

©2004, Acta Pharmacologica Sinica  
Chinese Pharmacological Society  
Shanghai Institute of Materia Medica  
Chinese Academy of Sciences  
<http://www.ChinaPhar.com>

# Simultaneous determination of quetiapine and three metabolites in human plasma by high-performance liquid chromatography–electrospray ionization mass spectrometry

Kun-yan LI, Ze-neng CHENG, Xin LI, Xue-lian BAI, Bi-kui ZHANG, Feng WANG, Huan-de LI<sup>1</sup>

*Clinical Pharmaceutical Research Institute, Xiangya Second Hospital, Central South University, Changsha 410011, China*

**KEY WORDS** quetiapine; high pressure liquid chromatography; electrospray ionization mass spectrometry; metabolism

## ABSTRACT

**AIM:** To develop a high performance liquid chromatography–electrospray mass spectrometry (HPLC-MS/ESI) method for simultaneous determination of quetiapine and its sulfoxide-, 7-hydroxy-, 7-hydroxy-*N*-dealkyl-metabolites in human plasma. **METHODS:** The HPLC separation of the compounds was performed on a Kromasil C<sub>18</sub>, (5 μm, 4.6 mm×150 mm) column, using water (formic acid: 1.70 mmol/L, ammonium acetate: 5.8 mmol/L)-acetonitrile (65:35) as mobile phase, with a flow-rate of 0.95 mL/min. The compounds were ionized in the electrospray ionization (ESI) ion source of the mass spectrometer and detected in the selected ion recording (SIR) mode. The samples were extracted using solid-phase extraction columns. **RESULTS:** The calibration curves were linear in the ranges of 10-2000 μg/L for quetiapine, 1-200 μg/L for its metabolites, respectively. The average extraction recoveries for all the four samples were above 85 %. The methodology recoveries were much higher than 95 %. The intra-day and inter-day RSD are less than 15 %. **CONCLUSION:** The method is accurate, sensitive, and simple for study of pharmacokinetics and metabolic mechanism of quetiapine in patients at therapeutic dose.

## INTRODUCTION

Quetiapine (QTP, Fig 1) is an antipsychotic drug used for the treatment of schizophrenia and other psychotic syndromes. It produces less extrapyramidal side effects, hyperprolactinmia, and agranulocytosis than other neuroleptics<sup>[1,2]</sup>.

*In vitro* experiments indicated CYP3A4 was the main isoenzyme involved in quetiapine sulfoxidation, and *N*- and *O*- dealkylation. The 7-hydroxylation of

quetiapine is thought to be partly mediated by CYP3A4, although CYP2D6 was shown to be involved as well<sup>[3]</sup>. The *in vivo* metabolic profile has not been clarified in detail, but previous studies showed the major *in vivo* metabolites of quetiapine were quetiapine sulfoxide (QTP-SF, Fig 1) and the parent acid metabolite, but without antipsychotic activity. 7-Hydroxy-quetiapine (QTP-H, Fig 1) and 7-hydroxy-*N*-desalkyl-quetiapine (QTP-ND, Fig 1) are active metabolites, but with relatively low concentrations in blood<sup>[4]</sup>.

In order to study the pharmacokinetics and metabolic mechanism of QTP in patients at therapeutic dose, it is necessary to establish an HPLC-MS method to determine QTP and its various metabolites. At present, HPLC-UV<sup>[5,6]</sup>, GC-MS<sup>[5]</sup>, and HPLC-MS/APCI<sup>[7]</sup> meth-

<sup>1</sup> Correspondence to Prof Huan-de LI.

Phn 86-731-552-4222, ext 2275. Fax 86-731-443-6720.

