

Validated HPLC–MS/MS method for determination of quetiapine in human plasma

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Abstract

A validated, highly sensitive and selective high-pressure liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed for the quantitative determination of quetiapine (QUE) in human Na₂EDTA plasma with mass spectrometry (MS) detection. Clozapine (CLO) was employed as an internal standard. Samples were extracted using solid phase extraction (SPE). Oasis HLB cartridges and the concentration of quetiapine was determined by isocratic HPLC–MS/MS. The SRM mode was used for MS/MS detection. The method was validated over a concentration range of 1.0–382.2 ng/mL. Inter- and intra-day precision and accuracy of the proposed method were characterized by relative standard deviation (R.S.D.) and the percentage of deviation, respectively; both were lower than 8%. The developed method was employed in the pharmacokinetic study of quetiapine.

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1. Introduction

Quetiapine (2-[2-(4-dibenzo[*b,f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]ethanol fumarate (2:1 salt)) is an atypical antipsychotic drug with a unique receptor-binding profile belonging to a new chemical class, the dibenzothiazepine derivatives [1–4]. Quetiapine is an antagonist at a broad range of neurotransmitter receptors [2,3]. Quetiapine is used in the treatment of schizophrenia or manic episodes associated with bipolar disorder. These antipsychotics have a low incidence of extrapyramidal side effects and tardive dyskinesias compared to older antipsychotics. The advantages of the therapeutic profile of quetiapine have led to increasing use in the clinical practice, which encourages the development of new pharmaceutical preparations. As a consequence, there is an increasing

demand for new analytical methods for determination of pharmacokinetic parameters in bioequivalence studies. Some of these methods could be also employed in therapeutic drug monitoring. Due to inter-individual pharmacokinetic variability the dose has to be carefully titrated depending on the clinical response and tolerability of the individual patient.

Quetiapine is metabolized by the liver and eleven confirmed metabolites of quetiapine have been identified [4]. Quetiapine appears to be the major circulating species in plasma. Unlike other antipsychotics such as Olanzapine no effect of cigarette smoking on quetiapine clearance was observed [4]. The pharmacokinetics of quetiapine are linear, and do not differ between men and women [2].

Several HPLC methods for the determination of QUE have been reported. Most of these require ultraviolet detection [7–9], as QUE is not electro active. However none of these methods is sensitive enough for determination of the expected drug levels and some of them are time-consuming and require complex sample pretreatment or long run times [10]. Some gas

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chromatography–mass spectrometry (GC–MS) methods have also been employed, however here QUE needs to be derivatized before analysis [11,12].

Rapid and effective ways for determination of drugs and metabolites in biological fluids are desirable. LC–MS/MS methods are suitable for the quantitative determination of drugs. MS/MS detection is sensitive and enables the effective elimination of interferences from endogenous components. Recently, two HPLC–MS methods have been published for determination of QUE [10,13].

The first paper [10] compares HPLC methods with ultraviolet and MS/MS detection. Although the sample preparation is fully automated, the run time is 35 min, thus the method allows determination of only 40 samples a day, which is not enough for routine analysis and commercial utilization in pharmacokinetic studies.

The next paper [13] describes HPLC–MS method for simultaneous determination of Clozapine, Olanzapine, Risperidone, and QUE in plasma. Nevertheless, this method requires two-step extraction and LOQ is too high for our purpose.

The goal of our work was to develop an HPLC–MS/MS method for determination of QUE in human plasma obtained in a pharmacokinetic study and to use the results for evaluating pharmacokinetic parameters. According to the literature, a QUE concentration between 1.5 and 350 ng/mL [5,6] in human plasma could be expected after the administration of a 100 mg QUE dose.

2. Experimental

2.1. Chemicals and reagents

Quetiapine fumarate was obtained from Medichem (Barcelona, Spain) and Clozapine from Sigma (Schnelldorf, Germany). Acetonitrile (MS grade), methanol (MeOH) (HPLC grade) and ammonium acetate were purchased from Sigma (Schnelldorf, Germany) and acetic acid from Fluka (Schnelldorf, Germany). Water was deionized and further purified for HPLC with a Milli-Q system (Millipore, Schwalbach, Germany).

Blank human plasma was obtained of healthy volunteers.

2.2. Mass spectrometry

Mass spectrometry was performed using a Quattro microTM triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an ESI source. The selected reaction monitoring (SRM) mode was employed for the determination of QUE due to its high selectivity. The specific precursor-to-ion transitions monitored were m/z 384.2 \rightarrow 253.1 for QUE and m/z 327.2 \rightarrow 270.3 for CLO. The dwell times used were 0.1 and 0.2 s, respectively. Collision-induced dissociation (CID) was carried out using 2.5×10^{-3} mbar argon. The collision energy was 25 eV for both compounds. The cone voltage was set at an optimized value (30 kV) in the positive-ion mode. The capillary voltage was 2.0 kV and the entrance and exit energies of the collision cell were set at 1 and 3 V, respectively. Nitrogen was used

as desolvation (400 L/h) and cone (40 L/h) gas. The source and desolvation temperatures were optimized and kept at 100 and 400 °C, respectively. The system was controlled by Masslynx V 4.0 software, Waters (Manchester, UK).

