631385), Khon Kaen, Thailand.

### Chromatographic conditions

The Agilent 1,260 Infinity liquid chromatography system (Agilent Technologies, Foster City, CA, USA) was used. The analytes were separated on an XBridge BEH C18 column (3.0×50 mm, 2.5 µm, Waters, USA) with an XBridge BEH C18 guard column (2.1×50 mm, 2.5 µm, Waters, USA) and maintained at 30°C. The analytes were eluted using a gradient mobile phase system consisting of mobile phase A (10 mM ammonium formate in 0.1% formic acid, pH 3.0) and mobile phase B (100% acetonitrile). After injection, the chromatogram at the start of the run consisted of 5% mobile phase A and 95% mobile phase B, which were held for 2.0 min. The composition then decreased to 5% mobile phase B at 2.2 min and was maintained for 2.5 min. It was then increased to mobile phase B at 10% from 2.5 to 3.0 minutes and maintained at 15% mobile phase B from 3.5 to 4.0 minutes. Finally, the solvent combination was reversed to 15% mobile phase A and 85% mobile phase B, and this composition was maintained until the total run time reached 6.0 min. The flow rate was 0.3 ml/min. The injection volume was 3 µL.

### Mass spectrometric conditions

An atmospheric pressure ionization (API) source (ABSciex API 3200TM) triple quadrupole mass spectrometer from Applied Biosystems (SCIEX, Framingham, MA, USA) with an electrospray ion source (ESI) interface operated in positive mode was used for multiple reaction monitoring (MRM) with an m/z transition of 384.4 → 253.4 for quetiapine, m/z 296.5 → 210.3 for norquetiapine, and m/z 412.0 → 191.1 for IS, respectively. The optimized spectrometric conditions, including ion fragments, declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell entrance potential, and collision cell exit potential (CXP) for QTP, NQTP, and IS, are listed in Table 1. The integration of peak area and concentrations was determined using Analyst software (version 1.5.2; SCIEX, Framingham, MA, USA).

### Preparation of stock and working solutions as well as standard and quality control samples

Primary stock solutions of QTP, NQTP, and IS for standard and quality control (QC) samples were prepared in methanol at a concentration of 1 mg/mL and stored at −20°C until use. The standard working solution was prepared by diluting a stock solution with a mixture of mobile phase A and B (0.1% formic acid in 10 mM ammonium formate at pH 3-acetonitrile; 50:50, v/v). The standards were prepared by spiking blank plasma with standard working solutions containing QTP and NQTP to obtain final concentrations of 2.0, 10.0, 25.0, 50.0, 100.0, 200.0, 400.0, and 600.0 ng/mL. The IS was diluted to 30.0 ng/mL. QCs working solutions were prepared at three different concentrations: low level (QCL=6.0 ng/mL), middle level (QCM=250.0 ng/mL), and high level (QCH=480.0 ng/mL). These calibration standards were treated in the same manner as the samples.

**Table 1.** Optimal MRM transition conditions for the targeted analytes and ion source

| Analyte | Q1 (m/z) | Q3 (m/z) | DP (Volts) | EP (Volts) | CE (Volts) | CXP (volts) | Dwell (msec) |
|---|---|---|---|---|---|---|---|
| Quetiapine | 384.4 | 253.4 | 28.54 | 2.52 | 30.24 | 3.81 | 300 |
| Norquetiapine | 296.5 | 210.3 | 76.23 | 3.74 | 35.10 | 2.73 | 300 |
| Risperidone | 412.0 | 191.1 | 41.40 | 2.01 | 35.28 | 2.46 | 300 |
| **Ion source** | | | | | | | |
| Ion Spray Voltage (IS) | 4,500 | | | | | | |
| Temperature (TEM) | 475 | | | | | | |
| Curtain Gas (CUR) | 30.0 | | | | | | |
| Collision Gas (CAD) | 6.0 | | | | | | |
| Polarity mode | Positive | | | | | | |
| Ion source Gas 1 (GS1) | 40.0 | | | | | | |
| Ion source Gas 2 (GS2) | 45.0 | | | | | | |
| Analyte-dependent | | | | | | | |

Q1=precursor ion mass; Q3=product ion mass; DP=declustering potential; EP=entrance potential; CE=collision energy; CXP=collision cell exit potential

Dwell time=refers to the time duration the mass spectrometer spends collecting data for a specific ion transition (or precursor-to-product ion) during each cycle.

