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ORIGINAL RESEARCH

# Sensitive liquid chromatography tandem mass spectrometry method for the quantification of Quetiapine in plasma

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ABSTRACT: A sensitive high-performance liquid chromatography–tandem mass spectrometry method was developed and validated for the quantification of quetiapine in rat plasma. Following liquid–liquid extraction, the analyte was separated using a gradient mobile phase on a reverse-phase column and analyzed by MS/MS in the multiple reaction monitoring mode using the respective $[M + H]^+$ ions, *m/z* 384 to *m/z* 221 for quetiapine and *m/z* 327 to *m/z* 270 for the internal standard. The assay exhibited a linear dynamic range of 0.25–500 ng/mL for quetiapine in rat plasma. The lower limit of quantification was 0.25 ng/mL with a relative standard deviation of less than 7%. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. The validated method was successfully used to analyze rat plasma samples for application in pre-clinical pharmacokinetic studies. This method in rodent plasma could be adapted for quetiapine assay in human plasma. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: quetiapine; liquid chromatography–tandem mass spectrometry; rat plasma

## INTRODUCTION

Quetiapine fumarate (Seroquel®) is a relatively new medication, released by AstraZeneca Pharmaceuticals in 1993. Quetiapine is an atypical antipsychotic medication indicated for the treatment of schizophrenia, and for the treatment of acute manic episodes associated with bipolar disorder (Burns, 2001). Recently quetiapine is also approved by the Food and Drug Administration (FDA) in October 2006 for the treatment of patients with depressive episodes associated with bipolar disorder (Seroquel, 2008). Seroquel is now the first and only single medication approved by the FDA to treat both depressive and manic episodes associated with bipolar disorder.

Quetiapine is a dibenzothiazepine derivative and is structurally similar to the prototype atypical antipsychotic, clozapine. It has been proposed that the quetiapine therapeutic activity in schizophrenia and its mood stabilizing properties in bipolar depression and mania are mediated through a combination of dopamine type 2 ($D_2$) and serotonin type 2 ($5HT_2$) antagonism (Cheer and Wagstuff, 2004).

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Quetiapine undergoes extensive metabolism that results in very low plasma concentrations (ng/mL levels), but the activity is primarily attributed to parent drug. The major *in-vivo* metabolites of quetiapine are quetiapine sulfoxide and the parent acid metabolite; both metabolites are pharmacologically inactive. 7-Hydroxy-quetiapine and 7-hydroxy-*N*-desalkyl-quetiapine are active metabolites, but with relatively low concentrations (<5%) in blood (DeVane and Nemeroff, 2001). Therefore quantification of quetiapine concentrations in plasma requires a bioanalytical method with high sensitivity (low ng/mL).

The bioanalytical component of a pharmacokinetic study requires a drug assay with simplicity, high sensitivity, selectivity, small volume requirements and rapid turnaround time. Several HPLC methods for the quantification of quetiapine in plasma have been reported (Davis *et al.*, 1999; Hasselstrom and Linnet, 2003; Mandrioli *et al.*, 2002; Mercolini *et al.*, 2007; Sachse *et al.*, 2006; Saracino *et al.*, 2006). However none of these methods is sensitive enough for quantification of low drug levels and some of them are time-consuming and require complex sample pretreatment or long chromatographic run times (Hasselstrom and Linnet, 2003). Gas chromatography–mass spectrometry methods have also been reported, but quetiapine needs to be derivatized before analysis (Anderson and Fritz, 2000; Flammia *et al.*, 2006; Pullen *et al.*, 1992). Generally for

analysis of large numbers of plasma samples, the derivatization step increases the time of sample preparation and the cost of the method.