2.3. Liquid chromatography

A Waters 2695 liquid chromatograph (Waters, Milford, USA) with an Atlantis dC18 column (100 mm \times 3.0 mm, 3 μ m) (Waters, Manchester, UK) and Pelliguard LC-18 (20 mm \times 4 mm) (Supelco, Schnelldorf, Germany) guard column was used for the separation of QUE and CLO. Other columns tested were Xterra MS C8 and C18—both 3.0 mm \times 10 mm, 3.5 μ m from Waters. The mobile phase was a mixture of acetonitrile–methanol–0.01 M ammonium acetate (31:19:50, v/v/v); pH was adjusted with acetic acid (pH 3.5). Before use, the mobile phase was degassed by vacuum filtration through a 0.45 μ m filter. The flow rate was set at 0.4 mL/min.

2.4. Preparation of standard and quality control solutions

The stock standard solutions of QUE were prepared by dissolving accurately weighed QUE standard in MeOH/H₂O (70:30, v/v). The stock standard solution was then diluted with MeOH/H₂O (70:30, v/v) to achieve a working standard solution at the concentration of 38218 ng/mL. Similarly the quality control (QC) working standard solution was prepared from QUE stock quality control solution at the concentration of 30401 ng/mL. Blank plasma samples (9.9 mL) were spiked by working solutions (100 μ L) to gain either the most concentrated calibration standard of QUE (S1) or quality control sample (QC1). All plasma samples were stored at -25 ± 5 °C.

The remaining plasma calibration standards (S7–S2) were prepared from S1 by sequential dilutions with blank plasma directly before sample processing. The final concentrations of plasma calibration standards were 1.0, 4.0, 11.9, 23.9, 95.5, 191.1, 382.2 ng/mL. Similarly the remaining quality control samples (QC3 and QC2) were prepared from the most concentrated quality control sample QC1 by sequential dilution with blank plasma to get the final concentrations of QCs 2.1, 152.0 and 304.0 ng/mL, respectively.

The stock internal standard solution was prepared by accurate weighing of CLO (0.0080 g), which was dissolved in MeOH/H₂O (70:30, v/v), into a volumetric flask. The working internal standard (WIS) was prepared by accurate dilution of stock internal standard with MeOH/H₂O (70:30, v/v) to get a final concentration of 4000.0 ng/mL. Stock IS was stored at 4 °C for 5 days. Volume of 50 μ L WIS was added to 0.50 mL plasma samples.

2.5. Preparation of plasma samples

Solid phase extraction (SPE) was used for sample pretreatment. Oasis HLB (hydrophilic-lipophilic balance) cartridges (30 mg, 1 mL) from Waters (USA) were activated with 2 mL

of MeOH and conditioned with 3 mL H₂O. The plasma sample (0.5 mL) was spiked with 50 μL of WIS, alkalized with 200 μL of 0.4 M NaOH, and vortex-mixed. The mixture was loaded on the prepared cartridges. The cartridge was washed with 3 mL H₂O, and the analyte was eluted with 200 μL of mobile phase. A 20-μL aliquote was then injected onto the HPLC system with MS/MS detection.

2.6. Pharmacokinetic study

A single center, open, randomized, bioequivalence study on quetiapine 100 mg in healthy volunteers was performed. A 36 h dose-titration period comprising three doses of 25 mg preceded the administration of the 100 mg dose. The titration period was incorporated into the study design to prevent sudden adverse effects of QUE in the 100 mg dose and to ensure the safety of the subjects involved in the study.

Plasma samples were obtained from 32 volunteers in various time intervals within 30 h after 100 mg drug administration. The analytical batch consisted of blank, blank with internal standard (S0), seven calibration standards (S7, S6, S5, S4, S3, S2, S1) and plasma samples gained from two volunteers involved in the study with six quality control (QC) samples interspersed (two series QC3, QC2 and QC1).

3. Results and discussion

3.1. Mass spectrometry

The chemical structures of QUE (molecular weight 383.5) and the internal standard (molecular weight 326.8) are presented in Fig. 1. The molecular weights of both compounds were confirmed by the presence of $[M + H]^+$ in the positive-ion mode and $[M - H]^-$ in the negative-ion ESI mass spectra without any fragmentation.

