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Original Article

A rapid LC-MS/MS method for simultaneous determination of quetiapine and duloxetine in rat plasma and its application to pharmacokinetic interaction study

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ABSTRACT

Combinations of new antidepressants like duloxetine and second-generation antipsychotics like quetiapine are used in clinical treatment of major depressive disorder, as well as in forensic toxicology scenarios. The drug–drug interaction (DDI) between quetiapine and duloxetine is worthy of attention to avoid unnecessary adverse effects. However, no pharmacokinetic DDI studies of quetiapine and duloxetine have been reported. In the present study, a rapid and sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed for simultaneous determination of quetiapine and duloxetine in rat plasma. A one-step protein precipitation with acetonitrile was applied for sample preparation. The analytes were eluted on an Eclipse XDB-C_{18} column using the mixture of acetonitrile and 2 mM ammonium formate containing 0.1% formic acid at a gradient elution within 6.0 min. Quantification was performed in multiple-reaction-monitoring mode with the ion transitions m/z 384.4 → 253.2 for quetiapine, m/z 298.1 → 154.1 for duloxetine and m/z 376.2 → 165.2 for IS (haloperidol), respectively. Good linearity was obtained in the range of 0.50–100 ng/mL for quetiapine ($r^2 = 0.9972$) and 1.00–200 ng/mL for duloxetine ($r^2 = 0.9982$) using 50 μL of rat plasma, respectively. The method was fully validated with accuracy, precision, matrix effects, recovery and stability. The validated data have met the acceptance criteria in FDA guideline. The method was applied to a pharmacokinetic interaction study and the results indicated that quetiapine had significant effect on the enhanced plasma exposure of duloxetine in rats under combination

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use. This study could be readily applied in therapeutic drug monitoring of major depressive disorder patients receiving such drug combinations.

1. Introduction

Major depressive disorder (MDD) is a severe mental disorder, and patients with MDD often show symptoms such as sadness, feelings of low self-worth, poor concentration, disturbed appetite and sleep, suicidal thoughts and even behaviors [1]. Various conventional antidepressant drugs are available for the treatment of MDD, but the monotherapy only work for 60–70% of these patients [2].

Combinations of new antidepressants and second-generation antipsychotics (SGAs) are frequently used in clinical treatment of MDD. Four SGAs namely brexiprazole, aripirazole, quetiapine and olanzapine, have been approved by the United States Food and Drug Administration (FDA) for the treatment of MDD [3]. Quetiapine is a multifunctional molecule acting as an antagonist against multiple types of receptors and has been demonstrated to be effective in clinical trials for generalized anxiety disorders, major depressive disorders [4,5]. Duloxetine is a novel inhibitor of the reuptake of serotonin and noradrenaline, and used for the treatment of MDD. There are several published reports about the combined use of quetiapine with duloxetine in clinical [6–9], which demonstrate to improve the efficiency and the side effects are mild. One case of severe urinary retention requiring urinary catheterization associated with treatment of depression with duloxetine and quetiapine had been reported [8]. Therefore, the drug–drug interaction (DDI) between quetiapine and duloxetine is worthy of attention to avoid unnecessary adverse effects without compromising the therapeutic benefits in combination therapy. However, to our knowledge, no pharmacokinetic DDI studies of quetiapine and duloxetine have been reported.

Several methods for analysis of quetiapine and its metabolites in biological sample have been reported, like GC-MS methods [10,11], HPLC-UV method [12], and LC-MS/MS methods [13–17]. However, GC-MS methods usually required derivatization before analysis; HPLC-UV method was simple in operation, but lacked good sensitivity. LC-MS methods provided high sensitivity and accuracy, but some of them used time-consuming sample preparation. A few methods have been reported for the determination of duloxetine, such as HPLC-UV method [18], LC-MS method [19] and LC-MS/MS method [20]. The published methods involved with a time-consuming and complicated SPE for sample pretreatment. All the above methods detected quetiapine and duloxetine separately. Only one published literature [21] reported an HPLC-UV method for determining the concentration of quetiapine and duloxetine in human plasma, but the method employed time-consuming liquid–liquid extraction and lacked sensitivity with LLOQ of quetiapine (25.0 μg/L) and duloxetine (10.0 μg/L). Thus, it is necessary to develop a more sensitive and efficient method to detect quetiapine and duloxetine simultaneously.

In this study, a rapid and sensitive LC-MS/MS method was developed and validated for simultaneous quantification of quetiapine and duloxetine in rat plasma. A one-step protein precipitation was adopted to prepare plasma samples. And the method was further applied to pharmacokinetic interaction study of quetiapine and duloxetine in rats.

