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# A validated method for the determination of quetiapine fumarate tablets in human plasma by UPLC-MS/MS and its application to a pharmacokinetic study in healthy Chinese subjects

**Kaiwei Luo[1], Haiming Zhao[1], Huina Zhang[1], Lihong Huang[1], Jie Lin[1], Qunan Cheng[1], Wenjuan Yang[2], Weiyong Lu[2], Pingping Sun[2] and Tingting Lou[2]***

[1]Fuan Pharmaceutical Group Ningbo Team Pharmaceutical Co., Ltd., Ningbo, China
[2]Tongde Hospital of Zhejiang Province, Hangzhou, China

**Abstract**: A rapid, highly specific and sensitive UPLC-MS/MS method was developed for the determination of Quetiapine Fumarate, a therapeutic drug for various psychiatric disorders, in human plasma. The samples were pretreated using a protein precipitation method, followed by chromatographic separation using a column (Kinetex C18, 2.6 µm 50*2.1 mm) equipped with an ESI source and MRM mode mass spectrometer. In the validation results of the method, the analyte quetiapine showed a peak at approximately 1.0 minute and exhibited good linearity within the concentration from 2.5 to 2000ng/mL. The intra- and inter-batch precision CV% were within the range of -1.3% to 7.7% and precision of intra- and inter-batch were below 15.0%. Furthermore, this method demonstrated low matrix effects and high recovery rates. The quetiapine plasma sample solution remained stable at room temperature for 25 hours and following 4 freeze-thaw cycles. The prepared samples remained stable in the autosampler (The temperature control of the autosampler was 5ºC) for 185 hours and after four freeze-thaw cycles at -20ºC and -70ºC for 40 days. The present work effectively employed this approach to investigate the pharmacokinetics of orally administered quetiapine fumarate tablets in a cohort of healthy Chinese individuals, both in a fasting state and after a meal.

**Keywords**: Quetiapine fumarate, UPLC-MS/MS, pharmacokinetic, validation

## INTRODUCTION

Quetiapine fumarate is an antipsychotic medication widely used for the treatment of schizophrenia and bipolar disorder. It belongs to the second-generation antipsychotic drug family and has multiple pharmacological actions (El-Khalili *et al*., 2012; Weisler *et al*., 2013; Muneer *et al*., 2015). Quetiapine fumarate mainly exerts its therapeutic effects by interacting with various neurotransmitter receptors (Bui *et al*., 2013; Cross *et al*., 2016; Ayed *et al*., 2021; Poyurovsky *et al*., 2021; Oruch *et al*., 2020). As a drug that targets multiple receptors, Quetiapine fumarate primarily acts on dopamine D2, serotonin 5-HT2A receptors and alpha-1 adrenergic receptors. Through these mechanisms, Quetiapine fumarate plays a crucial role in regulating the balance of neurotransmitters, particularly dopamine and serotonin, which can alleviate symptoms such as hallucinations, delusions and mood instability in patients (Zhang *et al*., 2021; Poyurovsky *et al*., 2023; Crapanzano *et al*., 2021; Stogios *et al*., 2022; Carr *et al*., 2016). The pharmacokinetic characteristics of quetiapine fumarate include good oral absorption, a relatively long half-life and high protein binding. It undergoes hepatic metabolism mainly involving the CYP3A4 enzyme, necessitating attention to potential drug interactions (Goodlet *et al*., 2019; Thomas et al., 2018; Sattar *et al*., 2020; Deutschmann *et al*., 2021). In clinical practice, Quetiapine fumarate is widely employed for treatment of schizophrenia and bipolardisorder. Additionally, it can also be used as an adjunctive therapy for other psychiatric disorders such as depression, anxiety and sleep disorders. Quetiapine fumarate can be used alone or in combination with other drugs, depending on the patient's specific condition and symptoms.

The absorption of quetiapine fumarate primarily occurs in the gastrointestinal tract and can be rapidly absorbed into the bloodstream following oral administration. The rate of drug absorption depends on several factors including the drug formulation, gastrointestinal motility and food intake. Generally, quetiapine fumarate is absorbed more quickly and completely when taken on an empty stomach. Furthermore, it is mainly distributed through plasma protein binding, particularly accumulating in tissues such as the brain and liver. This high degree of protein binding may result in interactions with other drugs or endogenous substances, affecting both efficacy and side effects (Ayed *et al*., 2021; Ji *et al*., 2021). Moreover, quetiapine fumarate undergoes multi-step enzyme-mediated metabolic reactions, especially metabolism mediated by the CYP3A4 enzyme (Shackleford *et al*., 2021). These metabolic reactions generate active metabolites, including Quetiapine, which play important roles in drug efficacy and adverse reactions.

Therefore, pharmacokinetic studies of quetiapine fumarate are of great significance for improving treatment efficacy, reducing adverse reactions and guiding

*Corresponding author: e-mail: lloutingting@126.com

individualized pharmacotherapy (Zhang *et al*., 2019; Abdel wahab *et al*., 2020). However, as our understanding of the pharmacokinetic mechanisms of this drug deepens, further research is needed to enhance our knowledge of quetiapine fumarate's pharmacokinetics and optimize its clinical application (Rezaei *et al*., 2018; Kaushik *et al*., 2018; Badhan *et al*., 2020). This paper presents a novel UPLC-MS/MS methodology for quantifying the concentration of quetiapine fumarate in human plasma. The method's efficacy has been verified by validation procedures including Chinese individuals who are in good health. The findings indicate that this approach exhibits a notable level of sensitivity and precision and has effectively been utilized in the examination of the pharmacokinetics of Quetiapine fumarate in human subjects.