Quantification of drugs in biological matrices by liquid chromatography/tandem mass spectrometry (LC-MS/MS) is becoming more common due to the improved sensitivity and selectivity of this technique. Recently few LC-MS/MS methods have been reported for quantification of quetiapine (Barrett *et al.*, 2007; Hasselstrom and Linnet, 2003; Li *et al.*, 2004; Lin *et al.*, 2004; Zhou *et al.*, 2004). Zhou *et al.* (2004) reported an LC-MS/MS method for simultaneous determination of clozapine, olanzapine, risperidone and quetiapine in 0.5 mL plasma with an lower limit of quantification (LLOQ) of 20 ng/mL. Hasselstrom and Linnet (2003) compared HPLC methods with ultraviolet and MS/MS detection. Although the sample preparation is fully automated, the runtime is 35 min. An LC-MS method was described for simultaneous determination of quetiapine and its sulfoxide-, 7-hydroxy-, 7-hydroxy-*N*-dealkyl-metabolites in human plasma (Li *et al.*, 2004). The calibration curve was linear with an LLOQ of 10 ng/mL. Lin *et al.* (2004) described an LC-MS/MS method for quantification of quetiapine in human plasma and liver microsomes. The method was validated over a concentration range of 1–500 ng/mL with LLOQ of 1 ng/mL. Recently Barrett *et al.* (2007) reported a validated LC-MS/MS method over a concentration range of 1–382.2 ng/mL using a solid-phase extraction procedure. According to the literature, a quetiapine concentration between 1.5 and 350 ng/mL (Diletti *et al.*, 1991; Thyrum *et al.*, 2000) in human plasma could be expected after the administration of a 100 mg quetiapine dose.

The purpose of the present investigation was to explore the high selectivity and sensitivity of a triple-quadrupole MS system operated in MS-MS mode with an electrospray interface for the development and validation of a robust reversed-phase LC-MS/MS method in multiple-reaction monitoring (MRM) mode for the quantification of quetiapine in rat plasma. It was essential to establish a method capable of quantifying quetiapine at concentrations down to 0.25 ng/mL. We believe that development of a method in rodent plasma would facilitate the ease of adaptability of quetiapine assay in human plasma.

## EXPERIMENTAL

**Chemicals.** Quetiapine fumarate and clozapine hydrochloride (internal standard, IS) were obtained from the R&D department of Suven Life Sciences Ltd (Hyderabad, India). Gradient-grade LiChrosolv acetonitrile and gradient-grade LiChrosolv methanol were purchased from Merck (Darmstadt, Germany). Diethyl ether, dichloromethane, ammonium acetate and *ortho*-phosphoric acid were purchased from Merck (Worli, Mumbai, India). Ultrapure type-1 water from Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

**LC-MS/MS instrument and conditions.** The HPLC SIL HTC system (Shimadzu Corporation, Kyoto, Japan) is equipped with LC-AD VP binary pump, a DGU20A5 degasser and a SIL-HTC auto sampler equipped with a CTO-10AS VP thermostated column oven. The chromatography was performed using Zorbax $C_8$, 50 × 4.6 mm at 30°C temperature. The analyte was eluted using a gradient mobile phase system consisting of mobile phase A (10 mM ammonium acetate) and mobile phase B (acetonitrile). After injection, a combination of 90% mobile phase A and 10% mobile phase B was held for 0.5 min, and then up to 2 min a linear change to 20% A and 80% B was performed. Then immediately the combination was reversed back to 90% A and 10% B and held for 5 min. The flow rate was 0.25 mL/min.

Mass spectrometric detection was performed on an API 4000 Q TRAP triple quadrupole instrument (MDS-SCIEX, Concord, Ontario, Canada) using MRM. A turboionspray interface operating in positive ionization mode was used. Typical source conditions were as follows: the turbo-gas temperature was set at 350°C, and the ion spray needle voltage was adjusted to 5500 V. The mass spectrometer was operated at unit resolution for both Q1 and Q3 in the MRM mode, with a dwell time of 200 ms per MRM channel. $MS^3$ analysis was performed through isolation of the desired precursor ion using a correlated sweep. AF2 (auxillary frequency/excitation energy) was ramped from 0 to 200 mV to find the most probable fragmentation pattern of the compound. The precursor/product ion pairs monitored were *m/z* 384 to *m/z* 221 for quetiapine and *m/z* 327 to *m/z* 270 for the IS. GS1 and GS2 were at 20 and 25 (arbitrary units), respectively. Collision gas, curtain gas and declustering potential were set at 6, 25 and 95 (arbitrary units), respectively. The collision energy, collision cell exit potential and entrance potential were set at 51, 5 and 9 for quetiapine and at 31, 7 and 9 for IS, respectively. Data acquisition was performed with analyst 1.4.1 software (MDS-SCIEX, Concord, Ontario, Canada).