2. Experimental

2.1. Chemicals and reagents

The reference standards of quetiapine fumarate (purity 98.0%) (Fig. 1) and haloperidol (purity 98.0%, IS) (Fig. 1) were obtained from National Institutes for Food and Drug Control (Beijing, China), and duloxetine hydrochloride (purity 98.0%) (Fig. 1) was purchased from Aladdin Corporation (Shanghai, China). Methanol and acetonitrile (HPLC grade) were supplied from Merck (Darmstadt, Germany). Formic acid and ammonium formate (HPLC grade) were purchased from CNW Technologies (Shanghai Anpu Co. Ltd., China). Purified water was obtained from an ELGA lab water purification system (Veolia Water Systems, UK).

2.2. Instrumentation and chromatographic conditions

A 1200 series HPLC instrument (Agilent Technologies, USA) equipped with CTC PAL autosampler (Agilent Technologies,

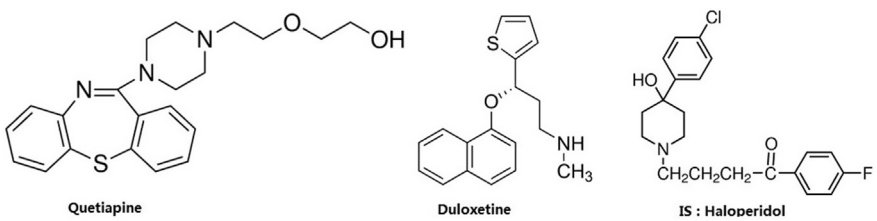

Fig. 1 – Chemical structures of quetiapine, duloxetine and haloperidol (IS).

USA) and API 4000 mass spectrometer (Applied Biosystems, USA) were operated for LC-MS/MS analysis.

Chromatographic separation was carried out on an Eclipse XDB-C_{18} (5 μm, 4.6 × 150 mm, Agilent Technologies, USA) and the column temperature was set at 40 °C. The mobile phase A was 2 mM ammonium formate and 0.1% formic acid in water, mobile phase B was 2 mM ammonium formate and 0.1% formic acid in acetonitrile (ACN). The mobile phase was pumped at a flow rate of 0.60 mL/min in a gradient method as follows (time/min, % mobile phase A): (0.00, 50), (4.00, 50), (4.01, 15), (5.00, 15), (5.01, 50), (6.00, 50), and 1 min 50% mobile phase A for equilibrium before gradient elution. The injection volume was 10 μL and the autosampler injection needle was washed with methanol after injection.

The triple quadrupole mass spectrometer was operated in positive ion ESI mode, and a multiple reaction monitoring (MRM) mode was applied for the analytes' quantification. The optimized parameters for the analytes and haloperidol (IS) were as follows. The ion transitions monitored were m/z 384.4 → 253.2 for quetiapine, m/z 298.1 → 154.1 for duloxetine and m/z 376.2 → 165.2 for IS. The declustering potential (DP) and collision energy (CE) were 100 V, 30 eV for quetiapine; 42 V, 9 eV for duloxetine; 90 V, 34 eV for IS. The entrance potential (EP) and collision cell exit potential (CXP) of the analytes were both 10 V. Other working parameters were summarized below: curtain gas (CUR) was 20 psi; ion spray voltage was 5500 V; temperature (TEM) was 550 °C; gas 1 was 50 psi, gas 2 was 65 psi; the dwell time was 150 ms. All data were acquired and analyzed by the Analyst 1.6.2 software (Agilent Technologies, USA).

2.3. Preparation of stock and working solutions

The primary stock solutions of quetiapine, duloxetine and IS were prepared in methanol at 1.00 mg/mL, respectively. Working solutions of the mixture of quetiapine and duloxetine were prepared by serial dilution of the stock solution with 50% methanol, with quetiapine ranged from 10.0 to 2000 ng/mL and duloxetine ranged from 20.0 to 4000 ng/mL. The working solution of IS (50.0 ng/mL) was obtained by diluting the IS stock solution with 50% methanol. All stock solutions and working solutions were stored at 4 °C and brought to room temperature before use.