Tandem mass spectrum (Fig. 2) shows the most important fragment ions arising by the cleavage in heterocyclic piperidine ring, i.e., the neutral loss of HOCH₂CH₂OCH₂

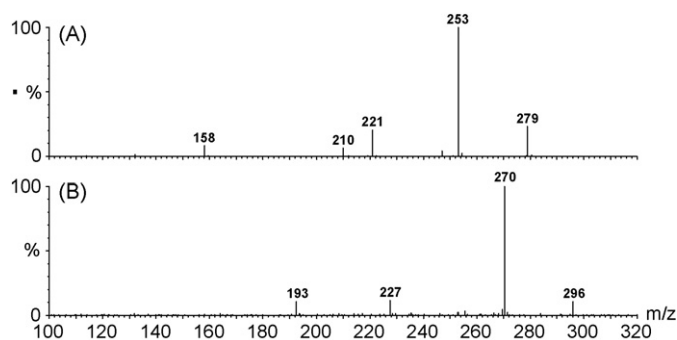


Fig. 2. ESI+ product ion scans of QUE (A) and CLO (B) obtained using the triple quadrupole spectrometer. The precursor ions were 384.2 and 327.2 ($[M + H]^+$) for QUE and CLO, respectively. Experimental parameters: cycle time 0.6 s, scan duration 0.5 min, interscan delay 0.1 s, mass range 100–400 (A) and 100–320 (B), collision energy 25 eV.

CH₂NHCH=CH₂ for quetiapine and of CH₃NHCH=CH₂ for clozapine. These transitions were used for SRM experiments and analyte quantitation.

3.2. HPLC separation

Complete chromatographic separation of analyte and internal standard was not necessary with MS/MS detection. LC was used mainly for pre-concentration of the analyte. Peak shape and intensity of the response were the main aspects for LC optimization. QUE is slightly basic and is well adsorbed on hydrophobic sorbents.

The Atlantis dC18 column was eventually selected for all assays because it exhibited excellent peak shape and had sufficient response for QUE.

The final composition of the mobile phase was methanol, acetonitrile and ammonium acetate buffer 0.01 M pH 3.5 (31:19:50, v/v/v). Increasing the percentage of the buffer in the mobile phase enhanced peak symmetry and the resolution between the analyte peak and the dead volume peak. Buffers with pH in the range 3–6 were tested. Peak symmetry and resolution increased with decreasing pH of buffer while simultaneously the retention

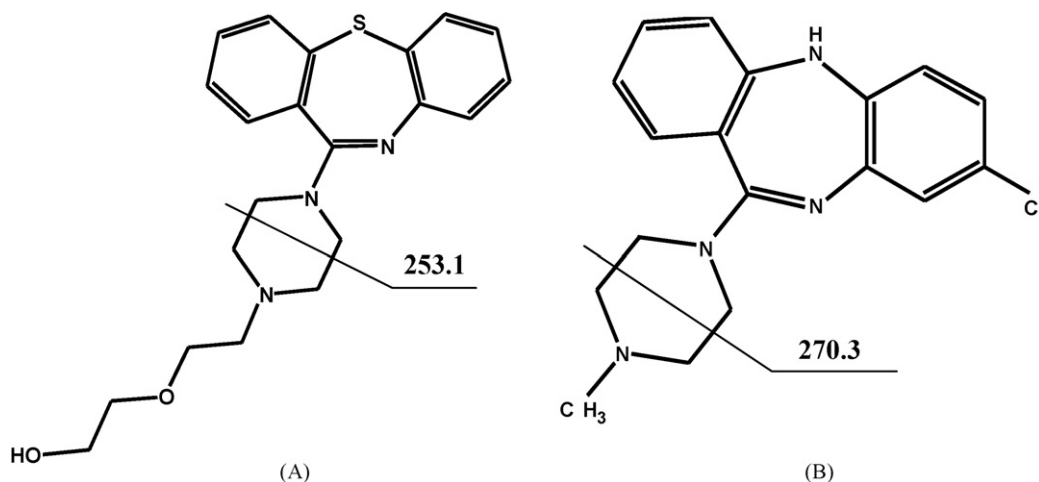


Fig. 1. (A) Quetiapine—molecular formula: C₂₁H₂₅N₃O₂S, 2-[2-(4-dibenzo[*b*,*f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]ethanol. (B) Clozapine (internal standard), molecular formula: C₁₈H₁₉ClN₄, 8-chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine.

times of analyte and internal standard were shortened. Eventually a buffer with pH 3.5 was chosen as a compromise between the discussed parameters.

A run time of 3 min could be achieved, contrary to the previously published method with UV detection, where the necessity of separation the analyte and internal standard and eluting most of the potential interferents acquired a run time of 35 min [10].

