

Development and Validation of a New HPLC Method for the Determination of Quetiapine and Its Metabolites 7-Hydroxy Quetiapine and Quetiapine Sulfoxide in Rat Plasma

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A new high performance liquid chromatography method was developed and validated for the determination of quetiapine and its metabolites 7-hydroxy quetiapine and quetiapine sulfoxide in rat plasma. Separation was performed on a C18 column (Zorbax Eclipse Plus 4.6 mm x100 mm, 3.5 µm particles) using a gradient elution at a flow rate of 1 mL/min. Mobile phase consisted of acetate buffer (10 mM, pH5) and acetonitrile. Analytes were detected with a DAD detector at 225 nm. Carbamazepine was used as internal standard in all analyses. Plasma samples were analyzed after a simple, one-step protein precipitation with acetonitrile. Separation time was 15 min including clean-up step. The method was validated in terms of precision, accuracy, recoveries, matrix effect and stability. It was found to be linear in the range of 0.065-130 µg/mL for quetiapine, 0.086-171 µg/mL for 7-hydroxy quetiapine and 0.042-83.35 µg/mL for quetiapine sulfoxide. All validation parameters were acceptable. This method was successfully applied to quantify the concentrations of the analytes in rat plasma.

Key words: Quetiapine, 7-Hydroxy Quetiapine, Quetiapine Sulfoxide, HPLC, Rat plasma

Sıçan Plazmasında Ketiapin ve Metabolitleri 7-Hidroksi Ketiapin ve Ketiapin Sülfoksit'in Tayini İçin Yeni Bir Yüksek Performanslı Sıvı Kromatografisi Yöntemi Geliştirilmesi ve Yöntemin Validasyonu

Sıçan plazmasında ketiapin ve metabolitleri 7-hidroksi ketiapin ve ketiapin sülfoksit'in tayini için yeni bir yüksek performanslı sıvı kromatografisi yöntemi geliştirilmiş ve valide edilmiştir. Ayrım, C18 kolon (Zorbax Eclipse Plus 4.6 mm x100 mm, 3.5 µm partiküller) üzerinde ve 1 mL/dak akış hızında gradient elüsyon kullanılarak yapılmıştır. Hareketli faz, asetat tamponu (10 mM, pH 5) ve asetonitrilden oluşmaktadır. Analitler DAD dedektör kullanılarak, 225 nm'de saptanmıştır. Tüm analizlerde karbamazepin iç standart olarak kullanılmıştır. Plazma numuneleri, asetonitrille gerçekleştirilen tek basamaklı basit bir protein çöktürme yöntemi sonrasında analiz edilmiştir. Analiz süresi, kolon temizleme basamağını da içerecek şekilde, 15 dakikadır. Yöntem kesinlik, doğruluk, geri kazanım, matris etkisi ve stabilite parametreleri incelenerek valide edilmiştir. Yöntem ketiapin için 0.065-130 µg/mL aralığında, 7-hidroksi ketiapin için 0.086-171 µg/mL aralığında ve ketiapin sülfoksit için 0.042-83.35 µg/mL aralığında doğrusal bulunmuştur. Tüm validasyon parametreleri kabul edilebilir sınırlar dahilindedir. Bu yöntem, sıçan plazmasındaki analit derişimlerini belirlemek için başarıyla uygulanmıştır.

Anahtar kelimeler: Ketiapin, 7-Hidroksi Ketiapin, Ketiapin sülfoksit, YPSK, Sıçan plazması

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INTRODUCTION

Schizophrenia, severely debilitating psychiatric disorder affecting approximately 1.5% of the world's population (1), is characterized by positive symptoms (e.g. hallucinations and delusions), disorganization, negative symptoms (e.g. poverty of speech, flat affectivity and apathy), cognitive deficits, mood and motor symptoms (2, 3).

Since first generation antipsychotics were developed in 1950s, drug treatment has become widespread in treatment of schizophrenia (1). These drugs were effective in treating positive symptoms of schizophrenia, however they have several side effects, like extrapyramidal side effects (EPS) and tardive dyskinesia (1). At 1990s, new drugs which are more effective and reliable were developed and were approved by FDA- risperidone, olanzapine, quetiapine, ziprasidone, aripiprazole, iloperidone, asenapine and paliperidone palmitate (4). They referred to as "atypical antipsychotics" (4).