2.4. Calibration standard and QC samples

Calibration standard samples and QC samples were prepared by spiking 20 μL aliquots of the appropriate working solution to 380 μL blank rat plasma. The final concentrations of calibration standard samples were 0.50, 1.00, 10.0, 20.0, 40.0, 80.0 and 100 ng/mL for quetiapine; 1.00, 2.00, 20.0, 40.0, 80.0, 160 and 200 ng/mL for duloxetine. The final three concentration levels of QC samples were 1.50, 30.0, 75.0 ng/mL for quetiapine and 3.00, 60.0, 150 ng/mL for duloxetine, respectively. All spiked samples were stored at −20 °C. Fresh calibration standard samples and QC samples were prepared each day for method validation.

2.5. Sample preparation

A one-step protein precipitation with acetonitrile was applied to prepare plasma samples. To an aliquot of 50 μL rat plasma sample, 20 μL of IS working solution and 430 μL acetonitrile were added into 1.5 mL centrifuge tube and vortexed. The mixture was then centrifuged at 11,000 rpm for 10 min. 250 μL of organic supernatant was transferred into a new centrifuge tube, and 250 μL purified water was dropped into the tube and vortexed. Finally, 100 μL of the mixed solution was transferred into vials and 10 μL was injected into the LC-MS/MS for analysis.

2.6. Method validation

The method was validated for selectivity, linearity, lower limits of quantification (LLOQ), precision, accuracy, extraction recovery, matrix effect and stability, according to the US FDA guidelines and the International Conference on Harmonization (ICH) [22,23].

2.6.1. Selectivity

To check the potential interference of endogenous substances for analytes and IS in rat plasma, the selectivity was investigated by analyzing six different sources of rat blank plasma samples (without analyte and IS) and compared with rat plasma samples spiked at the LLOQ and IS (n = 6).

2.6.2. Linearity and LLOQ

Linearity was assessed for quetiapine and duloxetine in the concentration range of 0.5–100 and 1–200 ng/mL at seven level concentration spiked plasma samples on three separate occasions. The calibration curve was constructed by plotting the peak area ratios (y) of the analytes to IS against the spiked concentrations of the analytes (x) with a $1/x^2$ weighted linear least squares regression.

LLOQ is defined as the lowest concentration of the calibration curve with the signal/noise ratio not less than 10. LLOQ was determined by the analysis of six replicates of LLOQ samples in three separate validation batches. The accuracy of each LLOQ samples should be within ±20% and the precision should not be greater than 20%.

2.6.3. Accuracy and precision

The intra-day of accuracy and precision were determined by analyzing six replicates of low, medium, high QC samples and LLOQ sample on one occasion. Whereas the inter-day of accuracy and precision were determined by analyzing the four level concentration samples on three consecutive separate occasions and with three separate calibration curves. The precision was expressed by relative standard deviation (RSD, %) and the accuracy by relative errors (RE, %), respectively.

2.6.4. Extraction recovery and matrix effect

The extraction recovery of quetiapine, duloxetine and IS were evaluated at low, medium and high QC concentration levels in six replicates by comparing the peak areas of the analytes and IS from regular extracted QC samples to the mean area of the analytes and IS from blank extracts spiked after extraction.

The matrix effect of quetiapine and duloxetine were tested in six different sources of rat plasma at three QC concentration levels (low, medium and high), respectively. And it was evaluated by comparing the peak areas of analytes and IS from blank extracts spiked after extraction to the mean area of

the analytes and IS from neat solutions at equivalent concentration.

2.6.5. Stability

The stability of the analytes were evaluated by analyzing low and high concentration QC samples in six replicates which were exposed to different storing and handling conditions. For short-term and long-term stability, QC samples were exposed at room temperature for 24 h and stored at −20 °C for 14 days, respectively. And freeze-thaw stability was assessed by analyzing samples through three freeze-thaw cycles, namely defrosted unassisted at room temperature and refrozen in a freezer at −20 °C for three times. These results were compared with the nominal values and were expressed in RSD (%) and RE (%).

2.7. Pharmacokinetic interaction study

Male Sprague–Dawley (SD) rats (weight 200–220 g) were provided by the Laboratory Animal Center of Second Military Medical University (Shanghai, China). Animals were bred in a breeding room for a week with the room temperature of 21–23 °C and humidity of 30–60%, then fasted overnight (12 h) before the experiment. Eighteen SD rats were randomly divided into three groups (6 rats per group) and received intragastric administration of a single dose of quetiapine (20 mg/kg), duloxetine (15 mg/kg) and the combination of quetiapine (20 mg/kg) and duloxetine (15 mg/kg), respectively. Blood samples were collected in heparinized tubes before dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24hr post-dosing. All blood samples were centrifuged immediately at 3500 rpm for 10 min and the collected plasma samples were stored at −20 °C until analysis. All procedures were in accordance with the National Institute of Health's guidelines regarding the principles of animal care (2004).