3.3. Preparation of plasma samples

Double liquid–liquid extraction was described by Zhou et al. [13] but the method was found not to be sensitive enough for the desired calibration range. Moreover complete removal of endogenous compounds interfering with QUE failed. In this light SPE procedure was tested as alternative sample pretreatment procedure. Hasselstrom and Linnert compared different types of SPE sorbents for QUE extraction. The best recovery was achieved using the endcapped CN cartridges but finally C2 sorbent was chosen because of the need of removing interference in blank serum samples [10]. In the cited article the choice of the most suitable SPE sorbent was complicated by the necessity of achievement of acceptable recovery of the analyte and the internal standard—of two substances that differ in their structure enough to be separated by HPLC in 15 min. When MS detection is used the chromatographic separation of QUE and IS is not so crucial as in case of UV detection and the choice of the internal standard can be better adjusted to the characteristics of the SPE sorbent optimal for the analyte.

We decided to test Oasis HLB cartridges for analyte extraction because of our good experience with this type of sorbent for extraction of a large amount of samples in the bioanalytical laboratory. The sorbent of these cartridges is a macroporous polymer made from two monomers, the lipophilic divinylbenzen and the hydrophilic *N*-vinylpyrrolidone. The Oasis cartridges are suitable for extraction of both non-polar and polar compounds, like parent drugs and metabolites. The extraction methods involving Oasis sorbent does not suffer from low reproducibility caused by sorbent drying, which is another important characteristic of a sorbent massively used in a bioanalytical laboratory with a large throughput of samples.

The sample was alkalized before loading onto the sorbent in order to depress the ionization of QUE and IS and to enhance their extraction. Furthermore the solubility of QUE in water decreases with the increase of pH of the solution [2], which can further enhance the extraction of the analyte from plasma. The pH of the plasma sample after the alkalization was 11.8, which was fairly enough to ensure the unionized form of the analyte and the internal standard compared to their pK_a values (QUE 6.8, CLO 7.57). The pH of samples was not measured routinely because of large amount of samples and their low volume, therefore we chose rather higher concentration of NaOH and a higher pH value of alkalized samples. After the removal of interferences by washing the cartridges with water the analyte was eluted with 200 μ L of mobile phase. The elution was enhanced by acid pH of mobile phase.

SPE sample pretreatment enabled analyte pre-concentration and was shown to have the advantages of simplicity and speed.

3.4. Validation of the QUE assay

3.4.1. Specificity and selectivity

Plasma samples from six different drug-free persons were tested for the presence of endogenous components, which might interfere with the detection of QUE or the internal standard (CLO). These samples were pre-treated according to the sample preparation procedure, apart from addition of the internal standard solution. Chromatograms of blank plasma and plasma sample spiked with QUE (1.0 ng/mL) and CLO (9600.0 ng/mL) were compared to show the specificity and selectivity of the proposed procedure. The chromatograms are presented in Fig. 3. The retention times of QUE and CLO were 2.0 and 2.1 min, respectively. No endogenous components interfering with the detection of QUE and CLO were found in the chromatograms of blank plasma samples.

In addition, the “cross-talk” between MS/MS channels used for monitoring QUE and CLO was assessed by the following procedure: separately injecting QUE (382.2 ng/mL) and monitoring the response in the CLO channel and by injecting a plasma sample spiked only with CLO and monitoring the response in the QUE channel. No “cross-talk” between channels was observed.