## Sample preparation

Plasma samples were prepared by liquid-liquid extraction. Plasma samples (150 µl) were processed by adding 25 µl of IS to each microtube and 300 µl of ethyl acetate. After vortex-mixing for 5 min, the sample was centrifuged at 13,200 rpm at 25°C for 5 min. The supernatant was transferred to a fresh microtube and evaporated to dryness for 20 min at 60°C. The dry residue was reconstituted in 50 µL of a 1: 1 mixture of mobile phase A and B (0.1% formic acid in 10 mM ammonium formate at pH3, acetonitrile; 50: 50, v/v), vortexed for 5 min, and centrifuged at 13,200 rpm at 25°C for 5 min. Finally, 45 µl of supernatant was transferred into the insert vial, and a 3-µl aliquot was injected into the LC-MS/MS system.

## Method validation

The methods were validated according to the US Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance for Industry[19], as detailed below. The ethics committee approved this study for Human Research, Khon Kaen University (HE631385), Khon Kaen, Thailand.

### 1) Selectivity, Specificity, and Carry-over

Selectivity was analyzed by comparing chromatograms of six different sources of blank human plasma samples and pooled plasma samples, with no interference from the QTP, NQTP, and IS peaks. Specificity was confirmed by analyzing six individual blank human plasma samples to ensure no interfering peaks were present at the retention times for QTP, NQTP, and IS at the LLOQ. The effect of carry-over for each ion was evaluated immediately after analyzing the highest calibration standard by injecting blank plasma.

### 2) Linearity and LLOQ

Linearity was evaluated by establishing a calibration standard curve with 8 concentration points on 3 different days of analysis, over a range of 2.0 to 600.0 ng/mL. Calibration curves were generated from the peak area ratios of the analyte to the internal standard (IS) against the concentrations analyzed. The slope, intercept, and correlation coefficient (r) regression parameters should be greater than 0.99 and were calculated using quadratic regression with 1/x weighting. The LLOQ was defined as the lower limit of quantification in the calibration curve, which is acceptable for precision and accuracy. To meet the validation criteria, the error of accuracy and coefficient of variation (CV) should be within ±15% of the nominal concentration value, except for LLOQ. In contrast, the error in accuracy should be within ±20% of the nominal value.

### 3) Precision and Accuracy

The intraday precision and accuracy were determined by analyzing six replicates of QTP and NQTP in a batch at QC level concentrations: LLOQ, QCL, QCM, and QCH (2.0, 6.0, 250.0, and 480.0 ng/ml, respectively). Interday precision and accuracy were determined using six replicates, consisting of five different batches of each QC concentration set over three days. The acceptance criteria for precision and accuracy were set at less than 15% of the variation coefficient, except for the LLOQ, which was set at less than 20% of the nominal value.

### 4) Matrix Effects and Extraction Recovery

The matrix effect was determined using six different lots of blank plasma spiked after extraction with the analyte and IS. For each analyte and IS, the matrix factor was calculated by comparing the ratio of peak areas present in the matrix (determined by analyzing blank matrix spiked with the analyte and IS after extraction) to the peak areas in the absence of the matrix (pure solution with the analyte and IS).

The extraction recovery represents the extraction efficiency of the method, which was defined as the ratio of

the peak areas of the extracted sample (before extraction) to the mean of the peak areas of the post-spiked sample (after extraction). Finally, the average percent recovery was calculated as follows:

Extraction recovery (%) = (before extraction/after extraction) × 100

All matrix effects and extraction recovery were performed using blank plasma samples from six different human donors at three concentrations of QCL, QCM, and QCH (6.0, 250.0, and 480.0 ng/mL, respectively), with IS at 30.0 ng/mL. As a result, the ratio measured by the coefficient of variation (CV) should be greater than 85% and less than 115%, whereas the coefficient of variation should be less than 15%.