2.8. Statistical analysis

The main pharmacokinetic parameters of quetiapine and duloxetine were calculated using Phoenix WinNonlin 6.2.1 software. All values were reported as mean ± standard deviation. The PK parameters between the combination group and the single drug group were compared in paired *t*-test by Statistic Package for Social Science (SPSS) 20.0 software. A value of $P < 0.05$ was considered statistically significant and $P < 0.01$ was very significant.

3. Results and discussion

3.1. Method development

3.1.1. Sample preparation

A one-step protein precipitation was used to prepare samples for its rapidness and easy operation. Protein precipitation solvent methanol and acetonitrile were studied in the preliminary experiments. The analytes had better chromatographic behavior using acetonitrile instead of methanol, thus acetonitrile was chosen as the protein precipitant. And equivalent volume of purified water was added to the samples in order to make the ratio of aqueous phase to organic phase in the mixed samples consistent with initial mobile phase.

3.1.2. Optimization of mass spectrometry

In order to optimize ESI conditions for quetiapine, duloxetine and haloperidol (IS), quadrupole full scans were carried out both in positive and negative ion detection mode and found that good response was achieved in positive ionization mode. In the Q1 full scan mode, the protonated precursor $[M+H]^+$ of quetiapine, duloxetine and haloperidol were m/z 384.4, 298.1 and 376.2, respectively. Then in the MS_2 scan mode, the ion m/z 253.2, 154.1 and 165.2 were selected as product ions of quetiapine, duloxetine and haloperidol, respectively. Therefore, the ion transitions monitored for quantification were m/z 384.4 → 253.2 for quetiapine, m/z 298.1 → 154.1 for duloxetine and m/z 376.2 → 165.2 for IS. Other parameters like DP and CE were optimized and shown in Section 2.2. Fig. 2 presented product ion mass spectra of $[M+H]^+$ ions of quetiapine, duloxetine and IS in the MS_2 scan mode.

3.1.3. Optimization of liquid chromatography

A common and practical Agilent C18 (5 μm, 4.6 × 150 mm) column was used in our study, and a pre-column Eclipse XDB was applied for protecting the column from biological matrix. The analytes and IS were eluted in isocratic elution for a short time, as 2.63 min, 2.99 min and 3.19 min for quetiapine, duloxetine and haloperidol, respectively. So we cut down the time to 6 min, first few minutes adopted an isocratic elution, and then change the proportion of the mobile phase for washing and balancing the column.

3.2. Validation of the analytical method

3.2.1. Selectivity

Typical MRM chromatograms of a blank plasma sample, a plasma sample spiked with quetiapine and duloxetine at LLOQ and IS, and a plasma sample from SD rat 1 h after intragastric administration of quetiapine and duloxetine are shown in Fig. 3. Under the above LC-MS/MS conditions, the retention time of quetiapine, duloxetine and haloperidol was 2.63 min, 2.99 min and 3.19 min, respectively. The results illustrated that no significant interference from endogenous substances were observed at the retention times of the analytes and IS.

3.2.2. Linearity and LLOQs

The calibration curves were validated at seven levels over the concentration range of 0.50–100 ng/mL for quetiapine and 1.00–200 ng/mL for duloxetine. Typical equations of the calibration curves and r^2 value were as follows: $y = 0.0287x + 0.0018$, $r^2 = 0.9972$ (for quetiapine) and $y = 0.0112x + 0.0015$, $r^2 = 0.9982$ (for duloxetine), where y represents the ratio of peak area of analytes to that of IS, and x represents the plasma concentration of analytes in ng/mL. The RSD on the slope of the calibration curves was 4.1% for quetiapine and 3.7% for duloxetine. The results demonstrated good linearity of quetiapine and duloxetine in the range.

The LLOQs for quetiapine and duloxetine were 0.50 ng/mL and 1.00 ng/mL, respectively. The accuracy and precision of LLOQs are shown in Table 1 and within the acceptance limit.