## MATERIALS AND METHODS

***Materials and reagents***

Reference preparation Quetiapine Fumarate tablets, supplier (AstraZeneca UK Limited); Reference to Quetiapine Fumarate: purity 99.6%, source (China Institute for Food and Drug Control); Internal standard Quetiapine fumarate-d8, (TLC PHARMACEUTICAL), the purity was 95.7%. Human heparin sodium (The First Affiliated Hospital of xiamen University), the batch number of the heparin sodium used was SBL2021041102. The supplier of CAN(HPLC) and MeOH(HPLC) was MERCK; Supplier of FA(ACS) was Sigma.

***Instrument***

Liquid chromatography was 30AD Series UHPLC System (SHIMADZU), Shimadzu Enterprise Management (China) Co., LTD.; MS: TurboIonSpray API 5500, Applied Biosystems/Sciex; Oscillator MX-S, Scilogex; DG-2500R, Shanghai Bajiu Industry; Balance(Sartorius), CPA225D; Trigger shake (Thermo); Centrifuge 5810R, Eppendorf; Ultrasonic cleaner(GoodUltrasonic) GT SONIC-D20.

***Conditions for UPLC and MS***

Conditions for UPLC: Mobile phase A: 0.2% aqueous solution of formic acid (FA), Mobile phase B: 0.2% FA solution in CAN:MeOH (5:5), respectively. Flow rate: 0.4mL/min, Injection volume: 3mL; Column temperature: 40ºC; Collection duration: 2 minutes. Sample injector was controlled at a temperature of 5ºC. Separation was carried out using an analytical column with the specifications of Kinetex C18 100Å, 50×2.1 mm, 2.6 µm.

Conditions for MS: ESI, Positive, MRM, Quetiapine Fumarate: 384.100→253.200, Quetiapine Fumarate-d8: 392.200→258.100; CAD: 8.00; IonSpray Voltage: 5500 V; TEM: 450.

***Preparation of standard solutions, stock solutions and quality control samples (QC sample)***

Prepared standard curve and working solutions for QC samples: Accurately weighed quetiapine fumarate, diluted with MeOH to form a stock solution with a concentration (1.0mg/mL). Dilute with a 50% methanol solution to obtain standard curve working solutions with concentrations of 50, 100, 400, 2000, 8000, 16000, 32000 and 40000ng/mL, as well as working solutions for LLOQ QC (50ng/mL), LQC (150ng/mL), GMQC (3000ng/mL), MQC (20000 ng/mL), HQC (30000ng/mL) and DQC (80000ng/mL).

Standard curve sample solutions: For convenience, diluted the standard curve working solution in blank matrix to obtain sample solutions with concentrations of 2.5, 5.00, 20.0, 100, 400, 800, 1600 and 2000ng/mL.

Internal standard (IS) working solution: Weighed an appropriate amount of quetiapine fumarate-d8, dissolved completely in 50% MeOH to create a stock solution with a desired final concentration of 1.0mg/mL. Subsequently, diluted to obtain working solution of IS with concentration of 8ng/mL.

QC sample solutions: Transfered corresponding working solutions (20µL) to blank matrix (380µL) and diluted to the following concentrations: LLOQ QC (2.50ng/mL), LQC (7.50ng/mL), GMQC (150ng/mL), MQC (1000ng/mL), HQC (1500ng/mL), DQC (4000ng/mL).

***Sample preparation***

Added 30µL of the sample (standard curve sample, QC sample, test sample) to the corresponding position in a 96-well plate and then added 30µL IS working solution. For the blank sample, added 30µL of 50% MeOH instead. Then added 540µL of 0.1% FA in ACN solution and mixed for 10 minutes. Centrifuged for 5 minutes under 4ºC. Transfered 50µL supernatant to a new 96-well plate. Added 700µL 0.1% FA in ACN, sealed the plate and shaked for 10 minutes.

***Method validation***

*Calibration procedure*

The chromatographic charts of the samples were obtained and analyzed using Analyst version 1.6.3. The integration of analytes and IS in the samples was performed automatically. Concurrently, a linear regression analysis was conducted using the data obtained from the standard curve, resulting in the determination of concentration values for each individual sample.

***System suitability***

For the system suitability samples, the s/n of the analyte and IS should be ≥5; and the CV% for the percentage of peak area and RT of the analyte and IS in 6 consecutive injections should be ≤15.0%.

***Selectivity***

Selectivity was evaluated by analyzing blank matrix plasma and LLOQ calibration curve samples. When the analyte's peak area in the blank matrix was ≤20% of LLOQ and the peak area of the IS was less than 5%, it can

be considered that the interference from endogenous substances had minimal quantitative influence on each analyte.

***Standard curve and LLOQ***

We needed to prepare six sets of standard curves, each containing 8 concentrations levels. At least 50% of the standard curve samples at each concentration level should have a deviation within ±15.0% of the theoretical value. The correlation coefficient ($R^2$) for the standard curve should be ≥0.99. For the LLOQ, the deviation requirement was ±20%, precision should be ≤20% and s/n should be ≥5.

***Accuracy and precision of intra-batch, inter-batch***

By analyzing a batch of six QC samples, the % CV of the measured concentrations was calculated to assess within-batch precision. The deviation between the actual and theoretical concentrations was calculated to evaluate within-batch accuracy. Inter-batch precision and accuracy are assessed using 3 batches. Deviation and precision must be within ±15.0%.