#### 5) Stability

The stability of QTP and NQTP in human plasma was evaluated by determining the low QC and high QC (6.0 and 480.0 ng/ml) values in six replicates under the following conditions. The short-term stability was determined by analyzing the QC samples exposed at room temperature (bench-top Stability) for 24 h. After liquid-liquid extraction, the samples were maintained in an autosampler for 6 hours and 12 hours at room temperature to assess post-preparative stability. Additionally, the stability of the freeze-thaw process was evaluated after three consecutive freeze-thaw cycles of the QC samples, which were taken from -80°C to room temperature on the same days. Additionally, long-term stability was assessed by storing samples at -20°C and -80°C for 2 months. The analyte was compared to the results from the first analysis day versus a defined analysis day of samples prepared from the same stock solution batch. The acceptable criteria for stability are within ±15% of their respective nominal concentration. Therefore, the sample was considered stable. Each analytical run consisted of standard samples and a series of QC samples.

#### 6) Statistical analyses

The data are presented as mean values and standard deviations. The Spearman rank correlation test was used to evaluate the association between two continuous random variables. The results of the lack-of-fit test were compared to pure error variances at a 95% confidence level to assess the acceptability of the results and the adequacy of the regression models. The zero-score test was used to determine whether something was true. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 28.0.1.0 (IBM Corporation, Armonk, New York, United States). A p-value less than 0.05 was considered statistically significant.

### Determination of plasma levels of quetiapine and NQTP in patient samples

Twenty psychiatric patients who were diagnosed with a Mini-Mental State Examination score and Neuropsychiatric Inventory (NPI Score) were enrolled. All patients received quetiapine in doses ranging from 25 to 400 mg for over 2 months. Blood samples were collected 12 to 16 hours after the last quetiapine administration to ensure measurement of trough drug concentrations at a steady state. The ethics committee approved this study for Human Research, Khon Kaen University (HE631385), Khon Kaen, Thailand. Blood samples from each patient were collected before the next QTP dose and placed into an EDTA tube. The plasma samples were separated and stored at −20°C until analysis.

## Results

### LC-MS/MS method validation

#### 1) Selectivity, Specificity, and Carry-over

Selectivity (n=6) was validated by analyzing different sources of blank human plasma and pooled plasma samples. The extraction ion chromatograms were compared between the same type of matrix to confirm that there were no interfering peaks from endogenous substances or other matrix components (Figure 1A). A standard spiked sample of 2.0 ng/mL QTP, NQTP, and 30.0 ng/mL of IS in human blank plasma exhibited retention times of 1.40 and 1.29 min, and 1.38 and 1.29 min, respectively (Figure 1B, C). The signal of the peak indicated well-separated symmetry with no overlap. Therefore, this LC-MS/MS method is highly selective for differentiating and quantifying analytes at a concentration of 2.0 ng/mL in plasma. The peaks for QTP, NQTP, and IS in the blank plasma samples, injected immediately after the upper limit of the quantitation sample, were less than 20% of the LLOQ, indicating negligible carry-over.

#### 2) Linearity and LLOQ

Linear eight-point calibration curves were constructed over a concentration range from 2.0 to 600.0 ng/ml for the analytes and 30.0 ng/ml for IS in human plasma. The analysis was conducted by establishing a three-day calibration curve, calculated using quadratic regression with 1/x weighting. Linearity was remarkably well-correlated with the mean calibration equation, and the range of value was y = -1.89e - 006x2 + 0.00542x + 0.00449, r = 0.9994 for QTP and y = -2.33e - 006x2 + 0.00241x + 0.00252, r = 0.9991 for NQTP. The precision and accuracy of all calibration points were within the acceptance of ±15% of the nominal concentration value, except for LLOQ, which was within ±20% of the nominal value. The LLOQ of QTP was 2.0 ng/ml with an accuracy (CV%) of 97.19% and a precision (%CV) of 11.16%. The LLOQ of NQTP was 2.0 ng/ml with an accuracy (CV%) of 97.08% and a precision (%CV) of 12.87%. This indicates that the sensitivity of this method is suitable for the therapeutic range observed for QTP.