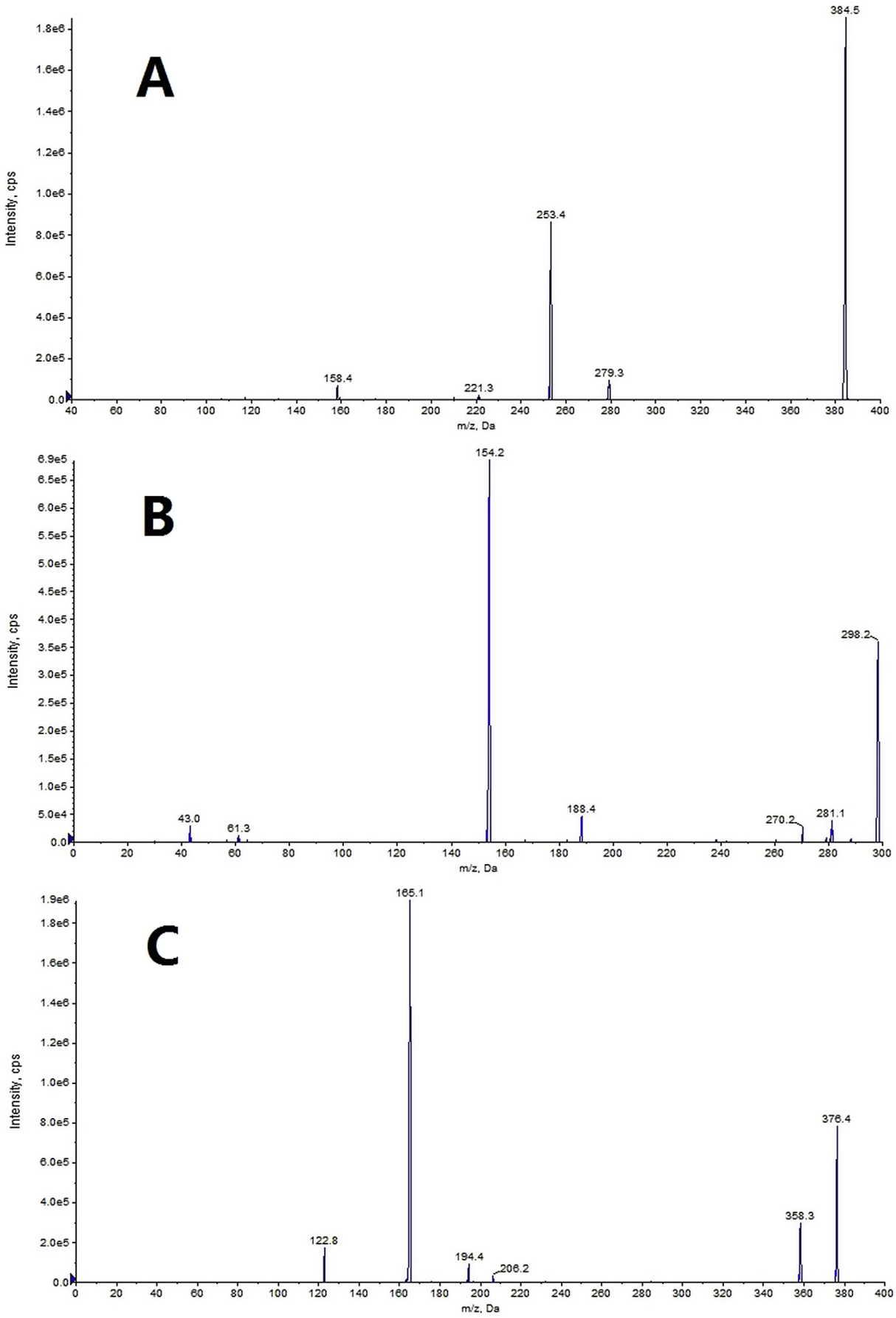

Fig. 2 – Product ion mass spectra of $[M+H]^+$ ions of quetiapine (A), duloxetine (B) and IS (C).