***Recovery rate of Analyte***

The analysis process was conducted using a mixed matrix to determine the concentrations within the range of the standard curve. Recovery rates of the analyte and IS were determined by using concentration levels in the analysis: LQC, MQC and HQC samples were prepared (prepared 6 of each concentration), along with extraction of 18 blank samples. Added analyte and IS to the extraction solution to obtain a solution with the same concentration as LQC, MQC and HQC samples. Precision was evaluated by comparing the average peak areas of individual QC samples and blank samples with added analyte and IS using the normalization method. The precision must be within 15.0%.

***Matrix effect***

We extracted individual human plasma from 6 batches to investigate the matrix effect (Deutschmann *et al*., 2021; Ayed *et al*., 2021; Yan *et al*., 2020). Analyte and IS were added to normal blank plasma samples to match the final concentrations of LQC, MQC and HQC injection concentrations, preparing reference solutions containing the same concentration of analyte and IS after extraction. Matrix effect was calculated by percentage of peak area of analyte to IS (Kaushik *et al*., 2018; Badhan *et al*., 2020; Galgatte *et al*., 2014; Patel *et al*., 2022). Matrix effect of hemolyzed plasma and high lipid plasma was evaluated by comparing the analyte concentration in LQC and HQC samples (6 each) added to hemolyzed plasma and high lipid plasma with the newly prepared standard curve and QC samples using regular plasma. The precision of the matrix effect should be within 15.0%.

***Multiple of dilution***

Prepared DQC with concentrations exceeding the ULOQ (4000ng/mL) and then diluted the DQC with blank sodium heparin plasma using a dilution factor of 1/10 to reach diluted quantities inside the standard curve's (n=6) range. Deviation should be within ±15% and the precision ≤15%.

***Resolution stability***

The stability of quetiapine fumarate human plasma samples was investigated under -20˚C and -70˚C, as well as long-term stability. The stability of human whole blood was also examined at room temperature. Throughout the validation process, within the analyte channel, interference peaks with peak areas exceeding 20.0% of the mean peak area of the LLOQ should not occur.

***Pharmacokinetic study design***

According to the "Guiding Principles for the Study of Human Bioequivalence of Chemical Generic Drugs with Pharmacokinetic Parameters as the Endpoint Evaluation Index" promulgated by NMPA, the recommendations for the inclusion of subjects are as follows: This study will select healthy volunteers, referring to the FDA's guidance on bioequivalence studies of the drug and it was recommended to conduct the experiment under fasting/postprandial conditions. The study adopted a single-center, randomized, open-label, two-period, two-sequence, single-dose fasting/postprandial oral administration design method. Fasting (or consuming a high-fat meal for the postprandial experiment) for at least 10 hours before administration. On the next morning, a blank blood sample was collected, followed by fasting/postprandial oral administration of 0.2g (1 tablet) of fenofibrate with 240mL of water. For the fasting/postprandial experiments, blood samples were collected at the following time points: 0h (within 60 minutes before administration), 0.25h, 0.5h, 0.75h, 1h, 1.33h), 1.67h (1 hour 40 minutes), 2h, 2.5h, 3h, 3.5h, 4h, 4.5h, 5h, 6h, 8h, 12h and 24h, totaling 18 time points. Approximately 4mL blood was collected into a heparin anticoagulant collection tube each time and all centrifuged plasma samples will be divided into two portions. After blood collection, centrifugation should be completed within 90 minutes and the pre-processing of blood samples should be completed within 120 minutes and stored in a freezer below -60°C. After collecting all blood samples from the fasting/postprandial experiments of the subjects, the plasma samples will be transported to the sample testing unit under appropriate conditions for blood drug concentration determination. A total of 48 subjects (32 males, 16 females) were selected for the fasting experiment, with an average age of 30.3±7.6 years, an average weight of 60.7±7.0kg, a body mass index of 22.3±2.3kg/m2 and an average height of 165.0±6.4 cm. A total of 52 subjects (35 males, 17 females) were selected for the postprandial experiment, with an average age of 27.1±5.9 years, an average weight of 61.5±7.2kg, a body mass index of 22.5±2.0kg/m2 and an average height of 165.5±8.1 cm.