E-mail lkunyan@163.net

Received 2002-10-18

Accepted 2003-05-23

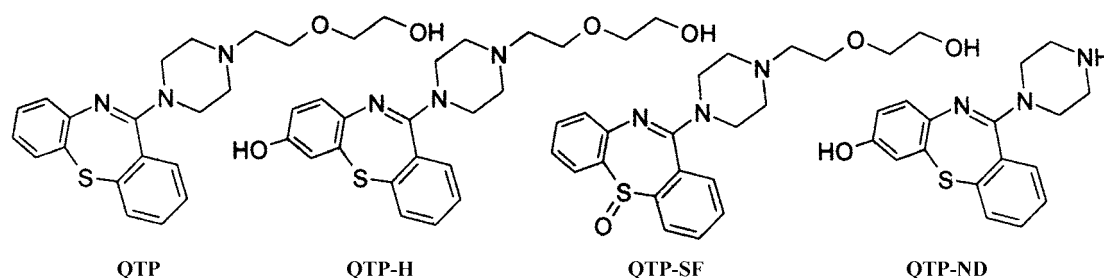


Fig 1. Chemical structures of QTP, QTP-H, QTP-SF, and QTP-ND

ods have been developed for determination of QTP. GC-MS<sup>[5]</sup> and HPLC-EC<sup>[6]</sup> methods have been used for determination of QTP-H and QTP-ND, but there is no report on simultaneous determination of the four compounds. The aim of this study is to develop a simple and selective HPLC-MS/ESI method for the determination of plasma levels of QTP and its three metabolites.

## MATERIALS AND METHODS

**Materials** Quetiapine (purity >99.6 %) was kindly provided by Hunan Dongting Pharmaceutical Co Ltd (Changde, Hunan, China). Quetiapine sulfoxide (purity =74 %), 7-hydroxy-*N*-dealkyl-quetiapine (purity=55 %) and 7-hydroxy-quetiapine (purity=77 %) were donated by AstraZeneca Pharmaceuticals (London, UK). Carbamazepine (IS, >99.9 %) was provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Other reagents at AR grade and HPLC grade are obtained from Chemical Reagent Factory of Hunan (Changsha, Hunan, China). Control human plasma (Catalog No 011020410014) was obtained from the Blood Center of Shanghai (Shanghai, China).

**Standard solution** The primary stock solutions of QTP (0.508 g/L), QTP-H (0.651 g/L), QTP-SF (0.617 g/L), QTP-ND (0.535 g/L), and carbamazepine (1.020 g/L) were prepared in methanol and serially diluted to working solution with methanol. All the stock and working solutions were stored at -10 °C.

**Chromatographic conditions** The HPLC analysis was performed on an Zirchrom reversed-phase column (Kromasil C<sub>18</sub>, 250 mm×4.6mm, 5 μm, Chenhang company, Shenzhen, Guangdong, China) with a column temperature of 40 °C. The mobile phase was water (containing formic acid 1.70 mmol/L and ammonium acetate 5.8 mmol/L) -acetonitrile (65:35). The

flow-rate was 0.95 mL/min, and the postcolumn splitting ratio was 3:1.

**MS/ESI detection conditions** A Micromass ZQ mass spectrometer (Wythenshawe, Manchester, UK) was equipped with an electrospray ionization (ESI) ion source. Sample cone and desolvation nitrogen were at the rate of 110 and 260 L/h, respectively. The vacuum running pressure was maintained at  $1.0 \times 10^{-4}$  mBar, the capillary voltage was 3.9 kV. The sample cone voltage was 45, 46, 38, 70, and 45 V for QTP, QTP-H, QTP-SF, QTP-ND, and carbamazepine, respectively. Extraction cone voltage was 6.1 V. The ion source temperature was 130 °C, and the desolvation temperature was 400 °C.

Selected ion recording (SIR) mode was used for quantification by the protonated molecular ions of each compound ( $m/z$  384.4 [QTP+H]<sup>+</sup>,  $m/z$  400.5 [QTP-SF+H]<sup>+</sup>,  $m/z$  400.5 [QTP-H+H]<sup>+</sup>,  $m/z$  312.4 [QTP-ND+H]<sup>+</sup>,  $m/z$  237.3 [IS+H]<sup>+</sup>).

**Plasma extraction** After addition of internal standard (IS: carbamazepine, 1.02 mg/L, 25 mL) to 0.5 mL aliquot of plasma sample, the sample was alkalinized by adding 0.3 mL sodium hydroxide (0.5 mol/L). After vortex-mixed for 2 min and centrifuged at 9500 rpm for 5 min, the mixture was immediately loaded onto the Oasis<sup>TM</sup> HLB extraction cartridge (1CC/10 mg, Waters Corporation, Milford, Massachusetts, USA), which had been activated with 1 mL of methanol and balanced with 1 mL of deionized water. Then the loaded plasma samples were drained away under vacuum (-40 kPa) and the columns were washed with 1 mL of ammonia water (2 %, v/v). The samples were subsequently eluted into glass tubes with 1 mL of methanol containing 2 % (v/v) acetic acid. The effluents were dried under nitrogen in a 40 °C water bath. The residue was reconstituted in 0.1 mL of mobile phase. Sample 50 μL was injected into the HPLC.