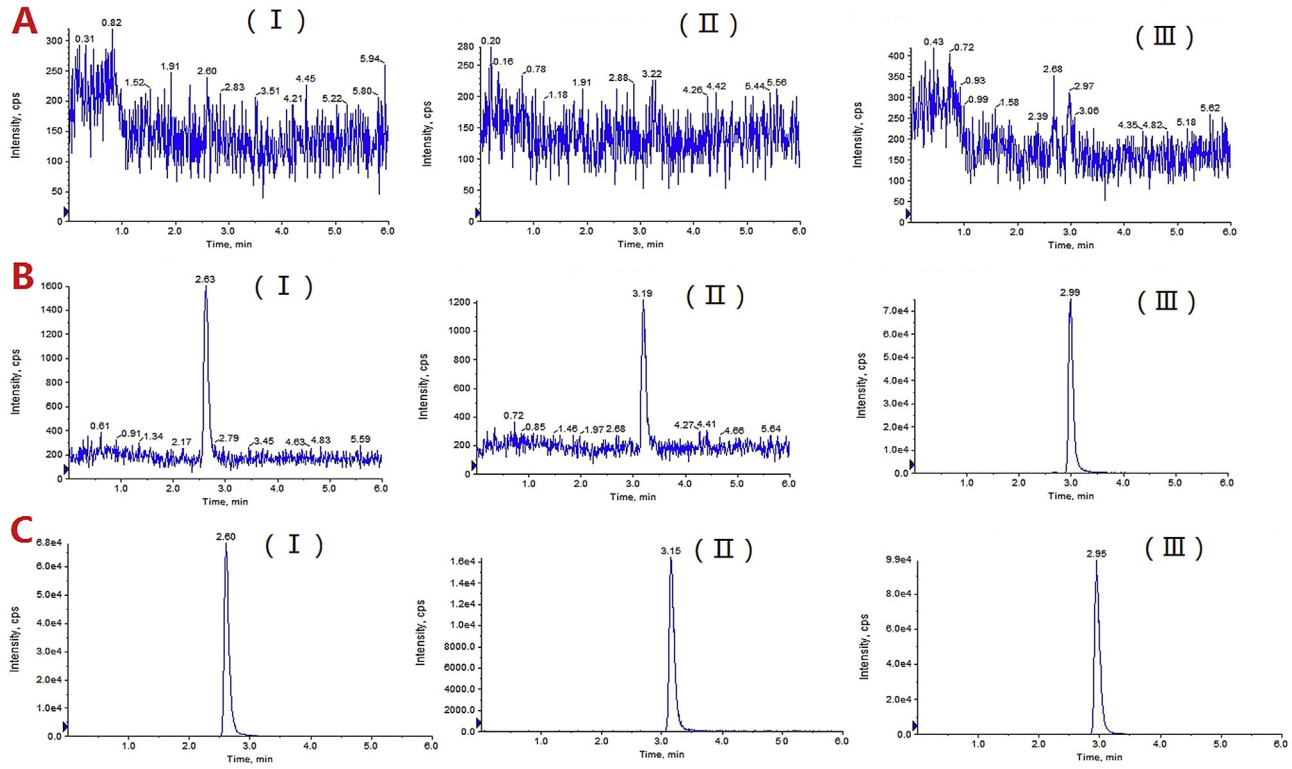

Fig. 3 – Typical MRM chromatograms of quetiapine (I), duloxetine (II) and IS (III) for (A) blank plasma sample, (B) extracted plasma sample at LLOQ and (C) extracted plasma sample at 1 h after combined administration of quetiapine and duloxetine.

3.2.3. *Accuracy and precision*

The results of accuracy and precision over LOQ, MOQ, HOQ and LLOQ samples are shown in Table 1. The accuracy (expressed by RE %) was in the range of −9.2–6.3% for quetiapine and duloxetine. The intra-day and inter-day precision (expressed by RSD %) were less than 6.9% for quetiapine and duloxetine. The results of accuracy, precision and dilution integrity met the acceptable criteria for bioanalytical purpose.

3.2.4. *Extraction recovery and matrix effect*

The extraction recoveries for quetiapine and duloxetine at LOQ, MOQ and HOQ levels are listed in Table 1. And the mean extraction recovery of IS was 99.3 ± 3.2% (n = 18). The results showed the developed method had high extraction efficiency and the recovery was not concentration dependent.

IS-normalized matrix effects of quetiapine were all 1.03 at LOQ, MOQ and HOQ levels, and the RSD were 4.9%, 2.9% and 1.9%, respectively. IS-normalized matrix effects of duloxetine were 0.84, 0.96 and 1.02 at LOQ, MOQ and HOQ levels, and the RSD were 6.0%, 4.2% and 2.9%, respectively. The above results are all within the acceptance limit and it illustrated that the rat plasma matrix had no interference for the analysis of quetiapine and duloxetine.

3.2.5. *Stability*

The results of stability are summarized in Table 2. The RSD % was in the range of 2.3–8.7%, and RE % was −8.0–8.7% for both quetiapine and duloxetine in the three conditions. The results demonstrated good stability of quetiapine and duloxetine throughout the experiment.

3.3. *Pharmacokinetic interaction of quetiapine with duloxetine*

The full validated method was successfully applied in drug–drug interaction study for the simultaneous

Table 1 – Accuracy, precision and extraction recovery for quetiapine and duloxetine in rat plasma by LC-MS/MS method.