**Sample preparation.** Standard stock solutions of quetiapine (1 mg/mL) and the IS (1 mg/mL) were separately prepared in methanol. Working solutions for calibration and controls were prepared by appropriate dilution in water–methanol (50:50, v/v; diluent). The IS working solution (1 μg/mL) was prepared by diluting its stock solution with diluent. Working solutions (100 μL) were added to drug-free rat plasma (900 μL) in bulk, to obtain quetiapine concentration levels of 0.25, 0.5, 1, 2, 5, 10, 50, 100 and 500 ng/mL as a single batch at each concentration. Quality control (QC) samples were also prepared in bulk on an independent weighing of standard drug, at concentrations of 0.25 (LLOQ), 0.75 (low), 200 (medium) and 400 ng/mL (high) as a single batch at each concentration. The calibration and control bulk samples were divided into aliquots in microcentrifuge tubes (Tarson, 1.5 mL) and stored in the freezer at below −50°C until analysis.

A plasma sample (100 μL) was pipetted into a 15 mL glass tube and then 10 μL of IS working solution (1 μg/mL) and 100 μL of 0.1% *ortho*-phosphoric acid were added. After vortex mixing for 10 s, 2.5 mL aliquot of the extraction solvent, diethyl ether:dichloromethane (70:30, v/v), was added and the



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sample was vortex-mixed for 3 min. The organic layer (2 mL) was transferred to a glass tube and evaporated to dryness using an evaporator at 40°C under a stream of nitrogen. Then the dried extract was reconstituted in 250 μL of reconstitution solvent (10 mM ammonium acetate:acetonitrile, 20:80) and a 10 μL aliquot was injected into the chromatographic system.

**Bioanalytical method validation.** A calibration curve was constructed from a blank sample (a plasma sample processed without the IS), a zero sample (a plasma processed with the IS) and nine non-zero samples covering the total range 0.25–500 ng/mL, including the LLOQ. The calibration curves were generated using the analyte to IS peak area ratios by weighted ($1/x^2$) least-squares linear regression on consecutive days. The acceptance criterion for a calibration curve was a correlation coefficient ($r$) of 0.99 or better, and that each back-calculated standard concentration must be within 15% deviation from the nominal value except at the LLOQ, for which the maximum acceptable deviation was set at 20%. At least 67% of non-zero standards were required to meet the above criteria, including acceptable LLOQ and upper limit of quantification.

The within-batch precision and accuracy were determined by analyzing three sets of QC samples (LLOQ, low, medium and high concentrations), each comprising five replicates in a batch. The between-batch precision and accuracy were determined by analyzing such five different batches. The acceptance criteria for within- and between-batch precision were 20% or better for LLOQ and 15% or better for the other concentrations, and the accuracy was 100 ± 20% or better for LLOQ and 100 ± 15% or better for the other concentrations.

Recovery of quetiapine from the extraction procedure was determined by a comparison of the peak area of quetiapine in spiked plasma samples (six each of low, medium and high QCs) with the peak area of quetiapine in samples prepared by spiking extracted drug-free plasma samples with the same amounts of quetiapine at the step immediately prior to chromatography. Similarly, recovery of IS was determined by comparing the mean peak areas of extracted QC samples ($n$ = 6) to mean peak areas of IS in samples prepared by spiking extracted drug-free plasma samples with the same amounts of IS at the step immediately prior to chromatography.

The stability of the analyte and IS in rat plasma under different temperature and timing conditions, as well as their stability in the stock solutions, was evaluated. QC samples were subjected to short-term room temperature conditions, to long-term storage conditions (–50°C), and to freeze–thaw stability studies. All the stability studies were conducted at two concentration levels (0.75 and 400 ng/mL as low and high values) with six replicates for each.