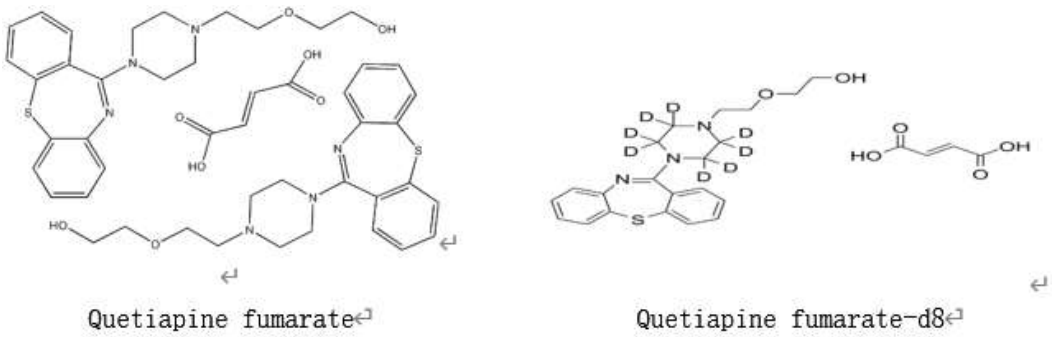


**Fig. 1**: Structural formula of Quetiapine fumarate and Quetiapine fumarate-d8

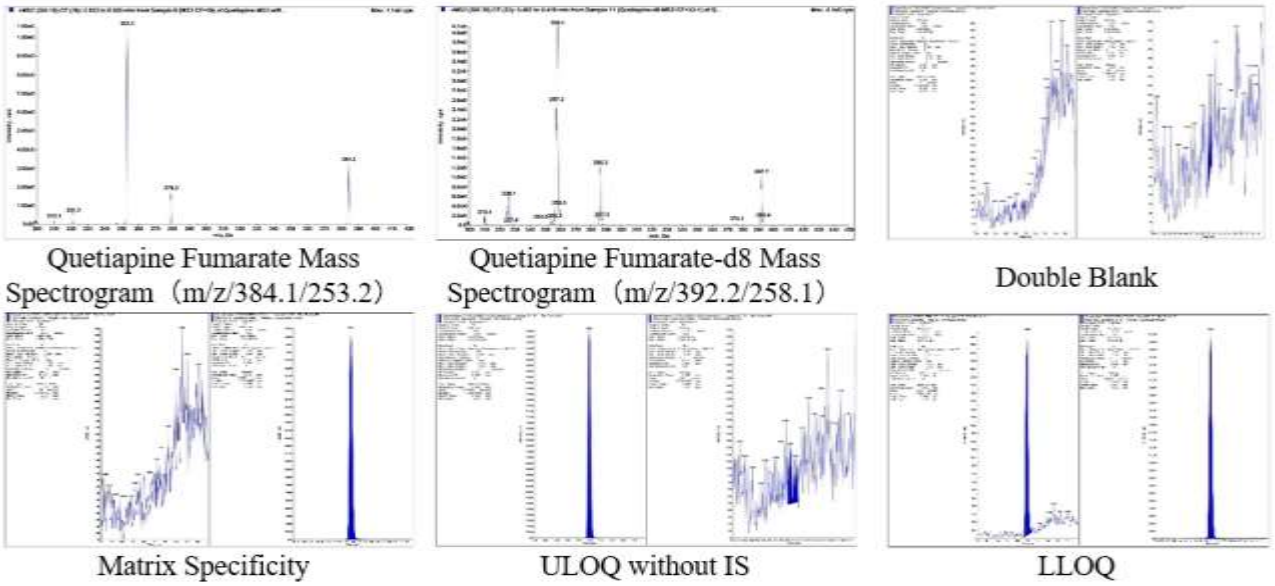


**Fig. 2**: Double Blank, Quetiapine Fumarate, Quetiapine Fumarate-d8, LLOQ Mass Spectrogram

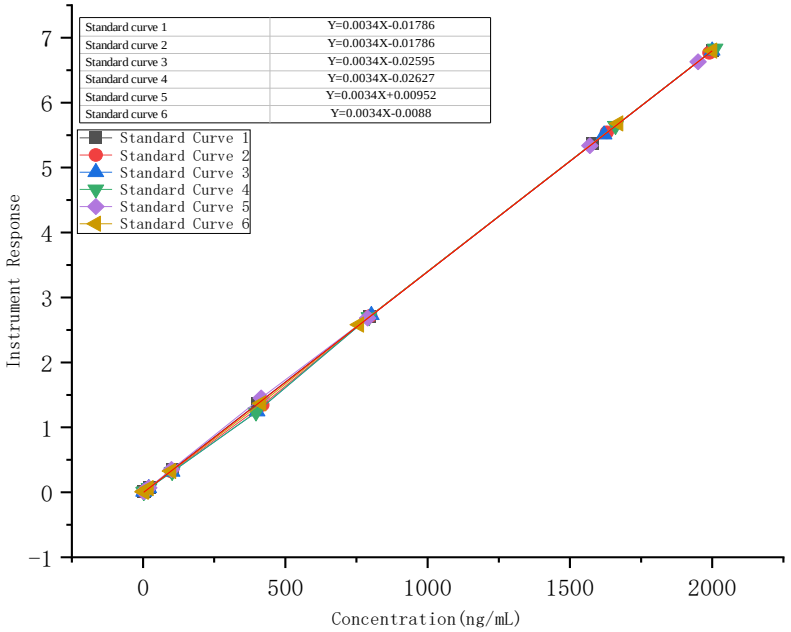


**Fig. 3**: Standard Curve of Quetiapine Fumarate

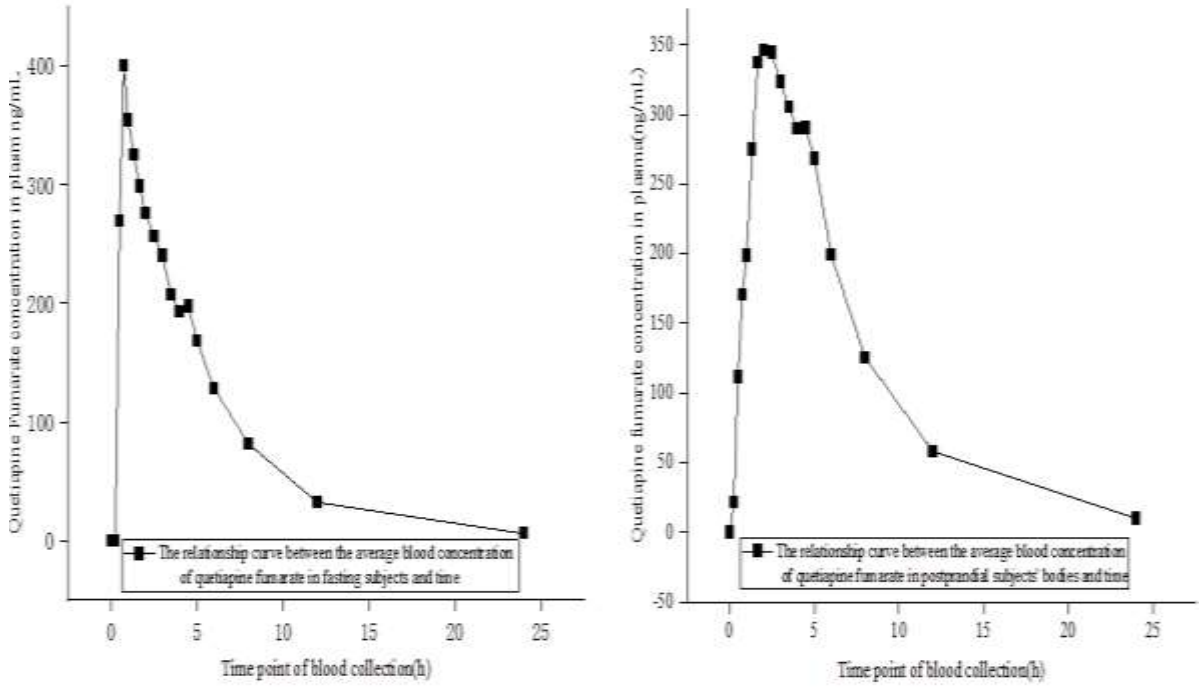


**Fig. 4**: Subject blood concentration-time curve (fasting and postprandial)

**Table 1**: System suitability result

| Sample ID | RT of quetiapine | RT of quetiapine CV% | IS RT | CV% of IS RT | Area ratio | Area ratio CV% |
|---|---|---|---|---|---|---|
| 1 | 0.986 | 0.3 | 0.969 | 0.3 | 1.791666 | 1.2 |
| 2 | 0.989 | | 0.971 | | 1.798831 | |
| 3 | 0.990 | | 0.970 | | 1.776716 | |
| 4 | 0.981 | | 0.965 | | 1.779185 | |
| 5 | 0.987 | | 0.964 | | 1.742002 | |
| 6 | 0.989 | | 0.966 | | 1.760920 | |

**Table 2**: The phenomenon of interference caused by a blank matrix on both the chemical and internal target

| Quetiapine Fumarate | | | | | Internal