Quetiapine (QTP, Figure 1) is a relatively new atypical antipsychotic used in the treatment of schizophrenia, acute mania and depression associated with bipolar disorder (5), affecting both positive and negative symptoms of schizophrenia with low potential for EPS (3, 6). It has affinity for serotonin 5HT_{2A} receptors, adrenergic (α_1), muscarinic, histaminergic receptors and minor affinity for dopamine D₂ and 5HT_{1A} receptors and very low affinity for 5HT_{2C}, α_2 and D₁ receptors (6, 7).

QTP is metabolized in liver, primarily by CYP3A. CYP2D6 is also responsible for hydroxylation of QTP (7, 8). Major metabolites are through sulfoxidation, N- and O- dealkylation and 7-hydroxylation (8). Quetiapine sulfoxide (QTP-SF) is a major metabolite of QTP, accounting for 15.1 % of total plasma radioactivity, and 7-hydroxy quetiapine (7-OH QTP) is one of two active metabolites of QTP accounting for 5 % of total plasma radioactivity. QTP is rapidly

absorbed after oral administration with mean time to reach maximum plasma concentration ranging from 1 to 2 hours following both single- and multiple dose administration (7).

Several studies for analysis of QTP in different biological fluids (rat plasma, human plasma and serum, microsomal matrix) were reported by using liquid chromatography (3, 9-14), liquid chromatography-mass spectrometry (15-25) and gas chromatography (26, 27).

Davis et al. developed a HPLC method for the determination of QTP and its 7-hydroxylated and 7-hydroxylated, N-dealkylated metabolites in human plasma (9). The same analytes were determined in rat plasma and rat cerebrospinal fluid by Tu et al. using UPLC-MS-MS method (19). Ansermot et al. and Peddio et al. determined QTP and norquetiapine in human plasma and microsomal matrix, respectively, using LC-MS methods (24, 25). Pullen et al. developed both HPLC and GC-MS methods for the analysis of QTP and 7-OH QTP in human plasma (26), whereas Li et al. developed UPLC-MS method for the analysis of QTP and QTP-SF in *in vitro* samples (17). Only two methods were reported for the simultaneous analysis of QTP, 7-OH QTP, and QTP-SF in biological fluids by LC-MS/MS (28, 29). Most of these methods involve liquid-liquid (9, 17, 19, 28, 29) and solid phase (26) extraction of the analytes from the plasma samples prior to analysis. Time-consuming and expensive extraction procedures increase the overall analysis time and the cost of the analysis. Although two methods including protein precipitation were developed, the use of mass spectrometry detection in these methods limits their simple and inexpensive characteristics (24, 25). In this study, one of the goals was to develop a simple and one step protein precipitation procedure to extract the analytes from rat plasma. Higher recovery values for three analytes, which are more than 87%, compared to other HPLC/MS/MS and HPLC-DAD assays (9, 17, 26, 28, 29) were obtained.