Analytes	Conc. (ng/mL)	intra-day (n = 6)		inter-day (n = 18)		Extraction recovery (n = 6) (Mean ± SD,%)
		RSD (%)	RE (%)	RSD (%)	RE (%)	
Quetiapine	0.50	6.1	−9.2	6.9	−5.6	–
	1.50	3.5	−4.0	3.2	−4.0	96.2 ± 2.1%
	30.0	2.9	−2.0	3.3	2.7	97.8 ± 1.6%
	75.0	2.9	−0.3	4.8	3.2	96.0 ± 3.1%
Duloxetine	1.00	5.6	1.0	6.3	−1.6	–
	3.00	1.6	3.7	4.1	2.0	94.1 ± 5.4%
	60.0	2.1	6.3	3.6	4.8	95.9 ± 1.5%
	150	1.1	6.0	5.5	4.0	87.7 ± 4.9%

Table 2 – Stability of quetiapine and duloxetine under three storage conditions.

Analytes	Nominal conc. (ng/mL)	Sample conditions					
		Room temperature for 24 h (n = 6)		Three freeze-thaw cycles (n = 6)		−20 °C for 14 days (n = 6)	
		RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)
Quetiapine	1.5	−4.0	3.5	5.3	3.2	8.7	2.5
	75	3.2	2.3	7.5	2.6	5.3	2.4
Duloxetine	3.0	−4.7	8.7	−2.0	2.4	−0.3	2.3
	150	−4.7	2.7	−8.0	2.5	−2.7	3.4

quantification of quetiapine and duloxetine. The plasma concentration below the LLOQ was treated as zero. The mean plasma concentration–time curves of quetiapine after intragastric administration of quetiapine alone and co-administration of quetiapine and duloxetine are shown in Fig. 4A. And the mean plasma concentration–time profiles of duloxetine after intragastric administration of duloxetine alone and co-administration of quetiapine and duloxetine are presented in Fig. 4B. The pharmacokinetic parameters such as $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, T_{max}, $t_{1/2}$ and C_{max} of quetiapine and duloxetine are shown in Table 3, which was estimated by Phoenix WinNonlin 6.2.1 and SPSS 20.0 software. Single quetiapine group displayed a little lower concentration compared with that of the combinational group from the concentration–time curve (Fig. 4A). Their main pharmacokinetic parameters were confirmed to have no statistically significant change ($P > 0.05$). However, concomitant use of quetiapine resulted in substantial increases in plasma concentrations of duloxetine as shown in Fig. 4B. Main pharmacokinetic parameters of duloxetine like C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ had statistically significant increase ($P < 0.05$ or $P < 0.01$). Quetiapine increased the C_{max} of duloxetine by 1.625-fold, from 34.4 ± 5.6 to 55.9 ± 13.0 ng/mL, and the $AUC_{0\text{-}t}$ of duloxetine by 1.528-fold, from 245.8 ± 48.8 to 375.6 ± 44.2 ng·h/mL. Both quetiapine and