**RESULTS**

**HPLC-MS/ESI** The HPLC-MS/ESI in the SIR mode provided a highly selective method for the determination of QTP and its metabolites. The retention times of QTP, QTP-ND, QTP-H, QTP-SF, and IS were approximately 15.5, 3.2, 4.1, 5.3, and 10.0 min, respectively. The chromatograms of control human plasma, standards in control human plasma and patient samples were shown in Fig 2. Full scan ESI<sup>+</sup> mass spectra of a patient plasma sample were illustrated in Fig 3. The protonated molecule [M+H]<sup>+</sup> was identified at *m/z* 384.4, 400.5, 400.5, 312.4, and 237.3 for QTP, QTP-SF, QTP-H, QTP-ND, and IS, respectively.

**Calibration curves** In the concentration range of 10 to 2000 µg/L for quetiapine, 1 to 200 µg/L for the three metabolites, the area ratio of each sample to IS was well related to the concentration (*r*<sup>2</sup>: 0.9992 for QTP, 0.9993 for QTP-ND, 0.9908 for QTP-H, and 0.9929 for QTP-SF).

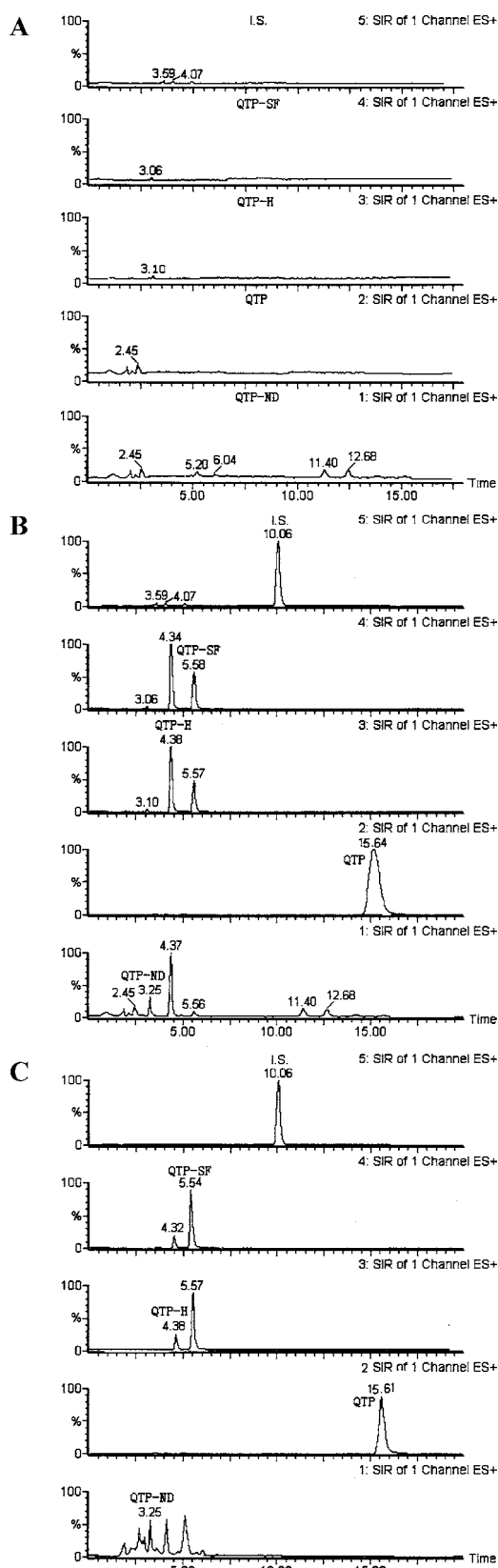
**Accuracy and precision** Extraction recoveries and precision assays were carried out in three concentrations for 3 times within the same day and over three different days. The mean extraction recoveries, methodology recoveries, intra-day and inter-day precision for the four analytes at the three concentrations were shown in Tab 1.