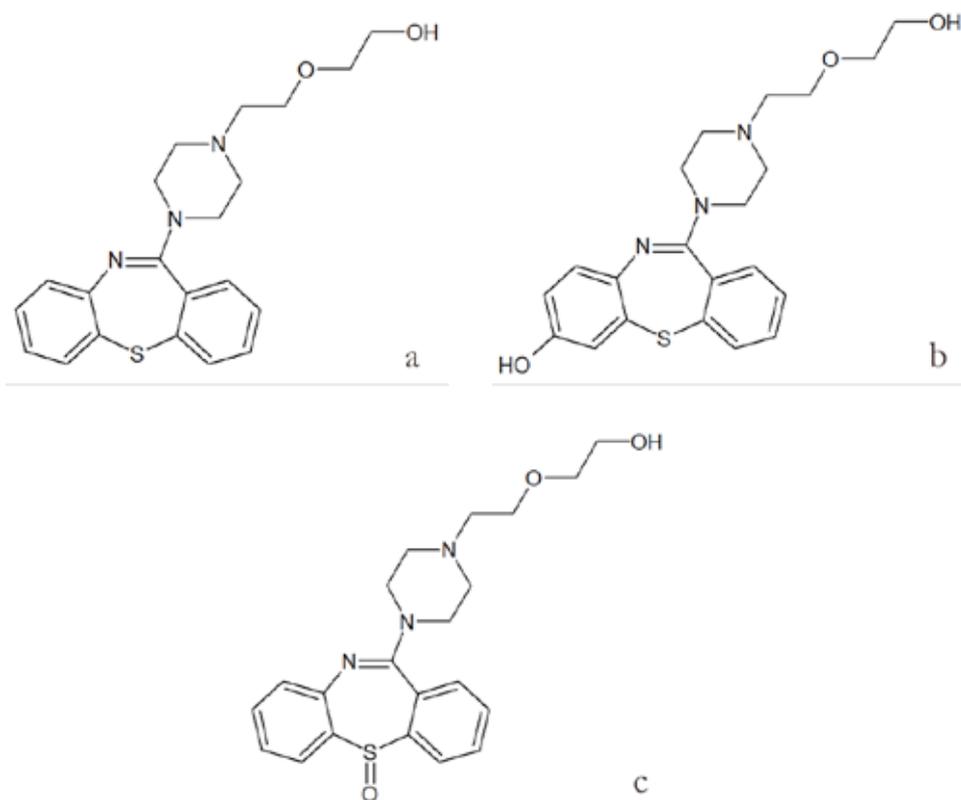


Figure 1. Chemical structures of QTP (a), 7-OH QTP (b), QTP-SF (c)

A new, simple and specific method for simultaneous determination QTP and its two metabolites, 7-OH QTP and QTP-SF, in rat plasma was developed using one step protein precipitation procedure.

EXPERIMENTAL

Materials

QTP and carbamazepine reference standards (>98.0% purity) were obtained from Sigma (St Louis, MO, USA). 7-OH QTP and QTP SF were purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). Acetonitrile (ACN), methanol (MeOH) and acetic acid were the products of Sigma (St Louis, MO, USA). ACN, MeOH and water were of high purity HPLC grade.

Instrumentation and chromatographic conditions

An Agilent 1290 Infinity Binary LC system (equipped with a binary pump, a degasser, an autosampler and a thermostatted column compartment) was employed (Agilent

Technologies, Waldbronn, Germany). Separation was obtained on a Zorbax Eclipse Plus C18 column (4.6 mm x100 mm, 3.5 μ m particles) using a gradient program. Mobile phase consists of acetate buffer (10 mM, pH5) and ACN. Optimum peaks were obtained at 35°C column temperature at a flow rate of 1 mL/min with an injection volume of 10 μ L. UV detection was monitored at 225 nm.

Preparation of stock solutions, calibration standards and quality control samples

1.30 mg/mL QTP, 1.71 mg/mL 7-OH QTP and 1.67 mg/mL QTP-SF stock solutions in MeOH were prepared individually. Aliquots of these stock solutions were mixed and diluted with water and a stock solution of 325 μ g/mL QTP, 427.5 μ g/mL 7-OH QTP and 436.75 μ g/mL QTP-SF was obtained. Working standard solutions were prepared by serial dilution of this stock solution with MeOH:water (1:1) and spiking them into drug-free rat plasma to obtain analyte concentrations in the range of 0.065-130 μ g/mL for QTP, 0.086-171 μ g/mL for 7-OH

QTP and 0.042-83.35 µg/mL for QTP-SF. Plasma proteins were precipitated by adding two fold ACN. After vortexing for 30 seconds, solutions centrifuged at 1699 x g for 10 minutes and clear supernatants were separated. All solutions were kept at -20°C until analysis and all solutions were filtered with 0.20 µm pore sized syringe filters prior to analysis.

Sample collection

Adult male Sprague–Dawley rats weighing about 250–350 g were obtained from the Laboratory Animal Center at Anadolu University. Rats were housed in a temperature-controlled laboratory and maintained under a 12 h light–dark cycle with free access to food and water at the beginning of the experiments. All animal experiments were performed in accordance with the principles of animal use and care approved by the ethical committee of Anadolu University (Approval File No. 22/2012). The rats were anesthetized with urethane (1.5 g/kg) by intraperitoneal (i.p.) injection and remained anesthetized throughout the experimental period (30). Tail vein was used for blood collection. 5 rats were used in the experiment and 50 mg/kg QTP in DMSO was given by i.p. injection. Following the dose, 0.2 mL blood was collected at 1.5 h following the administration of QTP. After the sampling, 0.2 mL SP was administered to sustain isotonic fluid balance.

Sample preparation

Collected rat blood was centrifuged at 1699 x g for 10 minutes at 25°C. 600 µL ACN was added to 300 µL clear supernatant to precipitate plasma proteins and vortexed for 30 seconds. Mixture was centrifuged at 1699 x g for 10 minutes. Clear supernatant was separated and all samples were kept at -20°C until the analysis. Prior to analysis the samples were kept at room temperature and filtered with 0.20 µm pore sized syringe filters.