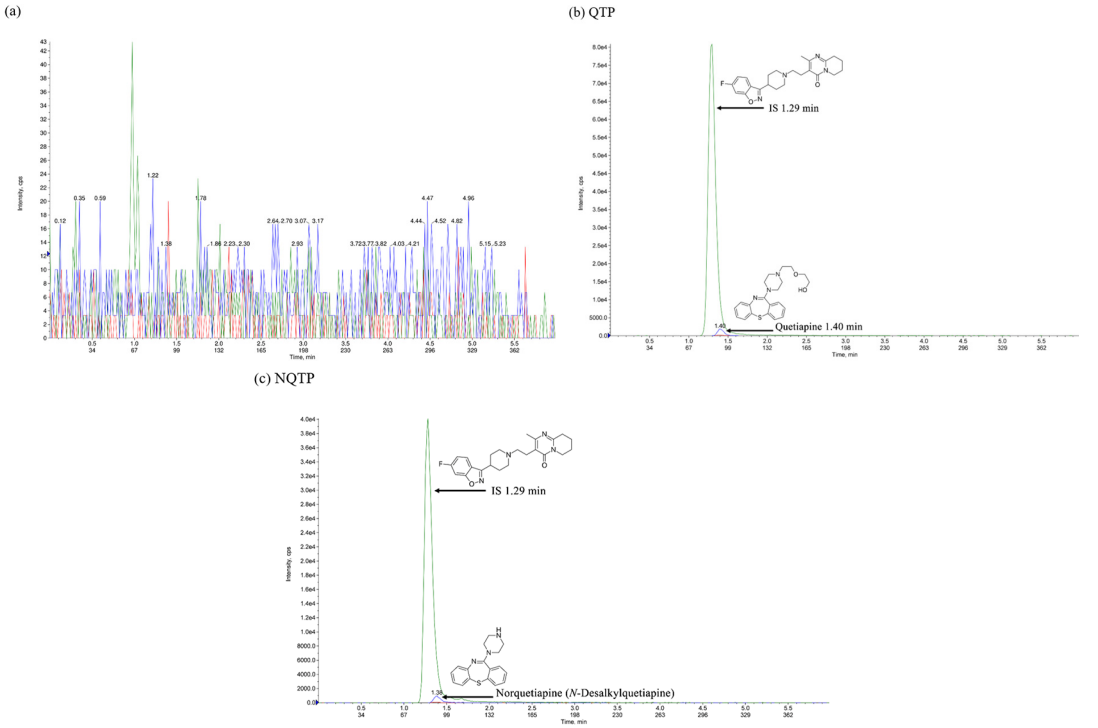


**Figure 1.** Representative LC-MS/MS mass transition chromatogram. A) Pooled blank plasma from six different lots; B) QTP blank plasma spiked with quetiapine at 2.0 ng/ml and IS at 30.0 ng/ml; C) Blank plasma spiked with norquetiapine at 2.0 ng/ml and IS at 30.0 ng/ml.

### 3) Precision and Accuracy

Intraday and interday precision and accuracy values for the analysis of six replicates for LLOQ, QCL, QCM, and QCH (2.0, 6.0, 250.0, and 480.0 ng/ml, respectively) from five different batches on three days are listed in Table 2. The precision and accuracy for the intraday LLOQ values were 8.16% CV and 99.58% for QTP, and 8.21% CV and 104.08% for NQTP, respectively. The interday precision and accuracy were 11.16% CV and 97.19% for QTP, and 12.87% CV and 97.08% for NQTP, respectively. The %CV for the QC values for QTP ranged from 4.23 to 10.29% for intraday precision and 3.57 to 6.82% for interday precision. For NQTP, the range was 6.46% to 7.95% for intraday precision and 5.25% to 7.99% for interday precision. The accuracy values for QTP were between 98.73% and 111.74% for intraday and 96.72% and 100.34% for interday, respectively. In contrast, NQTP was between 100.94% and 110.53% for intraday and 93.17% and 104.11% for interday, respectively, compared with the corresponding nominal concentrations. The intraday and interday precision and accuracy of QTP and NQTP were less than 15%, indicating that this method is both accurate and reproducible for analyzing QTP in human plasma samples.