standard(IS) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ID | Blank matrix peak area | LLOQ peak area-1 | LLOQ peak area-2 | Interference % | ID | Blank matrix peak area | peak area of LLOQ -1 | peak area of LLOQ -2 | Interference % |
| 1 | 32 | 8195 | 7553 | 0.4 | 1 | 168 | 899528 | 904864 | 0.0 |
| 2 | 65 | | | 0.8 | 2 | 0 | | | 0.0 |
| 3 | 62 | | | 0.8 | 3 | 329 | | | 0.0 |
| 4 | 51 | | | 0.6 | 4 | 82 | | | 0.0 |
| 5 | 56 | | | 0.7 | 5 | 291 | | | 0.0 |
| 6 | 63 | | | 0.8 | 6 | 75 | | | 0.0 |

**Table 3**: Mutual interference of internal standards and analytes

| Quetiapine Fumarate | | | | | Internal standard(IS) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ID | QC0 | LLOQ peak area-1 | LLOQ peak area-2 | Interference % | ID | ULOQ without IS | ULOQ peak area-1 | ULOQ peak area-2 | Interference % |
| 1 | 29 | 8195 | 7553 | 0.4 | 1 | 410 | 784538 | 771641 | 0.2<br>0.2<br>0.2 |
| 2 | 50 | | | 0.6 | 2 | 247 | | | |
| 3 | 68 | | | 0.9 | 3 | 213 | | | |
| 4 | 36 | | | 0.5 | | | | | |
| 5 | 88 | | | 1.1 | | | | | |
| 6 | 48 | | | 0.6 | | | | | |