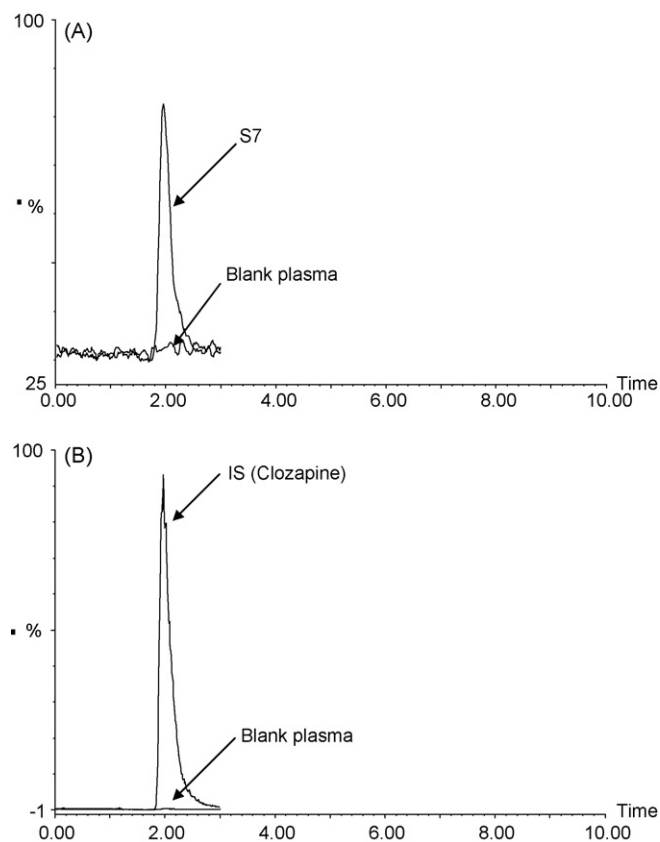


Fig. 3. SRM mass chromatograms depiction: (A) m/z 384.2 \rightarrow 253.1 in standard S7 (1.0 ng/mL QUE) and blank, and (B) m/z 327.2 \rightarrow 270.3 in standard S7 (9600 ng/mL CLO) and blank.

Table 1
Recovery, absolute matrix effect and process efficiency

Nominal concentration (ng/mL)	Mean peak area			ME (%)	RE (%)	PE (%)
	Set 1	Set 2	Set 3			
2.55	3,790	3,635	3,922	95.90	107.90	103.47
6.26	9,934	9,234	9,953	92.95	107.79	100.20
15.32	36,725	35,460	39,511	96.56	111.42	107.59
37.53	59,729	59,216	65,337	99.14	110.34	109.39
91.92	218,529	203,842	223,881	93.28	109.83	102.45
225.14	377,532	374,033	414,545	99.07	110.83	109.80
551.43	649,680	632,768	704,997	97.40	111.41	108.51

3.4.2. Recovery and matrix effect

Three sets of seven calibration standards and a blank with the internal standard (S0) were prepared for the evaluation of recovery, absolute ionization suppression or enhancement, and process efficiency. Set 1 was prepared to evaluate the MS/MS response of working standard solutions injected in the mobile phase. Working standard solutions were diluted 1:100 with acetonitrile/H₂O (70:30, v/v) to reach concentrations expected in plasma samples. Set 2 consisted of eight plasma samples spiked with 5 µL of working standard solutions *after* extraction. Plasma samples spiked *before* extraction and S0 were processed and analyzed to obtain Set 3. Three replications of each set were used for determination of recovery and absolute matrix effect. An internal standard was not added to standards.

The relative matrix effect was evaluated by analyte determination in six replicates (six different plasma sources) of two different concentrations (S7, S1). Samples were processed using the described sample pretreatment method and the relative matrix effect was assessed as the recovery to a nominal value of S7 and S1.

The absolute matrix effect (ME), the possibility of ionization suppression or enhancement, recovery (RE) and overall process efficiency (PE) were evaluated by comparing the results of analysis of three sets of samples as follows:

$$\text{ME}(\%) = \frac{B}{A} \times 100, \quad \text{RE}(\%) = \frac{C}{B} \times 100,$$

$$\text{PE}(\%) = \frac{C}{A} \times 100$$

where *A* is the mean peak area for single conc. of Set 1, *B* the mean peak area for single conc. of Set 2, and *C* is the mean peak area for single conc. of Set 3.

The results of the recovery, absolute matrix effect and process efficiency study are summarized in Table 1. The relative matrix effect was evaluated by calculation of % deviation (S7, 5.8%, S1, 2.2%) and RSD (S7, 5.6%, S1, 2.4%).

An absolute matrix effect of about 4% was found and it showed good consistency over the concentration range. No significant relative matrix effect was found in six different lots of plasma. Therefore it was concluded that the matrix does not affect the accuracy and precision of QUE determination. A recovery of 110% and an overall process efficiency of 106% with low variability were found to be consistent over the calibration range, consequently the published method was proved

to be reliable. Values of recovery and process efficiency exceeding 100% were supposed to be caused in part by evaporation of organic solvents and consequent analyte pre-concentration during the elution step of SPE, which was performed under vacuum. However, the influence of evaporation was not confirmed in another experiment, in which the eluate was completely evaporated after the elution step and the analyte was reconstituted in 200 µL of mobile phase before the injection. The results of recovery and process efficiency obtained in this experiment were similar to the presented values, hence we decided not to complicate and prolong the sample pretreatment step with the eluate evaporation.

Although the supernatant probably contained endogenous compounds, it did not affect the determination (Section 3.4.1); MS/MS detection ensured the selectivity of the method.

3.4.3. Limit of detection and quantification

The limit of detection (LOD) was estimated as the amount of QUE, which caused a signal that was three times the noise (S/N = 3/1). The value of LOD was calculated according to the equation $\text{LOD} = (3N/S) \times \text{amt. found}$ [14]. The data used for the calculation of LOD of the proposed method are summarized in Table 2. They were obtained by analyzing six different samples with the nominal concentration of 1.0 ng/mL. The S/N prints of respective chromatograms are presented in Fig. 4. The obtained value of LOD was 0.3 ng/mL.