RESULTS AND DISCUSSION

Method optimization

A Zorbax Eclipse Plus C18 column (4.6 mm x100 mm, 3.5 µm particles) was used for

all experiments. Different mobile phases including water, ACN and MeOH were evaluated and the best retention time and peak shape results were obtained with water:ACN mixture. A buffer was needed to improve peak morphology. Phosphate buffer was tested at pH3, pH5, pH6, pH7, pH8 and acetate buffer at pH4 and pH5. Acetate buffer at pH5 was selected because of optimum peak shapes and resolution. Then, it was tested at the concentrations of 10 mM and 20 mM. Finally the mixture of acetate buffer (10 mM, pH5) and CAN was decided as mobile phase. A gradient program was used to protect column from plasma impurities and to decrease the analysis time (Table 1). In order to improve resolution, different column temperatures between 25°C-45°C and flow rates between 0.45-1mL/min were tested. The best peak shapes with short analysis time were obtained when the column temperature was set at 35°C and flow rate as 1 mL/min.

Maximum UV absorbances for the analytes were obtained at 225 nm. Therefore analyses were performed at this wavelength.

Aripiprazole, bupropion HCl, carbamazepine and atomoxetine were tested as internal standard. Carbamazepine was chosen because of its proper retention time with no peak overlap.

Method was validated in terms of precision, accuracy, specificity, recoveries, matrix effect and stability in accordance with bioanalytical validation guidelines (31, 32). Peak area ratios of the analytes to that of the IS were used while evaluating data.

Linearity

Calibration solutions were prepared by diluting a solution containing 325 µg/mL QTP, 427.5 µg/mL 7-OH QTP and 436.75 µg/mL QTP-SF. Rat plasma samples were spiked with these solutions and analyzed as described above. GraphPad Prism v6.0 program was used to generate linear regression equations for the calibration curves. The method was found to be linear in the concentration range of 0.065-130 µg/mL for QTP, 0.086-171 µg/mL for 7-OH QTP and 0.042-83.35 µg/mL for QTP-SF. Statistical data are shown in Table 2. LLOQ, defined as the lowest concentration of the calibration graph with acceptable accuracy (RSD and bias

% less than 20 %) (31), was found to be 0.065, 0.086 and 0.042 µg/mL for QTP, 7-OH QTP and QTP-SF, respectively, in rat plasma.

described above to obtain the concentrations of 1.625 µg/mL QTP, 2.138 µg/mL 7-OH QTP, 2.084 µg/mL QTP-SF; 8.125 µg/mL QTP, 10.69 µg/mL 7-OH QTP, 10.42 µg/mL

Table 1. The gradient elution program of the method

Minute	%B
0	35
2	35
3.5	55
5.5	55
7	70
8	70
10	35
15	35

Accuracy and precision

The intra- and inter-day assay precision and accuracy results were evaluated using three different concentrations of the analytes. Three quality control (QC) samples were prepared as

QTP-SF and 65 µg/mL QTP, 85.50 µg/mL 7-OH QTP, 83.35 µg/mL QTP-SF. % Relative standard deviation and % recovery values were calculated and shown in Table 3. The intra- and inter-day assay