### 4) Matrix Effects and Extraction Recovery

A summary of the matrix effect and extraction recovery results is listed in Table 3. The mean extraction recovery of six replicates, spanning a concentration range of 6.0 to 480.0 ng/mL for QTP, NQTP, and 30.0 ng/mL of IS, was determined by comparing the peak responses of pre- and post-extraction samples. The extraction recoveries were between 101.04% and 107.18% for the QC sample of QTP, whereas for NQTP, they were between 57.33% and 69.54%, with a CV of less than 15%. The mean recovery of the IS was 95.74%, with a 6.65 %CV. The mean matrix effect range for QTP was 103.52% to 112.15% and 73.20% to 86.89% for NQTP, which were observed for QLC, QCM, and QCH, respectively. All coefficients of variation were less than 15%. These results indicated that plasma samples pretreated by liquid-liquid extraction yielded appropriate and stable extraction efficiencies for all analytes and IS without significant interference from the plasma matrix.

### 5) Stability

The QTP and NQTP sample stabilities were examined by storing in an autosampler at 25°C for 6 h and 12 h after extraction, whereas the short-term stability was determined on a bench-top at 25°C for 24 h. Plasma samples were stable for no more than three freezing steps. Thaw cycles should be performed on plasma samples during storage and transportation, indicating that they may be frozen and thawed at least three times before analysis. The freeze-thaw

**Table 2.** Intraday and Interday Precision and Accuracy for Quetiapine and Norquetiapine in Human Plasma

| Analyte | Nominal concentration (ng/ml) | Intraday (n=6) | | | Interday (n=18) | | |
|---|---|---|---|---|---|---|---|
| | | Measured conc. (ng/ml ± SD) | Accuracy (%) | Precision (%CV) | Measured conc. (ng/ml ± SD) | Accuracy (%) | Precision (%CV) |
| Quetiapine | 2.0 | 1.99±0.16 | 99.58 | 8.16 | 1.94±0.21 | 97.19 | 11.16 |
| | 6.0 | 6.09±0.26 | 101.44 | 4.23 | 5.98±0.36 | 100.34 | 6.20 |
| | 250.0 | 246.83±25.40 | 98.73 | 10.29 | 250.44±16.46 | 100.16 | 6.82 |
| | 480.0 | 434.33±20.54 | 111.74 | 4.73 | 464.28±16.07 | 96.72 | 3.57 |
| Norquetiapine | 2.0 | 2.08±0.17 | 104.08 | 8.21 | 1.94±0.24 | 97.08 | 12.87 |
| | 6.0 | 6.07±0.40 | 101.11 | 6.64 | 6.03±0.48 | 100.45 | 7.99 |
| | 250.0 | 276.33±21.98 | 110.53 | 7.95 | 260.28±15.74 | 104.11 | 6.26 |
| | 480.0 | 484.50±31.30 | 100.94 | 6.46 | 447.22±23.68 | 93.17 | 5.25 |

Measured concentrations were shown as means ± SD

SD=The standard deviation of the mean; CV=The error of accuracy and coefficient of variation

**Table 3.** Recovery and Matrix effects of quetiapine, norquetiapine, and IS in human plasma (n=6)

| Analyte | Nominal concentration (ng/ml) | Extraction recovery (%) | CV (%) | Matrix effect (%) | CV (%) |
|---|---|---|---|---|---|
| Quetiapine | 6.0 | 106.14 | 7.14 | 103.52 | 2.93 |
| | 250.0 | 107.18 | 4.00 | 107.88 | 4.27 |
| | 480.0 | 101.04 | 7.31 | 112.15 | 2.06 |
| Norquetiapine | 6.0 | 69.54 | 4.38 | 73.20 | 5.76 |
| | 250.0 | 68.02 | 4.62 | 80.01 | 8.15 |
| | 480.0 | 57.33 | 8.22 | 86.89 | 12.12 |
| Risperidone (IS) | 30.0 | 95.74 | 6.65 | 96.73 | 2.66 |

IS=internal standard; CV=The error of accuracy and coefficient of variation

stability assay results at low and high concentrations are provided in Table 4, indicating that the analytes remain considerably stable under the above conditions. Moreover, the long-term stability indicated that both compounds were stable for 2 months when stored at -20°C and -80°C. In addition, the error in the precision of the peak area of QTP and NQTP was less than 15%, and the accuracy was within 85% to 115%.