## RESULTS AND DISCUSSION

### Mass spectrometry and method development

In order to develop a method with the desired LLOQ (0.25 ng/mL), it was necessary to use MS/MS detection, as MS/MS methods provide improved limit of detection and selectivity for trace-mixture analysis. The inherent selectivity of MS/MS detection was also expected to be beneficial in developing a selective and sensitive method. $[M + H]^+$ was the predominant ion in the Q1 spectrum and was used as the precursor ion to obtain product ion spectra (Fig. 1). The characteristic product ions $m/z$ 279, 253, 247, 221, 210 and 158 are obtained from precursor ions of quetiapine. The most intense ion at $m/z$ 253 was selected as mass transition from $m/z$ 384 for quetiapine. With this transition we only obtain an LLOQ of 1 ng/mL (Fig. 2). To develop a more sensitive method, the mass spectrometric parameters were optimized. The product ion $m/z$ 221 was intense with increasing the collision energy from 32 to 51 mV and mass transition from $m/z$ 384 to $m/z$ 221 was chosen to obtain a sensitive method with an LLOQ of 0.25 ng/mL. Figure 2 shows the signal-to-noise ratios obtained at LLOQ for the transition (a) $m/z$ 384 to $m/z$ 253 (LLOQ = 1 ng/mL) and (b) $m/z$ 384 to $m/z$ 221 (LLOQ = 0.25 ng/mL).

The fragmentation of quetiapine was studied with collision-induced dissociation (CID) as a function of collision energy by using nitrogen as collision gas. The cleavage of the piperazine ring takes place relatively easily, which is similar in appearance to that obtained by Nuutinen *et al.* (2001). The proposed fragmentation pathways for the fragment ions formed from quetiapine are shown in Scheme 1. The lowest energy fragmentation was the loss of 131 Da from the $[M + H]^+$ ion, which requires extensive rearrangement in combination with the cleavage of the piperazine ring. The formation of the $[M + H - 105]^+$ ion can be rationalized in terms of the breaking of two C–N bonds at the ethoxyethanol group attached piperazine nitrogen. With increased collision energy the ion at $m/z$ 253 showed a loss of 43 Da to give the production ion at $m/z$ 210, as well as an easy loss of S atom to produce ions at $m/z$ 221. Similarly the ion at $m/z$ 279 showed a facile loss of S atom to give the production ion at $m/z$ 247 and no other significant fragmentation channels were observed. $MS^3$ analysis confirms the formation of these ions from the respective precursor ions (Fig. 3). Loss of S usually is diagnostic of sulfur heterocycles (Garin *et al.*, 2003). The dissociation products at $m/z$ 221 and $m/z$ 247 are very stable, because both of them cannot dissociate into small pieces. This observation could be rationalized by the formation of a very stable conjugated system (Scheme 1).

The characteristic product ions of clozapine are $m/z$ 296, 270, 227, 192 and 84 (Fig. 1). Similar to quetiapine, the product ions $m/z$ 270 and ion $m/z$ 296 are formed with extensive rearrangement in combination with the cleavage of the piperazine ring (Scheme 2). Unlike quetiapine, the ions $m/z$ 227 and $m/z$ 192 are formed directly from the protonated clozapine but not from the ion $m/z$ 270 ($MS^3$ analysis data not shown). The product ion mass spectra and their proposed rationalizations in terms of fragmentation patterns of quetiapine and IS