**Sensitivity** The limit of quantification (LOQs) was 10 µg/L for QTP and 1 µg/L for the three metabolites (S/N=10). The precision and reproducibility for QTP and the three metabolites are presented in Tab 1. The limits of detection (LODs) were 0.30 µg/L, 0.15 µg/L, 0.15 µg/L, and 0.4 µg/L for QTP, QTP-SF, QTP-H, and QTP-ND, respectively (S/N=3).

**Analysis of patient plasma** Plasma samples were obtained from 5 schizophrenia patients (25±4 a, male) at 1 h and 12 h after oral administration of quetiapine fumarate (Seroquel) (200 mg each time, two times daily). The steady-state concentrations of QTP, QTP-ND, QTP-H, and QTP-SF determined by the method were shown in Tab 2.

**DISCUSSION**

Caccia reported that quetiapine and quetiapine sulfoxide were the major circulating species in plasma. However, the plasma concentrations of 7-hydroxy-quetiapine and 7-hydroxy-*N*-desalkyl-quetiapine are about 5 % and 2 %, respectively, of that of quetiapine. Thus, the two active metabolites are not thought to



**Fig 2. Chromatograms of QTP, QTP-SF, QTP-H, QTP-ND, and IS. A) Control human plasma; B) Standard QTP, QTP-SF, QTP-H, QTP-ND and IS in control human plasma; C) Patient plasma sample after 1 h of oral administration of QTP (200 mg each time, two times daily).**

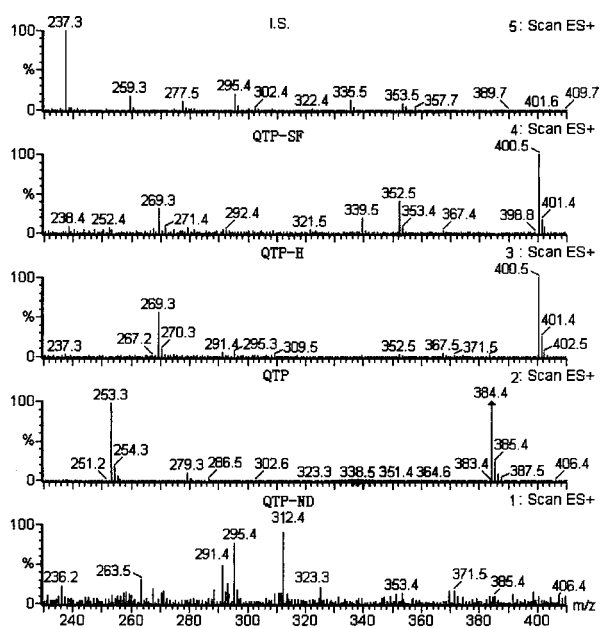


Fig 3. Full scan ESI<sup>+</sup> mass spectra of one patient plasma sample.

Tab 1. The mean extraction recoveries, methodology recoveries, intra-day and inter-day precision for the four compounds at the three concentrations. *n*=3. Mean±SD.

Compound	Conc. added/ μg·L <sup>-1</sup>	Extraction recovery/ %	RSD/ %	Methodology recovery/ %	Precision (RSD)/%	
					Intra-day	Inter-day
QTP	10	93±8	10.4	95±6	5.9	11.57
	100	102±3	3.7	97.5±2.3	2.4	7.35
	500	100±5	11.3	100±8	8.5	12.79
	1000	105±3	3.6	100±6	6.2	5.32
QTP-SF	1	104±8	9.4	106±9	8.5	13.34
	10	96±7	7.0	97.5±2.3	2.3	2.51
	50	95±4	7.4	99.5±2.9	2.9	2.81
	100	94±3	7.6	96±4	3.9	9.75
QTP-H	1	97±8	11.2	97±4	4.6	15.24
	10	99±6	2.4	95.7±2.7	2.9	4.98
	50	97±4	6.0	97.9±2.0	2.1	13.96
	100	99±7	7.2	102±3	3.4	10.32
QTP-ND	1	82±10	9.6	89±14	13.2	14.35
	10	85±5	9.1	100±4	4.3	2.07
	50	89±3	7.4	98.7±1.7	1.8	8.06
	100	84±6	7.6	96±10	11.9	2.94

contribute to the pharmacological effects of administered quetiapine in Caucasian<sup>[3]</sup>. But the expression of CYP3A4 varies up to a 30-fold difference between individuals, so it is necessary to measure the concen-

Tab 2. Plasma concentrations of QTP and its metabolites in 5 schizophrenia patients (25±4 a, male) at 1 h and 12 h after oral administration of quetiapine fumarate (Seroquel) (200 mg each time, two times daily).