The lower limit of quantification (LLOQ) (defined as the lowest concentration that could be analyzed with acceptable accuracy and precision (20%)) was 1.0 ng/mL, which was sufficient for the purpose of the pharmacokinetic study.

Table 2
Signal to noise ratio used for calculation of LOD

Sample name	Signal/noise, S/N	Amount found (ng/mL)	LOD (ng/mL)
LOQ_A	7.92	1.1	0.4
LOQ_B	8.01	1.1	0.4
LOQ_C	8.29	1.1	0.4
LOQ_D	21.33	1.1	0.2
LOQ_E	19.54	1.1	0.2
LOQ_F	24.15	1.1	0.1

Average LOD = 0.3 ng/mL.

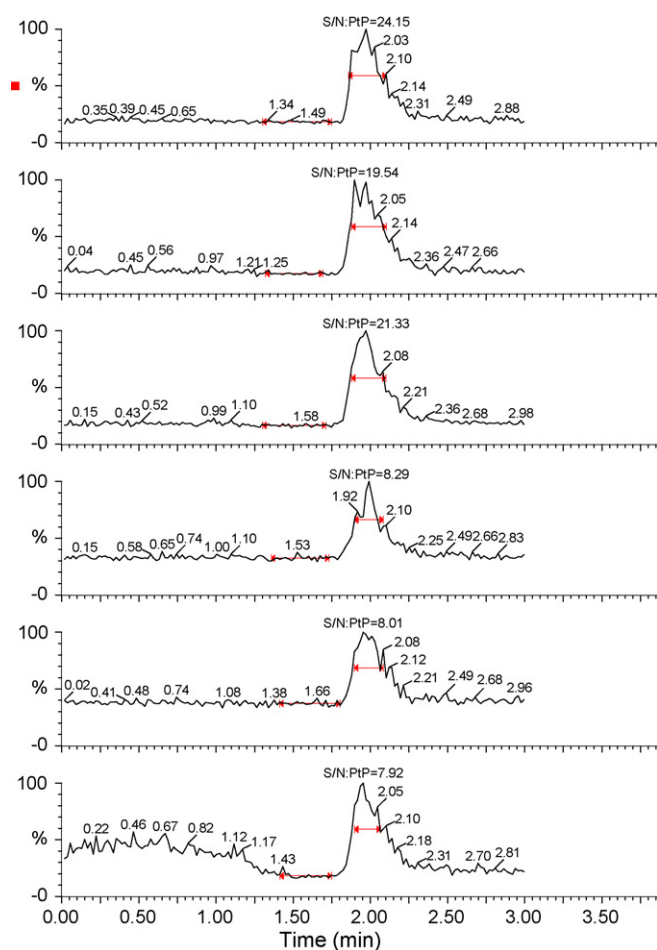


Fig. 4. S/N print of chromatograms used for determination of LOD.

3.4.4. Linearity, accuracy and precision

The seven point calibration curve obtained by weighted linear regression ($1/X$) showed good linearity over the whole concentration range (1.0–382.2 ng/mL), which covered the concentrations typically found in human plasma after administration of QUE in the pharmacokinetic study. The correlation coefficient was better than 0.999 ($n = 5$). Table 3 gives a summary of the response linearity.

Inter-day and intra-day assay were performed to evaluate precision (R.S.D.) and accuracy (% deviation). Intra-day precision

Table 3
Linearity summary

Run number	Equation form: $Y = BX + A$		Correlation coefficient, r
	B	A	
1	0.00580	0.00065	0.9994
2	0.00576	0.00125	0.9993
3	0.00554	0.00107	0.9996
4	0.00548	0.00036	0.9996
5	0.00563	0.00095	0.9995
n	5	5	5
Mean	0.00564	0.00086	0.9995
S.D.	0.00014	0.00035	0.0003

Table 4
Intra-day assay summary

	Amount added (ng/mL)			
	1.0	11.9	95.5	382.2
Amount found (ng/mL)	1.1	11.0	92.3	378.3
	1.0	12.6	90.8	363.5
	1.1	12.2	96.8	385.6
	1.0	12.9	98.3	363.4
	1.1	12.5	95.9	421.3
	0.9	11.7	93.6	392.4
Mean	1.03	12.15	94.62	384.08
S.D.	0.08	0.69	2.86	21.64
% Deviation (a)	3.33	2.10	-0.92	0.49
% R.S.D. (b)	7.90	5.72	3.02	5.63
Overall % deviation	1.25			
% R.S.D.	3.02–7.90			

(a) Accuracy; (b) precision.

and accuracy were assessed by the analysis of four plasma samples (S7, S5, S3 and S1) in six series. Inter-day precision and accuracy was determined by analyzing six series of four plasma samples (S7, S5, S3 and S1) in 4 runs within 4 days. Intra- and Inter-day assays results are summarized in Tables 4 and 5, respectively, and prove acceptable precision and accuracy of the proposed method.