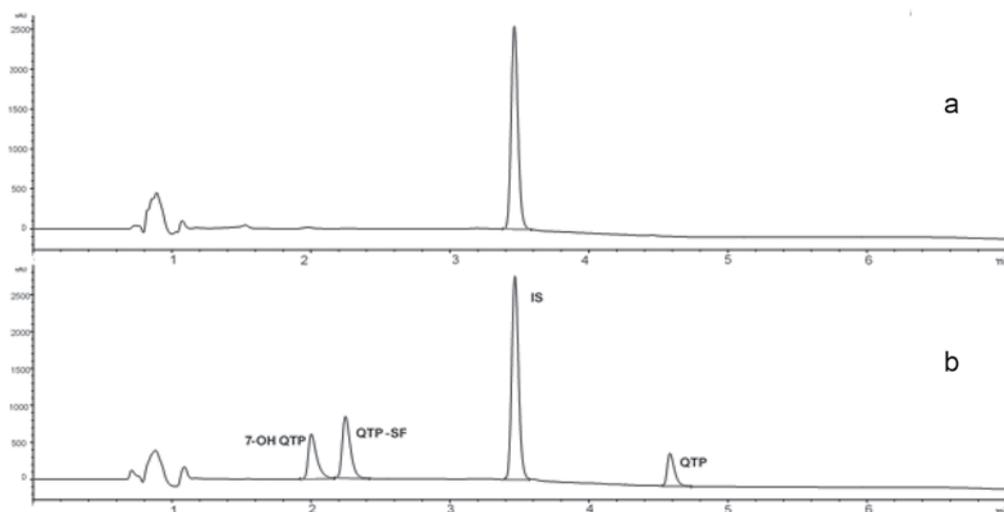


Figure 2. Chromatograms of blank plasma (a), 1.625 µg/mL QTP, 2.138 µg/mL 7-OH QTP and 2.084 µg/mL QTP-SF spiked plasma (b) (The concentration of IS is 360 µg/mL)

Table 2. Statistical data for the linearity of QTP, 7-OH QTP and QTP-SF in rat plasma

Parameters (Units)n=8	QTP	7-OH QTP	QTP-SF
Linear Range ($\mu\text{g/mL}$)	0.065-130	0.086-171	0.042-83.35
Slope \pm SD	0.0154 \pm 0.0002	0.0156 \pm 0.0002	0.0215 \pm 0.0002
Intercept \pm SD	-0.0223 \pm 0.0116	-0.0006 \pm 0.0110	-0.0058 \pm 0.0053
Determination Coefficient (r^2)	0.9988	0.9994	0.9997
95% Confidence Limits (CL)	0.0149-0.0159	0.0152-0.0160	0.0212-0.0219

Specificity

Specificity was indicated by comparing the chromatograms of blank rat plasma and rat plasma spiked with the analytes. The peaks of analytes were separated well and no interference was observed at retention times of the analytes. Chromatograms of blank plasma and rat plasma spiked with 1.625 $\mu\text{g/mL}$ QTP, 2.138 $\mu\text{g/mL}$ 7-OH QTP and 2.084 $\mu\text{g/mL}$ QTP-SF under described conditions are shown in Figure 2.

Stability

Stability of the analytes were indicated with two different concentrations, 1.625 $\mu\text{g/mL}$ QTP, 2.138 $\mu\text{g/mL}$ 7-OH QTP, 2.084 $\mu\text{g/mL}$ QTP-SF and 8.125 $\mu\text{g/mL}$ QTP, 10.688 $\mu\text{g/mL}$ 7-OH QTP, 10.419 $\mu\text{g/mL}$ QTP-SF. Solutions were prepared by spiking rat plasma with the analytes as described before. Analyses were performed after storing solutions under three different conditions: at room temperature for 24 hours, at -20°C for 2 weeks and after 3 cycles of freeze- thaw. Results are shown in Table 4. All results for the stability of the analytes were found acceptable.

Matrix effect

The matrix effect was evaluated by comparing the peak responses of analytes dissolved in blank sample extracts (the final solution obtained from blank rat plasma after protein precipitation) with those of standard analyte solutions as references (33). For standard solutions, 150 μL water and 300 μL ACN were added to 300 μL stock solution, the solution was vortexed for 30 seconds and

filtered with 0.20 μm pore sized syringe filters prior to analysis. For plasma solutions, drug-free plasma sample was precipitated with two fold ACN, vortexed for 30 seconds, centrifuged at 1699 x g for 10 minutes and supernatant was separated. 150 μL clear supernatant and 300 μL ACN were added to 300 μL stock solution, the solution was vortexed for 30 seconds and filtered with 0.20 μm pore sized syringe filters prior to analysis. Both standard and plasma samples were prepared at two concentrations; 1.625 $\mu\text{g/mL}$ QTP, 2.138 $\mu\text{g/mL}$ 7-OH QTP, 2.084 $\mu\text{g/mL}$ QTP-SF and 8.125 $\mu\text{g/mL}$ QTP, 10.688 $\mu\text{g/mL}$ 7-OH QTP, 10.419 $\mu\text{g/mL}$ QTP-SF.

As seen in Table 5, the peak area ratios for the post-spiked standards versus references at the QC concentrations were within the acceptable limits (85–115%) and no matrix effect was observed.