**Table 4**: Accuracy and precision of intra-batch, inter-batch

| Experiment number | LLOQ QC (ng/mL) | Accuracy deviation % | LQC (ng/mL) | Accuracy deviation % | GMQC (ng/mL) | Accuracy deviation % | MQC (ng/mL) | Accuracy deviation % | HQC (ng/mL) | Accuracy deviation % |
|---|---|---|---|---|---|---|---|---|---|---|
| 1# | 2.35 | -6.0 | 8.05 | 7.3 | 155 | 3.3 | 974 | -2.6 | 1470 | -2.0 |
| | 2.52 | 0.8 | 8.22 | 9.6 | 146 | -2.7 | 989 | -1.1 | 1480 | -1.3 |
| | 2.53 | 1.2 | 8.04 | 7.2 | 155 | 3.3 | 1030 | 3.0 | 1480 | -1.3 |
| | 2.49 | -0.4 | 8.18 | 9.1 | 147 | -2.0 | 976 | -2.4 | 1510 | 0.7 |
| | 2.48 | -0.8 | 7.81 | 4.1 | 142 | -5.3 | 1010 | 1.0 | 1530 | 2.0 |
| | 2.38 | -4.8 | 8.20 | 9.3 | 140 | -6.7 | 1040 | 4.0 | 1590 | 6.0 |
| Average | 2.46 | NA | 8.08 | NA | 148 | NA | 1000 | NA | 1510 | NA |
| intra-batch SD | 0.0752 | NA | 0.154 | NA | 6.35 | NA | 28.0 | NA | 45.2 | NA |
| intra-batch %CV | 3.1 | NA | 1.9 | NA | 4.3 | NA | 2.8 | NA | 3.0 | NA |
| intra-batch Accuracy deviation % | -1.6 | NA | 7.7 | NA | -1.3 | NA | 0.0 | NA | 0.7 | NA |
| 2# | 2.47 | -1.2 | 8.06 | 7.5 | 151 | 0.7 | 1050 | 5.0 | 1530 | 2.0 |
| | 2.46 | -1.6 | 7.44 | -0.8 | 153 | 2.0 | 977 | -2.3 | 1500 | 0.0 |
| | 2.41 | -3.6 | 7.82 | 4.3 | 149 | -0.7 | 1050 | 5.0 | 1490 | -0.7 |
| | 2.60 | 4.0 | 7.02 | -6.4 | 147 | -2.0 | 1080 | 8.0 | 1560 | 4.0 |
| | 2.52 | 0.8 | 7.71 | 2.8 | 153 | 2.0 | 969 | -3.1 | 1450 | -3.3 |
| | 2.46 | -1.6 | 7.44 | -0.8 | 151 | 0.7 | 987 | -1.3 | 1470 | -2.0 |
| Average | 2.49 | NA | 7.58 | NA | 151 | NA | 1020 | NA | 1500 | NA |
| intra-batch SD | 0.0656 | NA | 0.363 | NA | 2.34 | NA | 46.8 | NA | 40.0 | NA |
| intra-batch %CV | 2.6 | NA | 4.8 | NA | 1.5 | NA | 4.6 | NA | 2.7 | NA |
| intra-batch Accuracy deviation % | -0.4 | NA | 1.1 | NA | 0.7 | NA | 2.0 | NA | 0.0 | NA |
| 3# | 2.42 | -3.2 | 7.83 | 4.4 | 150 | 0.0 | 1020 | 2.0 | 1520 | 1.3 |
| | 2.47 | -1.2 | 7.53 | 0.4 | 150 | 0.0 | 1030 | 3.0 | 1530 | 2.0 |
| | 2.57 | 2.8 | 7.51 | 0.1 | 148 | -1.3 | 1010 | 1.0 | 1520 | 1.3 |
| | 2.72 | 8.8 | 7.75 | 3.3 | 151 | 0.7 | 1010 | 1.0 | 1510 | 0.7 |
| | 2.43 | -2.8 | 7.61 | 1.5 | 157 | 4.7 | 1050 | 5.0 | 1540 | 2.7 |
| | 2.51 | 0.4 | 7.98 | 6.4 | 155 | 3.3 | 1040 | 4.0 | 1550 | 3.3 |
| Average | 2.52 | NA | 7.70 | NA | 152 | NA | 1030 | NA | 1530 | NA |
| intra-batch SD | 0.112 | NA | 0.185 | NA | 3.43 | NA | 16.3 | NA | 14.7 | NA |
| intra-batch %CV | 4.4 | NA | 2.4 | NA | 2.3 | NA | 1.6 | NA | 1.0 | NA |
| intra-batch Accuracy deviation % | 0.8 | NA | 2.7 | NA | 1.3 | NA | 3.0 | NA | 2.0 | NA |
| inter-batch SD | 0.0856 | NA | 0.323 | NA | 4.52 | NA | 32.4 | NA | 35.8 | NA |
| inter-batch %CV | 3.4 | NA | 4.1 | NA | 3.0 | NA | 3.2 | NA | 2.4 | NA |
| inter-batch Accuracy deviation % | -0.4 | NA | 3.9 | NA | 0.0 | NA | 2.0 | NA | 0.7 | NA |

**Table 5**: Recovery rate of Analyte

| Experiment number | HQC peak | | | MQC peak | | | LQC peak | | |
|---|---|---|---|---|---|---|---|---|---|
| | area after extraction | Pre-extraction | Recovery% | area after extraction | Pre-extraction | Recovery% | area after extraction | Pre-extraction | Recovery% |
| 1 | 2753364 | 2682960 | 101.7 | 1848814 | 1802978 | 104.6 | 15048 | 13093 | 109.9 |
| 2 | 2786859 | 2718284 | 102.9 | 1842024 | 1753674 | 104.2 | 13949 | 13819 | 101.9 |
| 3 | 2746451 | 2770093 | 101.4 | 1828406 | 1740603 | 103.4 | 14032 | 13623 | 102.5 |
| 4 | 2747305 | 2678584 | 101.5 | 1806698 | 1755781 | 102.2 | 14408 | 13796 | 105.3 |
| 5 | 2788887 | 2665862 | 103.0 | 1835429 | 1787132 | 103.8 | 14104 | 13960 | 103.0 |
| 6 | 2747408 | 2729733 | 101.5 | 1810129 | 1768554 | 102.4 | 14739 | 13829 | 107.7 |
| Average | 2761712 | 2707586 | NA | 1828583 | 1768120 | NA | 14380 | 13687 | NA |
| SD | 20400 | 39200 | 0.743 | 17100 | 23200 | 0.967 | 438 | 310 | 3.20 |
| %CV | 0.7 | 1.4 | 0.7 | 0.9 | 1.3 | 0.9 | 3.0 | 2.3 | 3.0 |
| Overall recovery% | 103.5 | | | | | | | | |
| Overall %CV | 1.5 | | | | | | | | |