3.4.5. Stability

Freeze-thaw stability ($-75 \pm 5^\circ\text{C}$) was determined as percent recovery compared to the nominal value of QC3 and QC1 (2.1 and 304.0 ng/mL) in triplicate. The test was carried out within 4 days in 4 runs. Every day the samples were thawed for analysis and frozen again. The difference of the nominal value varied between -1.3 and 10.9% in the fourth cycle being 8.9% for QC3 and -1.1% for QC1. It was concluded that four cycles of freeze-thaw could be carried out with no loss of QUE.

Long-term stability at $-75 \pm 5^\circ\text{C}$ was performed in 4 runs over 3 months. Per-cent recovery of QC3 and QC1 (2.1 and 304.0 ng/mL) in triplicate was determined and compared to the nominal value. The obtained data showed no loss of QUE. The results are summarized in Table 6.

Table 5
Inter-day assay summary

	Amount added (ng/mL)			
	1.0	11.9	95.5	382.2
Amount found (ng/mL)				
Run 1	1.0	11.3	92.2	385.4
Run 2	1.0	11.7	93.3	388.3
Run 3	1.1	11.5	93.2	385.9
Run 4	1.1	11.5	92.9	383.3
Mean	1.04	11.50	92.90	385.74
S.D.	0.01	0.14	0.50	2.06
% Deviation (a)	4.17	-3.33	-2.73	0.93
% R.S.D. (b)	0.92	1.20	0.54	0.53
Overall % deviation	-0.24			
% R.S.D.	0.53–1.20			

(a) Accuracy; (b) precision.

Table 6
Long-term stability

Day	Concentration	Mean amount found (ng/mL)	Nominal value (ng/mL)	Difference (%)
0	Low	2.1	2.1	2.2
	High	301.9	304.0	-0.7
15	Low	2.2	2.1	-6.5
	High	287.6	304.0	-5.4
39	Low	2.0	2.1	-6.5
	High	280.9	304.0	-7.6
91	Low	2.2	2.1	4.3
	High	322.4	304.0	6.1

Room temperature stability was assessed by analyte determination in five sets of QC2 (152.0 ng/mL) in triplicate. Each set was left at room temperature for various times (0, 15, 30, 60, 120 min) before sample processing. Since the difference of the nominal value of QUE found was lower than 7%, it was concluded that QUE is stable up to 2 h at room temperature before sample processing.

In-process stability of QUE was evaluated by determination of five sets of QC2 (152 ng/mL) in triplicate. After alkalization by NaOH the sets were left for various times (0, 15, 30, 60, 120 min). A difference of less than 9% of the nominal value of QUE was observed, therefore it was concluded that QUE in plasma was stable for 2 h after alkalization.

Autosampler stability (stability of QUE eluate) was estimated by analysis of QC samples (three series of QC3, QC2 and QC1). Samples were analyzed at the beginning of the test and after 24 h while stored in autosampler at 10°. The results of both sets of data differed by less than 10% from the nominal value, which proved the desired stability of the analyte during storage in autosampler.

All plasma samples for stability evaluation were prepared as described in Section 2. To verify reliability of the method, the measured concentrations should not differ by more than 15% from the nominal value. All results of stability tests implied good stability of QUE over all steps of determination; therefore the method was proved to be applicable for routine analyses.

3.5. Pharmacokinetic study

Fig. 5 shows the pharmacokinetic profile obtained after 36 h dose-titration period comprising three doses of 25 mg quetiapine followed by a single dose administration of 100 mg. The limit of quantification (1.0 ng/mL) was low enough to assess QUE over 30 h after the drug administration.

According to the literature the pharmacokinetic parameters of quetiapine are linear [2] and the results obtained at steady states suggest that they are time- and dose-independent up to 400 mg twice a day [4].