Method application

The concentrations of unbound analytes in rat plasma were determined by administrating 50 mg/kg QTP to five individual rats and collecting rat plasma samples after 1.5 hours. Data for the concentrations of QTP and its two metabolites in rat plasma are presented in Table 6. Plasma levels were in the range of 140.96-587.13 ng/mL for QTP, 373.32-909.87 ng/mL for 7-OH QTP and 91.62-179.82 ng/mL for QTP-SF. Plasma levels of 7-OH QTP and QTP-SF for rat 3 and plasma level of QTP-SF for rat 2 were lower than LLOQ values.

CONCLUSION

LC is a common and powerful analytical technique for the determination of drugs in biological fluids. This HPLC method is rapid, reproducible and accurate to quantify QTP and its two metabolites, 7-OH QTP and QTP-SF. It involves a simple plasma deproteination technique using one step protein precipitation

with ACN. The procedure has minimal sample transfer steps and it does not harm the analytes. Thus, it represents an alternative procedure for routine therapeutic drug monitoring of patients treated with QTP. This assay was successfully applied for the analysis of QTP, 7-OH QTP and QTP-SF in rat plasma samples.

Table 3. Precision and accuracy of QTP, 7-OH QTP and QTP-SF in rat plasma

Added ($\mu\text{g/mL}$)	Conc.	Intra day (n=7)			Inter day(n=21)		
		Measured Conc. (mean \pm SD)	Accuracy (%)	RSD (%)	Measured Conc. (mean \pm SD)	Accuracy (%)	RSD (%)
QTP	1.625	1.70 \pm 0.06	104.54 \pm 3.53	3.38	1.60 \pm 0.05	98.39 \pm 3.22	3.28
	8.125	9.01 \pm 0.15	110.83 \pm 1.86	1.68	8.68 \pm 0.10	106.78 \pm 1.19	1.11
	65.00	66.21 \pm 1.15	101.86 \pm 1.77	1.74	64.51 \pm 0.83	99.242 \pm 1.28	1.29
7-OH QTP	2.138	1.91 \pm 0.04	89.26 \pm 1.81	2.03	1.87 \pm 0.04	87.66 \pm 1.68	1.91
	10.69	10.79 \pm 0.06	100.98 \pm 0.60	0.60	10.66 \pm 0.07	99.731 \pm 0.70	0.70
	85.50	91.52 \pm 0.92	107.04 \pm 1.07	1.00	90.52 \pm 0.51	105.88 \pm 0.60	0.56
QTP-SF	2.084	1.96 \pm 0.04	93.85 \pm 1.72	1.83	1.90 \pm 0.03	91.13 \pm 1.46	1.60
	10.42	11.27 \pm 0.11	108.12 \pm 1.07	0.99	11.36 \pm 0.04	106.232 \pm 0.39	0.36
	83.35	83.51 \pm 0.68	100.19 \pm 0.81	0.81	81.408 \pm 0.33	97.671 \pm 0.39	0.40

Table 4. The stability of the analytes under different storage conditions

Theoretical Concentration ($\mu\text{g/mL}$)	Short-term stability (24 h, room temperature)		Long-term stability (2 weeks, -20°C)		Freeze-thaw stability (3 cycles)		
	Recovery (%) (mean \pm SD)	RSD (%)	Recovery (%) (mean \pm SD)	RSD (%)	Recovery (%) (mean \pm SD)	RSD (%)	
QTP	1.625	100.71 \pm 5.58	5.54	85.96 \pm 3.04	3.53	87.28 \pm 0.76	0.87
	8.125	90.85 \pm 1.13	1.25	90.15 \pm 1.31	1.46	88.64 \pm 2.27	2.56
7-OH QTP	2.138	99.71 \pm 5.24	5.26	94.28 \pm 1.54	1.64	103.37 \pm 2.92	2.82
	10.688	93.52 \pm 1.60	1.71	104.60 \pm 0.99	0.95	102.30 \pm 2.63	2.57
QTP-SF	2.084	106.67 \pm 2.85	2.67	92.37 \pm 1.39	1.51	85.61 \pm 1.85	2.16
	10.419	112.28 \pm 2.32	2.07	92.83 \pm 1.24	1.34	89.25 \pm 1.08	1.20

Table 5. The matrix effect of the analytes

	Theoretical Concentration (µg/mL)	The matrix effect (%) (mean±SD)	RSD (%)
QTP	1.625	96.52±2.05	2.12
	8.125	101.02±0.21	0.21
7-OH QTP	2.138	91.74±2.32	2.53
	10.688	96.74±2.07	2.14
QTP SF	2.084	106.69±2.55	2.39
	10.419	95.42±1.83	1.92