Pati-ent	Time/h	Coadmini-stered drugs	Concentration/μg·L <sup>-1</sup>			
			QTP	QTP-H	QTP-ND	QTP-SF
1	1	N	780.93	87.75	13.56	623.11
	12		75.08	11.93	6.44	75.31
2	1	N	511.12	75.85	20.17	497.86
	12		50.08	12.41	9.85	61.48
3	1	N	528.35	51.21	13.64	436.08
	12		64.14	9.91	5.09	101.70
4	1	N	137.79	14.80	8.41	108.27
	12		54.04	4.62	4.22	34.59
5	1	Propranolol 10 mg	233.53	8.75	8.79	188.03
	12		53.73	5.28	11.45	38.82

N: no coadministered drugs

trations of quetiapine and some of its metabolites in Chinese.

At present, high performance liquid chromatography-electrospray mass spectrometry (HPLC-MS/ESI) has emerged as a powerful analytical technique for determination of drugs and metabolites in biological fluids. It can detect the concentration by using one *m/z* or several *m/z* of one compound. There is an individual detecting channel for each compound in the selected ion recording mode, so the compounds with different *m/z*, which had not been separated under HPLC condition, can be separated and not interfere each other through different detecting channels. Compared with the HPLC method, HPLC-MS/ESI improved the specificity, shortened the analysis time, and simplified the preparation of the sample. However compounds with the same *m/z*, like QTP-H and QTP-SF, must be separated completely under HPLC condition to avoid the interference with each other, thus there was a choice of the mobile phase during the experiment. Using water (formic acid: 1.70 mmol/L, ammonium acetate: 5.8 mmol/L)-acetonitrile (65:35) as mobile phase, with a flow-rate of 0.95 mL/min, QTP-H and QTP-SF had been well separated in HPLC system. Formic acid was added in the mobile phase to adjust the pH to about 6, which can increase the sensitivity.

In this assay, the extraction rate can be influenced by the concentration of alkaline solution added to the

samples during the solid phase extraction. When the concentration of sodium hydroxide added in the samples is between 0.15 and 0.20 mol/L, the extraction rate is high. When the concentration is less than 0.15 mol/L, the extraction rate is low, possibly because of the incomplete alkalization of the compounds. When the concentration is more than 0.2 mol/L, the extraction rate decreases, which is attributed to the destruction of the compounds. Above all, when sodium hydroxide solution (0.3 mL) 0.5 mol/L is added in 0.5 mL plasma sample, the extraction rate is high and the precision is very good.

In conclusion, the HPLC-MS/ESI method described in this report was highly sensitive and specific enough for simultaneous determination of quetiapine and its metabolites in human plasma.

**ACKNOWLEDGEMENTS** Thanks are due to Astra-Zeneca Pharmaceutical (London, UK) for providing us with pure compounds and the funds to develop this work, and also to Dr Ze-lin LIU and Nurse Meng-xian DENG for their clinical assistance.

## REFERENCES

- 1 Richelson E. Receptor pharmacology of neuroleptics: relation to clinical effect. *J Clin Psychiatry* 1999; 60 (Suppl 10): 5-14.
- 2 Arvanitis LA, Miller BG. Multiple fixed doses of "Seroquel" (quetiapine) in patients with acute exacerbation of schizophrenia: a comparison with haloperidol and placebo. *Biol Psychiatry* 1997; 42: 233-46.
- 3 Caccia S. New antipsychotic agents for schizophrenia: pharmacokinetics and metabolism update. *Curr Opin Investing Drugs* 2002; 3: 1073-80.
- 4 DeVane CL, Nemeroff CB. Clinical pharmacokinetics of quetiapine. *Chin Pharmacokinet* 2001; 40: 509-22.
- 5 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* 1992; 573: 49-57.
- 6 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* 1999; 20: 271-82.
- 7 McConville BJ, Arvanitis LA, Thyrum PT, Yeh C, Wilkinson LA, Chaney RO, *et al*. Pharmacokinetics, tolerability and clinical effectiveness of quetiapine fumarate: an Open-label trial in adolescents with psychotic disorders. *J Clin Psychiatry* 2000; 61: 252-60.