### Application of the LC-MS/MS method

The goals of this study were to develop and validate a highly efficient LC-MS/MS method for measuring quetiapine and NQTP in psychiatric patients, and to determine the association between the genetic polymorphism of *CYP3A5*, quetiapine, and NQTP levels, and clinical outcomes.

## Discussion

The authors developed an LC-MS/MS method for detecting QTP and NQTP in human plasma, which was fully validated according to US FDA guidelines. CYP3A4 metabolizes QTP to NQTP, which is a major metabolite. The mechanism of action of QTP is mediated through an antagonizing combination of both serotonin type 2 (5-HT2A) and dopamine type 2 (D2) receptors[20]. NQTP inhibits norepinephrine transporter (NET) reuptake and, therefore, mediates the antidepressant effect of QTP. NQTP binds to the serotonin type, including the 5-HT2C and 5-HT1A receptors. NQTP accounts for most of the pharmacological effects of QTP with similar potency in anti-depressive patients[21]. Previous studies were limited to measuring both QTP and NQTP in human plasma. Therefore, we measured both QTP and NQTP in human plasma to improve the therapeutic monitoring of QTP treatment of psychiatric patients. The objective of developing this method is not only to facilitate research purposes but also to apply it in the clinic for future patient monitoring. Therefore, we established a simple, sensitive, rapid, and low-cost method to measure QTP and its metabolite NQTP in human plasma. The liquid-liquid extraction method exhibited high sensitivity, simplicity, and a shorter preparation time compared with previous studies[18,22]. Previously, a longer retention time was reported compared to the present study (10 to 35 min vs. 6 min) for QTP, and NQTP was limited to the analysis of large samples simultaneously, which was impractical for a clinical service laboratory. Different extraction methods were performed, including solid-phase extraction and protein precipitation.

**Table 4.** Stability of QC Samples for Quetiapine (QTP) and Norquetiapine (NQTP) in Human plasma

| Stability | Quetiapine | | | Norquetiapine | | |
|---|---|---|---|---|---|---|
| | Nominal conc. (ng/ml) | Measured conc. (ng/ml) | Accuracy (%) | Nominal conc. (ng/ml) | Measured conc. (ng/ml) | Accuracy (%) |
| Autosampler (room temperature for 6 h) | 6.0 | 6.51±0.29 | 108.56±4.86 | 6.0 | 6.40±0.36 | 106.58±6.00 |
| | 480.0 | 475.00±5.31 | 98.96±7.13 | 480.0 | 445.33±30.51 | 92.78±6.36 |
| Autosampler (room temperature for 12 h) | 6.0 | 6.74±0.17 | 112.36±2.85 | 6.0 | 6.42±0.24 | 106.92±4.04 |
| | 480.0 | 469.33±34.76 | 97.78±7.24 | 480.0 | 432.67±23.90 | 90.14±4.98 |
| Freeze-thaw (3 cycles) | | | | | | |
| −80°C | 6.0 | 6.39±0.31 | 106.42±5.10 | 6.0 | 6.85±0.30 | 114.17±4.95 |
| | 480.0 | 439.00±25.46 | 91.46±5.30 | 480.0 | 439.67±27.98 | 91.60±5.83 |
| Bench-top (room temperature for 24 h) | | | | | | |
| | 6.0 | 6.75±0.32 | 112.42±5.31 | 6.0 | 6.50±0.19 | 108.28±3.13 |
| | 480.0 | 473.50±17.16 | 98.65±3.57 | 480.0 | 445.67±167.91 | 92.85±10.31 |
| Long-term (2 months) | | | | | | |
| −20°C | 6.0 | 6.28±0.30 | 104.58±5.04 | 6.0 | 6.01±0.26 | 100.14±4.31 |
| | 480.0 | 459.5±28.03 | 95.73±5.84 | 480.0 | 459.33±20.53 | 95.69±4.28 |
| −80°C | 6.0 | 6.10±0.21 | 101.64±3.46 | 6.0 | 6.35±0.25 | 105.86±4.14 |
| | 480.0 | 431.33±18.18 | 89.86±3.79 | 480.0 | 466.17±23.67 | 97.12±4.93 |

Measured concentration and accuracy are shown as Means ± SD.