Table 6. The concentration data for QTP, 7-OH QTP and QTP-SF in rat plasma

	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
QTP (ng/mL)	274.01	140.96	346.57	587.13	488.69
7-OH QTP (ng/mL)	627.05	373.32	ND	770.83	909.87
QTP SF (ng/mL)	105.06	ND	ND	91.62	179.82

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REFERENCES

- Bishara D, Taylor D, Upcoming Agents for the Treatment of Schizophrenia Mechanism of Action, Efficacy and Tolerability, *Drugs* 68(16), 2269-2292, 2008.
- Bruijnzeel D, Suryadevara U, Tandon R, Antipsychotic treatment of schizophrenia: an update, *Asian J Psychiatr* 11, 3-7, 2014.
- Mandrioli R, Fanali S, Ferranti A, Raggi MA, HPLC analysis of the novel antipsychotic drug quetiapine in human plasma, *J Pharm Biomed Anal* 30(4), 969-977, 2002.
- Ellenbroek BA, Psychopharmacological treatment of schizophrenia: What do we have, and what could we get? *Neuropharmacology* 62(3), 1371-1380, 2012.
- Dando TM, Keating GM, Quetiapine - A review of its use in acute mania and depression associated with bipolar disorder, *Drugs* 65(17), 2533-2551, 2005.
- Kasper S, Tauscher J, Heiden A, Quetiapine: efficacy and tolerability in schizophrenia, *Eur Neuropsychopharm* 11, 405-413, 2001.
- DeVane CL, Nemeroff CB, Clinical pharmacokinetics of quetiapine: an atypical antipsychotic, *Clin Pharmacokinet* 40(7), 509-522, 2001.
- Bakken GV, Rudberg I, Christensen H, Molden E, Refsum H, Hermann M, Metabolism of quetiapine by CYP3A4 and CYP3A5 in presence or absence of cytochrome B5, *Drug Metab Dispos* 37(2), 254-258, 2009.
- Davis PC, Wong J, Gefvert O, Analysis and pharmacokinetics of quetiapine and two metabolites in human plasma using reversed-phase HPLC with ultraviolet and electrochemical detection, *J Pharm Biomed Anal* 20(1-2), 271-282, 1999.
- Frahnert C, Rao ML, Grasmader K, Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring, *J Chromatogr B* 794(1), 35-47, 2003.
- Hasselstrom J, Linnet K, Fully automated on-line quantification of quetiapine in human