**Table 6**: IS recovery rate

| ID | Concentration: 500ng/mL | | |
|---|---|---|---|
| | IS peak area after extraction | Pre-extraction IS peak area | Recovery% |
| 1 | 607550 | 606875 | 101.5 |
| 2 | 584946 | 589267 | 97.7 |
| 3 | 589856 | 594926 | 98.6 |
| 4 | 587593 | 587572 | 98.2 |
| 5 | 584805 | 598347 | 97.7 |
| 6 | 583570 | 600905 | 97.5 |
| 7 | 585771 | 597153 | 97.9 |
| 8 | 577414 | 584058 | 96.5 |
| 9 | 586289 | 595067 | 98.0 |
| 10 | 577685 | 595076 | 96.5 |
| 11 | 567934 | 580048 | 94.9 |
| 12 | 565118 | 606557 | 94.4 |
| 13 | 586325 | 612872 | 98.0 |
| 14 | 592318 | 604028 | 99.0 |
| 15 | 586195 | 622117 | 98.0 |
| 16 | 589470 | 601558 | 98.5 |
| 17 | 585800 | 605416 | 97.9 |
| 18 | 574283 | 589811 | 96.0 |
| Average | 584051 | 598425 | NA |
| SD | 9450 | 10500 | 1.58 |
| %CV | 1.6 | 1.8 | 1.6 |
| Average Recovery % | 97.6% | | |

**Table 7**: Results of matrix effect

| Normal plasma matrix effect | | | | | |
|---|---|---|---|---|---|
| Concentration | Granisetron matrix effect | SD% | CV% of matrix effect | IS working solution concentration level | Precision of IS normalized matrix effect mean |
| LQC | 1.011 | 0.00942 | 0.9 | 2.1 | 0.8 |
| MQC | 1.002 | 0.00643 | 0.6 | | |
| HQC | 0.994 | 0.01340 | 1.3 | | |

| Hemolytic plasma matrix effect | | | Hyperlipidemic matrix effect | |
|---|---|---|---|---|
| Concentration | Accuracy deviation of concentration% | Precision deviation concentration% | Accuracy deviation concentration% | Precision deviation concentration% |
| LQC | 0.0 | 2.0 | -0.1 | 2.0 |
| HQC | 0.0 | 2.5 | 3.3 | 1.3 |

**Table 8**: Results of Multiple of dilution

| Experiment number | DQC detection concentration (ng/mL) (prepare a concentration of 400ng/mL) | Accuracy deviation |
|---|---|---|
| 1 | 4160 | 4.0 |
| 2 | 4140 | 3.5 |
| 3 | 4110 | 2.8 |
| 4 | 4080 | 2.0 |
| 5 | 4190 | 4.8 |
| 6 | 4160 | 4.0 |
| Average | 4140 | N/A |
| %CV | 1.0 | N/A |
| Accuracy deviation% | 3.5% | N/A |

**Table 9**: Pharmacokinetic parameters of Quetiapine Fumarate

| Parameters | Mean±SD | |
|---|---|---|
| Fasting | Postprandial | |
| Tmax (h) | 1.37±1.07 | 2.92±2.02 |
| Cmax (ng/mL) | 675.71±274.84 | 585.44±248.43 |
| $AUC_{0-t}$ (h*ng/mL) | 2278.23±922.42 | 2656.68±1007.46 |
| $AUC_{0-\infty}$ (h*ng/mL) | 2325.57±954.90 | 2716.57±1051.02 |
| $\%AUC_{\%Extrap}$ | 1.93±0.93 | 2.04±1.01 |
| $\lambda_z$ ($h^{-1}$) | 0.18±0.03 | 0.17±0.02 |
| $T_{1/2}$( h) | 4.06±0.70 | 4.16±0.48 |

## STATISTICAL ANALYSIS OF PHARMACOKINETIC PARAMETERS

The ethical approval process of this study followed "Drug Administration Law of the People's Republic of China," the Helsinki Declaration and relevant domestic laws and regulations. The software used for calculating pharmacokinetic parameters is WinNonlin 8.2. This study adopted a single-center approach with a randomized, open-label design. The experiment consists of two periods and two sequences, using a crossover method. The focus of the research was to evaluate the pharmacokinetic parameters of healthy adult volunteers using a validated method. A graphical representation was created to illustrate the correlation between the average plasma concentration of quetiapine fumarate and time. Subsequently, considering the precise timing of sample collection, non-compartmental models (Chaudhrya *et al*., 2021) were used to determine the pharmacokinetic parameters of the participants. The parameter $AUC_{0-\infty}$ denotes the definite integral of the concentration-time curve throughout the interval from 0 to ∞. The parameter $AUC_{0-t}$ denotes the definite integral of the concentration-time curve throughout the time interval from the initial time to the final time. The term "$C_{max}$" denotes the measured maximum concentration, whereas "$T_{max}$" refers to the time taken to achieve this maximum concentration. The term "$t_{1/2}$" denotes the duration necessary for the concentration of a drug to diminish by 50% during the elimination phase. The symbol λz denotes the apparent terminal elimination rate constant.

## RESULTS

***System suitability result***

It can be seen in table 1 that the RT time CV% of quetiapine was 0.3%, the RT time CV% of the IS was 0.3% and Ratio of peak area of CV% was 1.2%, all of the aforementioned entities adhere to the established criterion, which stipulates a maximum threshold of 15.0%. Furthermore, the validation results was satisfactory.