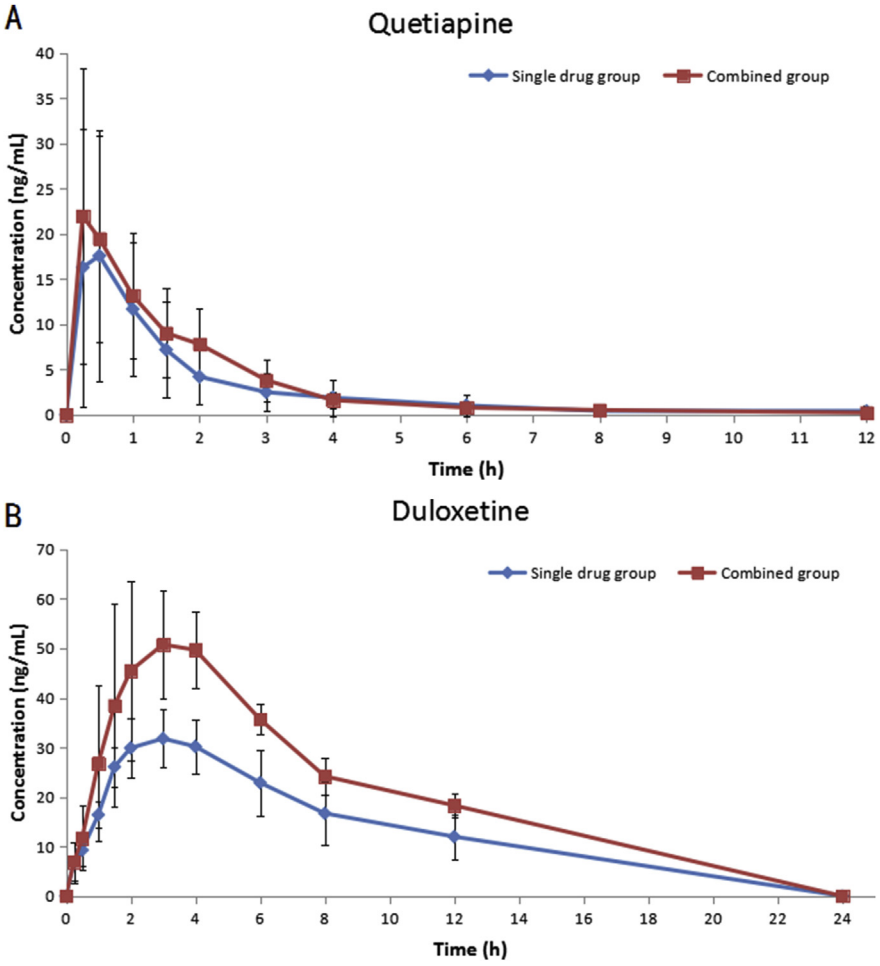

Fig. 4 – Mean plasma concentration–time profiles of (A) quetiapine after intragastric administration of quetiapine alone and co-administration of quetiapine and duloxetine; (B) duloxetine after intragastric administration of duloxetine alone and co-administration of quetiapine and duloxetine.

Table 3 – Main pharmacokinetic parameters of quetiapine and duloxetine.

Parameter	Quetiapine		Duloxetine	
	Single drug group	Combined group	Single drug group	Combined group
C_{max} (ng/mL)	18.1 ± 14.6	22.8 ± 15.5	34.4 ± 5.6	55.9 ± 13.0*
t_{max} (h)	0.4 ± 0.1	0.8 ± 0.8	3.2 ± 0.7	3.1 ± 1.0
$t_{1/2}$ (h)	2.1 ± 1.2	2.0 ± 0.9	6.5 ± 2.8	6.2 ± 2.1
AUC_{0-t} (ng h/mL)	32.2 ± 28.1	38.9 ± 19.8	245.8 ± 48.8	375.6 ± 44.2**
$AUC_{0-\infty}$ (ng h/mL)	35.2 ± 31.0	41.2 ± 21.1	372.7 ± 105.9	544.0 ± 56.0*

$P < 0.05$ was statistically significant, indicated by *; $P < 0.01$ was significantly difference, indicated by **.

duloxetine are metabolized by cytochrome P450 (CYP) enzymes. CYP3A4, CYP3A5, CYP2D6, and CYP2C19 are vital members of the CYP superfamily and they metabolize various drugs [24]. Duloxetine and its major metabolite, 4-hydroxy duloxetine, are metabolized primarily by CYP1A2 and partially by CYP2D6 [25]. At the same time, duloxetine is a moderate inhibitor of CYP2D6, while has minimal or no effect on the activity of CYP1A2 and CYP3A4 [26]. Quetiapine and its main active metabolite, N-desalkylquetiapine, are extensively metabolized in the liver predominantly by CYP3A4 and 7-hydroxy-N-desalkylquetiapine (secondary metabolite of N-desalkylquetiapine) is exclusively formed by CYP2D6 [27–29]. Therefore, duloxetine would not affect the pharmacokinetics of quetiapine in the combinational group. And it might be speculated that the metabolism of quetiapine's secondary metabolite inhibits the metabolism of duloxetine, leading to a significant increase in the plasma concentration of duloxetine. Further studies are necessary to elucidate the mechanism of pharmacokinetic interaction of quetiapine and duloxetine with combination dosage.

4. Conclusions

A rapid and sensitive LC-MS/MS method was developed and validated for simultaneous quantitation of quetiapine and duloxetine in a small volume of 50 μL rat plasma. A simple one-step protein precipitation method was used for sample pretreatment and the analysis process was within 10 min. The method was successfully applied in the pharmacokinetic interaction study of quetiapine and duloxetine for the first time. The results revealed that quetiapine would significantly increase the plasma concentration of duloxetine in rats when combination used. The pharmacokinetic information about these two drugs might be valuable to the combination therapy and thus TDM of these drugs under combination use is very important for the sake of public health.

Conflicts of interest

The authors have declared no conflicts of interest.

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