- serum by solid phase extraction and liquid chromatography, *J Chromatogr B* 798(1), 9-16, 2003.
12. Saracino MA, Mercolini L, Flotta G, Albers LJ, Merli R, Raggi MA, Simultaneous determination of fluvoxamine isomers and quetiapine in human plasma by means of high-performance liquid chromatography, *J Chromatogr B* 843(2), 227-233, 2006.
 13. Belal F, Elbrashy A, Eid M, Nasr JJ, Stability-indicating HPLC method for the determination of quetiapine: Application to tablets and human plasma, *J Liq Chromatogr R T* 31(9), 1283-1298, 2008.
 14. Madej K, Biedron A, Garbacik A, Study of separation and extraction conditions for five neuroleptic drugs by an LLE-HPLC-DAD method in human plasma, *J Liq Chromatogr R T* 32(20), 3025-3037, 2009.
 15. Kirchherr H, Kuhn-Velten WN, Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: A multi-level, single-sample approach, *J Chromatogr B* 843(1), 100-113, 2006.
 16. Barrett B, Holcapek M, Huclova J, Borek-Dohalsky V, Fejt P, Memec B, Jelinek I, Validated HPLC-MS/MS method for determination of quetiapine in human plasma, *J Pharm Biomed Anal* 44(2), 498-505, 2007.
 17. Li KY, Zhou YG, Ren HY, Wang F, Zhang BK, Li HD, Ultra-performance liquid chromatography-tandem mass spectrometry for the determination of atypical antipsychotics and some metabolites in in vitro samples, *J Chromatogr B Analyt Technol Biomed Life Sci* 850(1-2), 581-585, 2007.
 18. Nirogi R, Bhyrapuneni G, Kandikere V, Mudigonda K, Ajjala D, Mukkanti K, Sensitive liquid chromatography tandem mass spectrometry method for the quantification of Quetiapine in plasma, *Biomed Chromatogr* 22(10), 1043-1055, 2008.
 19. Tu JY, Xu P, Xu DH, Li HD, UPLC-MS-MS analysis of quetiapine and its two active metabolites, 7-hydroxyquetiapine and 7-hydroxy-N-dealkylquetiapine, in rat plasma and cerebrospinal fluid, *Chromatographia* 68(7-8), 525-532, 2008.
 20. Kundlik ML, Kambli S, Shah V, Patel Y, Gupta S, Sharma R, Zaware B, Kuchekar SR, Quantification of Quetiapine in Human Plasma by LC-MS-MS, *Chromatographia* 70(11-12), 1587-1592, 2009.
 21. Hasselstrom J, Quantification of antidepressants and antipsychotics in human serum by precipitation and ultra high pressure liquid chromatography-tandem mass spectrometry, *J Chromatogr B* 879(1), 123-128, 2011.
 22. Pan RN, Kuo BPC, Pao LH, Validated LC-MS-MS Method for the Determination of Quetiapine in Human Plasma: Application to a Pharmacokinetic Study, *J Chromatogr Sci* 50(3), 277-282, 2012.
 23. Urinovska R, Brozmanova H, Sistik P, Silhan P, Kacirova I, Lemr K, Grundmann M, Liquid chromatography-tandem mass spectrometry method for determination of five antidepressants and four atypical antipsychotics and their main metabolites in human serum, *J Chromatogr B* 907, 101-107, 2012.
 24. Ansermot N, Brawand-Amey M, Kottelat A, Eap CB, Fast quantification of ten psychotropic drugs and metabolites in human plasma by ultra-high performance liquid chromatography tandem mass spectrometry for therapeutic drug monitoring, *J Chromatogr A* 1292, 160-172, 2013.
 25. Peddio G, Pittau B, Manca I, Salis R, Pani L, Pira L, Validated method to determine quetiapine and norquetiapine in microsomal matrix by LC MS-MS: Implication in quetiapine metabolism, *Chromatographia* 77(1-2), 75-82, 2014.
 26. Pullen RH, Palermo KM, Curtis MA, Determination of an antipsychotic agent (ICI 204,636) and its 7-hydroxy metabolite in human plasma by high-performance liquid chromatography and gas chromatography-mass spectrometry, *J Chromatogr* 573(1), 49-57, 1992.
 27. Da Fonseca BM, Moreno IE, Barroso M, Costa S, Queiroz JA, Gallardo E, Determination of seven selected antipsychotic drugs in human plasma using microextraction in packed sorbent and gas chromatography-tandem mass spectrometry, *Anal Bioanal Chem* 405(12), 3953-3963, 2013.
 28. Davis PC, Bravo O, Gehrke M, Azumaya CT. Development and validation of an LC-MS/MS method for the determination of quetiapine and four related metabolites in human plasma, *J Pharm Biomed Anal* 51(5), 1113-1119, 2010.
 29. Fisher DS, Handley SA, Taylor D, Flanagan RJ, Measurement of quetiapine and four quetiapine metabolites in human plasma by LC-MS/MS, *Biomed Chromatogr* 26(9), 1125-1132, 2012.
 30. Tuncel N, Sener E, Cerit C, Karasu U, Gurer F, Sahinturk V, Baycu C, Ak D, Filiz Z, Brain mast cells and therapeutic potential of vasoactive intestinal peptide in a Parkinson's disease model in rats: Brain microdialysis, behavior, and microscopy, *Peptides* 26(5), 827-836, 2005.

31. Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ, McKay G, Miller KJ, Patnaik RN, Powell ML, Tonelli A, Viswanathan CT, Yacobi A, Bioanalytical method validation--a revisit with a decade of progress, *Pharm Res* 17(12), 1551-1557, 2000.
32. Gonzalez O, Blanco ME, Iriarte G, Bartolome L, Maguregui MI, Alonso RM, Bioanalytical chromatographic method validation according to current regulations, with a special focus on the non-well defined parameters limit of quantification, robustness and matrix effect, *J Chromatogr A* 1353, 10-27, 2014.
33. Song M, Xu XX, Hang TJ, Wen AD, Yang L, Development of an LC-MS/MS method for the simultaneous quantification of aripiprazole and dehydroaripiprazole in human plasma, *Anal Biochem* 385(2), 270-277, 2009.

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