In our study the peak concentration C_{\max} geometric mean was evaluated to be 213.4 $\mu\text{g/L}$ ranging from 88.7 to 459.7 $\mu\text{g/L}$. These values are lower than the value of $391 \pm 59.4 \mu\text{g/L}$ published in adolescent patients at steady state [15]. The $\text{AUC}_{0-\infty}$ geometric mean value was found to be 813.2 $\mu\text{g L/h}$ ranging

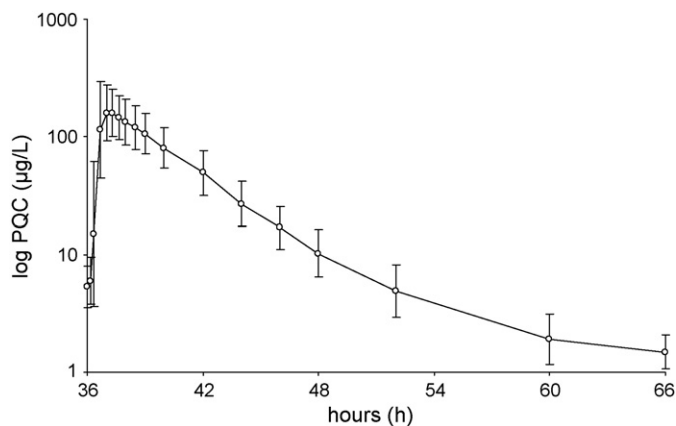


Fig. 5. Log geometric means of plasma quetiapine concentration—log PQC ($\mu\text{g/L}$) vs. time curve following a single oral 100 mg dose of quetiapine subsequent to a titration dose of $3 \text{ mg} \times 25 \text{ mg}$ in 32 subjects.

between 415.5 and 1686.9 $\mu\text{g L/h}$ after 100 mg QUE administration, which is lower than the published mean values at steady state during dosing interval ($1322.6 \pm 223.0 \mu\text{g L/h}$) [15] but the ranges of values found in literature overlap. Moreover, the group of patients was considerably smaller than ours, younger, and possibly with lower lean body weight. The results in patients could also be influenced by concomitant medication.

The median value of quetiapine t_{\max} found was 1.00 h (0.33–3.00 h). These values fit well with the published range of 0.5–2.0 [15]. The median elimination half-life was calculated to be 4.75 h (range from 2.69 to 7.99). These values are in good agreement with values of about 6–7 h seen with a clinical dosing range of 250 mg and higher [2,4].

4. Conclusion

The method for the determination of quetiapine in human Na_2EDTA plasma covering the concentration range 1.0–382.2 ng/mL, using 0.5 mL of plasma was proposed and validated. No interferences from endogenous plasma components or other sources were found and no “cross-talk” was observed in plasma samples. The assay showed good precision and accuracy. A simple preparation procedure and short retention time could allow determination of more than 250 samples per day.

The analytical method presented here has been proved useful for the investigation of the characteristics of QUE in human plasma in pharmacokinetic studies.

References

- [1] The Complete Drug Reference (Monograph on CD-ROM), Pharmaceutical Press, London, 2003.
- [2] Seroquel™, Summary of Product Characteristics, vol. 23, AstraZeneca UK Limited, 2003.
- [3] http://www.rxlist.com/cgi/generic2/quetiap_pi.htm, 2003, p. 31.
- [4] C.L. DeVane, C.B. Nemeroff, Clin. Pharmacokinet. 40 (2001) 509–522.
- [5] P.T. Thyrum, Y.W. Wong, C. Yeh, Prog. Neuropsychopharmacol. Biol. Psychiatry 24 (2000) 521–533.
- [6] E. Diletti, D. Hauschke, V.W. Steijns, Int. J. Clin. Pharmacol. Ther. Toxicol. 29 (1991) 1–8.

- [7] P.C. Davis, J. Wong, O. Gefvert, *J. Pharm. Biomed. Anal.* 20 (1999) 271–282.
- [8] R. Mandrioli, S. Fanali, A. Ferranti, M.A. Raggi, *J. Pharm. Biomed. Anal.* 30 (2002) 969–977.
- [9] C. Frahnert, M.L. Rao, K. Grasmaeder, *J. Chromatogr. B* 794 (2003) 35–47.
- [10] J. Hasselstrom, K. Linet, *J. Chromatogr. B* 798 (2003) 9–16.
- [11] D.T. Anderson, K.L. Fritz, *J. Anal. Toxicol.* 24 (2000) 300–304.
- [12] A. Pelander, I. Ojanpera, J. Sistonon, I. Rasenen, E. Vuori, *J. Anal. Toxicol.* 27 (2003) 226–232.
- [13] Z. Zhou, X. Li, K. Li, Z. Xie, Z. Cheng, W. Peng, F. Wang, R. Zhu, H. Li, *J. Chromatogr. B* 802 (2004) 257–262.
- [14] U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Veterinary Medicine, Guideline No. 135, Validation of Analytical Procedures for Type C Medicated Feeds, November 7, 2005.
- [15] B.J. McConville, L.A. Arvanitis, P.T. Thyrum, C. Yeh, L.A. Wilkinson, R.O. Chaney, K.D. Foster, M.T. Sorter, L.M. Friedman, K.L. Brown, J.E. Heubi, *J. Clin. Psychiatry* 61 (2000) 252–260.