***Selectivity***

As shown in table 2 and table 3, the interference of blank plasma matrix on quetiapine ranged from 0.4% to 0.8%, with an average interference of 0.0% for the IS. The interference of the IS on the analyte ranges from 0.4% to 1.1%. The analyte exhibits an interference of 0.2% on the internal standard. The validation results were in accordance with the acceptance criteria.

***Results of standard curve and LLOQ***

The linear correlation between the peak area signal of the analyte and its corresponding concentration was seen in the standard calibration curve of Quetiapine Fumarate, as depicted in fig. 2. The lower limit of quantification (LLOQ) was determined to be 2.5ng/mL. In order to enhance the credibility of the calibration, we conducted six calibration curves, all of which exhibited R2 values surpassing 0.999. Additionally, the deviation of each concentration level from the standard concentration falls within ±15.0% of the theoretical value.

***Accuracy and precision of intra-batch, inter-batch***

Based on the data presented in table 4, it can be shown that the maximum precision within batches, excluding the LLOQ QC samples, was 4.8%. Additionally, the intra-batch accuracy deviation ranged from -1.3% to 7.7%. The LLOQ QC samples had a maximum intra-batch precision of 4.4%, whereas the intra-batch accuracy deviation ranged from -1.6% to 0.8%. The inter-batch precision, with the exception of the LLOQ QC samples, reached a maximum value of 4.1%. The inter-batch accuracy

deviation ranged from 0.0% to 3.9%. The LLOQ QC samples had a maximum inter-batch precision of 3.4%, with an inter-batch accuracy deviation of -0.4%. The aforementioned findings shown a high level of precision and accuracy in the methodology.

***Recovery rate of Analyte***

The results from tables 5 and 6 indicated that the extraction recovery rate of Quetiapine Fumarate was 103.5% and the extraction recovery rate of the internal standard was 97.6%. The maximum precision values were 3.0% and 1.6% respectively, the results indicate that the method has a good recovery rate.

***Matrix effect***

Table 7 showed that the normalized matrix effect of the IS in normal plasma at low concentration was 1.011, at medium concentration was 1.002 and at high concentration was 0.994. The matrix effect of the IS was 1.006, with a matrix effect CV% ranging from 0.6% to 1.3%, which met the acceptance criteria. The accuracy deviation of the LQC and HQC in hemolyzed plasma was 0.0% and the precision was 2.0% to 2.5%, which met the acceptance criteria. The accuracy deviation of the high and low concentration QC samples in hyperlipidemic plasma was -0.1% to 3.3% and the precision was 1.3% to 2.0%, which met the acceptance criteria.

***Multiple of dilution***

As shown in table 8, the average deviation between the detected concentration and the theoretical concentration in the diluted samples with a dilution factor of 10 was 3.5%, within ±15.0%. The precision was 1.0%, which was less than 15.0%, meeting the acceptance criteria.

***Resolution stability***

The short-term stability of the test substance stock solution in methanol at room temperature was 28 hours and it remained stable for 40 days under the conditions of 2-8ºC using a 50% methanol solution as the solvent for the working solution of the test substance. The stability of the whole blood matrix was 2 hours at room temperature, while the stability of the plasma matrix was 25 hours at room temperature, with long-term stability of 17 hours at -20ºC and 40 hours at -70ºC. The prepared samples remained stable after 4 freeze-thaw cycles and are stable for 185 hours under the condition of 5ºC in an autosampler. In this experiment, the test substance was used as an isotopic IS due to its similarity in properties with the test substance and it had been confirmed that the IS had no interference with the compound in each analysis batch. Therefore, no stability test for IS related solutions was conducted separately.

***Pharmacokinetic study***

Fig. 4 and table 9 presented the pharmacokinetic parameters of healthy subjects under fasting and postprandial administration. The elimination half-lives ($T_{1/2}$) for fasting and postprandial conditions were 4.06±0.70hours and 4.16±0.48hours, respectively. The $AUC_{0-t}$ were 2278.23±922.42ng·h/mL and 2656.68±1007.46ng·h/mL, while the $AUC_{0-\infty}$ were 2325.57±954.90ng·h/mL and 2716.57±1051.02ng·h/mL. The respective Tmax values were 1.37±1.07 hours and 2.92±2.02 hours. Moreover, the Cmax were 675.71±274.84ng/mL and 585.44±248.43ng/mL.

## DISCUSSION

This method demonstrated high selectivity, precision and accuracy, with coefficient of variation (CV%) below 15%. It showed good linearity within the range of 2.5-800ng/mL and matrix effect results indicated no interference from the matrix. We observed a multi-phasic decline in the drug concentration over time, suggesting the existence of multiple metabolic pathways. Quetiapine fumarate undergoes metabolism in the liver via cytochrome P450 enzyme system and is eliminated through the kidneys. However, further research is still needed for validation and deeper exploration. By fitting the concentration-time curve of the drug, we calculated the pharmacokinetic parameters of quetiapine fumarate. Consistent results were obtained for elimination half-life ($t_{1/2}$) under fasting and postprandial conditions, both being 4 hours.

## CONCLUSION

The UPLC-MS/MS method validated in this study can quickly and accurately determine the concentration of quetiapine fumarate in human plasma. In the future, this method will also be applied to the bioequivalence study of quetiapine fumarate.

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