CV=The error of accuracy and coefficient of variation

**Table 5.** Plasma Concentrations of Quetiapine and Norquetiapine and C/D Ratios in Psychiatric Patients

| Quetiapine plasma concentrations, daily dose, and C/D ratio | |
|---|---|
| n | 20 |
| Sex M/F | 10/10 |
| Age, years | 61.95±17.85 |
| Dose, (mg/day) | 231.25±133.99 |
| Mean Concentration of Quetiapine (ng/mL) | 76.24±63.96 |
| Mean Concentration of Norquetiapine (ng/mL) | 64.62±64.36 |
| C/D Quetiapine ratio, [(ng/mL)/(mg)] | 0.36±0.28 |
| C/D Norquetiapine ratio, [(ng/mL)/(mg)] | 0.28±0.27 |

Data are shown as Means ± SD.

C/D ratios=Dose-adjusted plasma concentrations of Quetiapine and N-desalkylquetiapine were calculated by dividing the plasma concentration by the total daily dose of quetiapine. (C/D ratios, ng per milliliter per milligram per day).

Solid-phase extraction is expensive and time-consuming, making it unsuitable for limited research budgets[18,22]. Moreover, protein precipitation requires time for evaporation and a large volume of solvent[23]. Therefore, we devised a simple one-step liquid-liquid extraction and rapid sample preparation method. Previous studies used large plasma sample volumes (500 µl) with a low chance of performing replicates and re-analysis[18,22]. Our method requires a small amount of plasma sample (150 µl). Moreover, a previous study reported quantifying QTP and NQTP in human plasma at an LLOQ of 5 ng/ml[12], and the LLOQ for QTP and NQTP was 1 ng/ml in rat plasma[24]. We measured an LLOQ of 2.0 ng/ml for QTP and NQTP in psychiatric patients treated with QTP. Therefore, we developed a method to measure QTP and NQTP for use in the research laboratory and the clinic. Therapeutic drug monitoring for QTP and NQTP using our assay may improve efficacy and reduce toxicity in psychiatric patients.

## Conclusion

The authors developed and validated a simple, sensitive, rapid, and low-cost assay to measure QTP and NQTP, which was validated in accordance with US FDA guidelines. The method showed high accuracy, precision, and linearity for measuring QTP and NQTP within the 2.0 to 600.0 ng/ml range. It requires a smaller amount of plasma sample (150 µl) and includes a simple one-step liquid-liquid extraction to prepare blood samples. This method can measure QTP and NQTP levels in psychiatric patients.

### What is already known on this topic?

QTP and NQTP have very low plasma concentrations. The current HPLC methods are limited to measuring both blood levels. The LC-MS/MS method had better sensitivity and specificity for measuring. Previous studies on measuring QTP and NQTP using the LC-MS/MS method have described complex, low-sensitivity, and expensive extraction methods, as well as long analysis times for each sample. Therefore, these methods did not apply to clinical service laboratories. Therefore, we developed and validated an LC-MS/MS method for measuring QTP and NQTP, aiming to

simplify, reduce costs, and shorten analytical time.

### What this study adds?

The present study employed a validated LC-MS/MS method to measure QTP and its metabolite, NQTP, in patients with psychiatric disorders. Interestingly, both QTP and NQTP were stable for 2 months under various conditions, including freeze-thaw cycles and long-term storage at −20°C and −80°C. The present study presents a more efficient, sensitive, and clinically applicable QTP and NQTP measurement method, offering advantages in terms of cost, time, and sample volume. It provides a practical tool for pharmacokinetic studies and therapeutic drug monitoring in psychiatric settings, making it a valuable addition to the field of psychiatric pharmacology research.


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## Conflicts of interest

The authors declare no conflict of interest.

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