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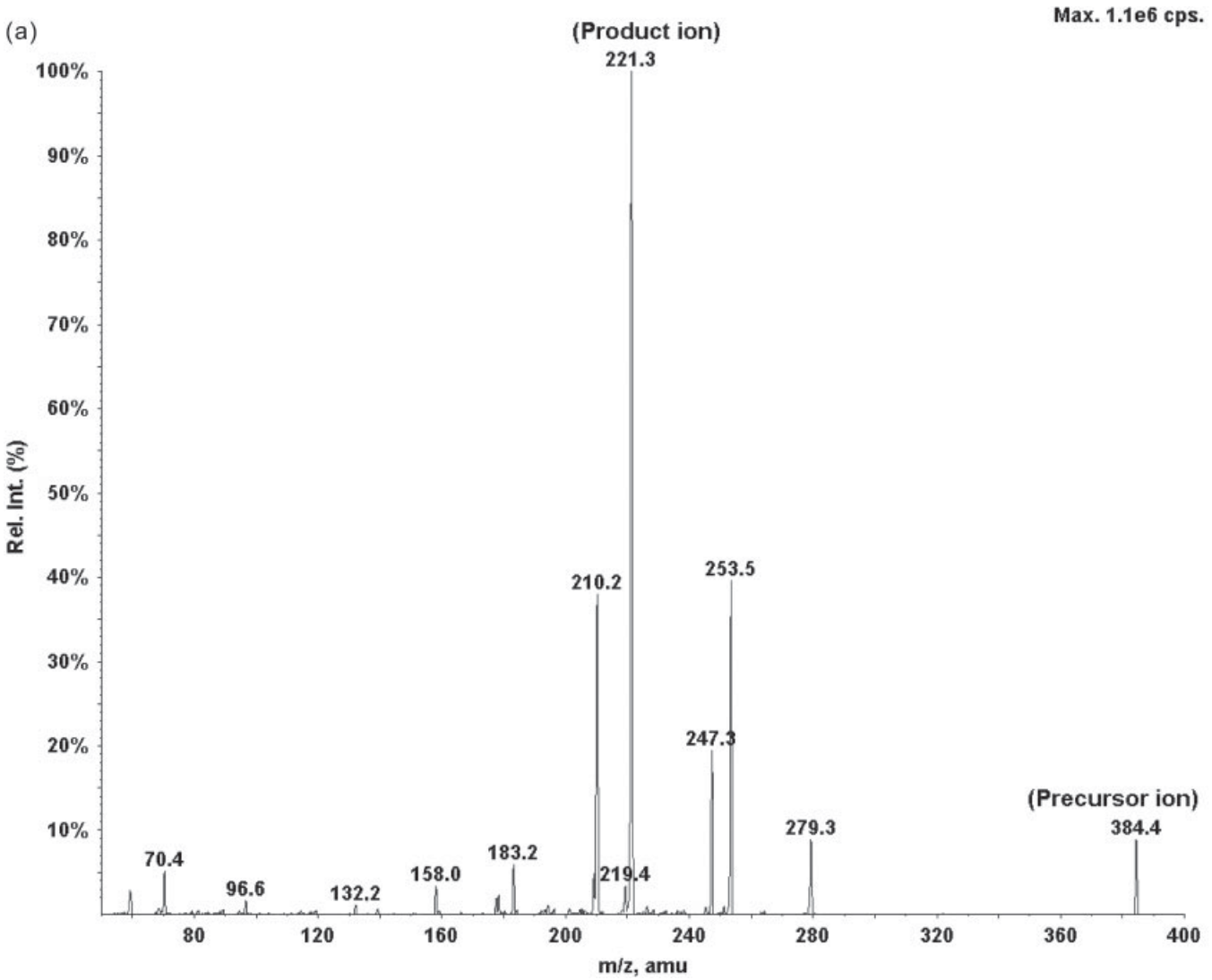


**Figure 1.** ESI/MS$^2$ spectrum of (a) protonated quetiapine (*m/z* 384) and (b) protonated clozapine (internal standard) (*m/z* 327).

are illustrated in Fig. 1 and Schemes 1 and 2. The most sensitive mass transition was from *m/z* 384 to 221 for quetiapine and from *m/z* 327 to 270 for the IS.

Liquid–liquid extraction (LLE) was used for the sample preparation in this work. LLE can be helpful in producing a spectroscopically clean sample and avoiding the introduction of non-volatile materials onto the column and MS system. Clean samples are essential for minimizing ion suppression and matrix effect in LC-MS/MS analyses. Diethyl ether:dichloromethane (70:30) was found to be optimal, which can produce a clean chromatogram for a blank plasma sample. The average absolute recovery of quetiapine from spiked plasma samples was 83.3 ± 2.4 and the recovery of the IS was 69.9 ± 2.8% at the concentration used in the assay (1 μg/mL). Recoveries of the analyte and IS were good, and were consistent, precise and reproducible. The assay has proved to be robust in high-throughput bioanalysis.

A good internal standard should mimic the analyte in the entire sample extraction, chromatographic elution and mass spectrometric detection. It should track the analyte during the extraction and compensate for any potential recovery inconsistency. It will elute together with the analyte on the column and compensate for any potential inconsistent response due to matrix effects. It will not cause interference to the analyte and vice versa. Stable isotope internal standards (deuterated and $C_{13}$ labeled analyte) are ideal candidates for meeting the above criteria. However, isotopes are not always easily accessible to bioanalysis laboratories due to the prohibitively high cost or the technical difficulty in synthesizing them. Several compounds were investigated to find a suitable IS, and finally clozapine, a commercially



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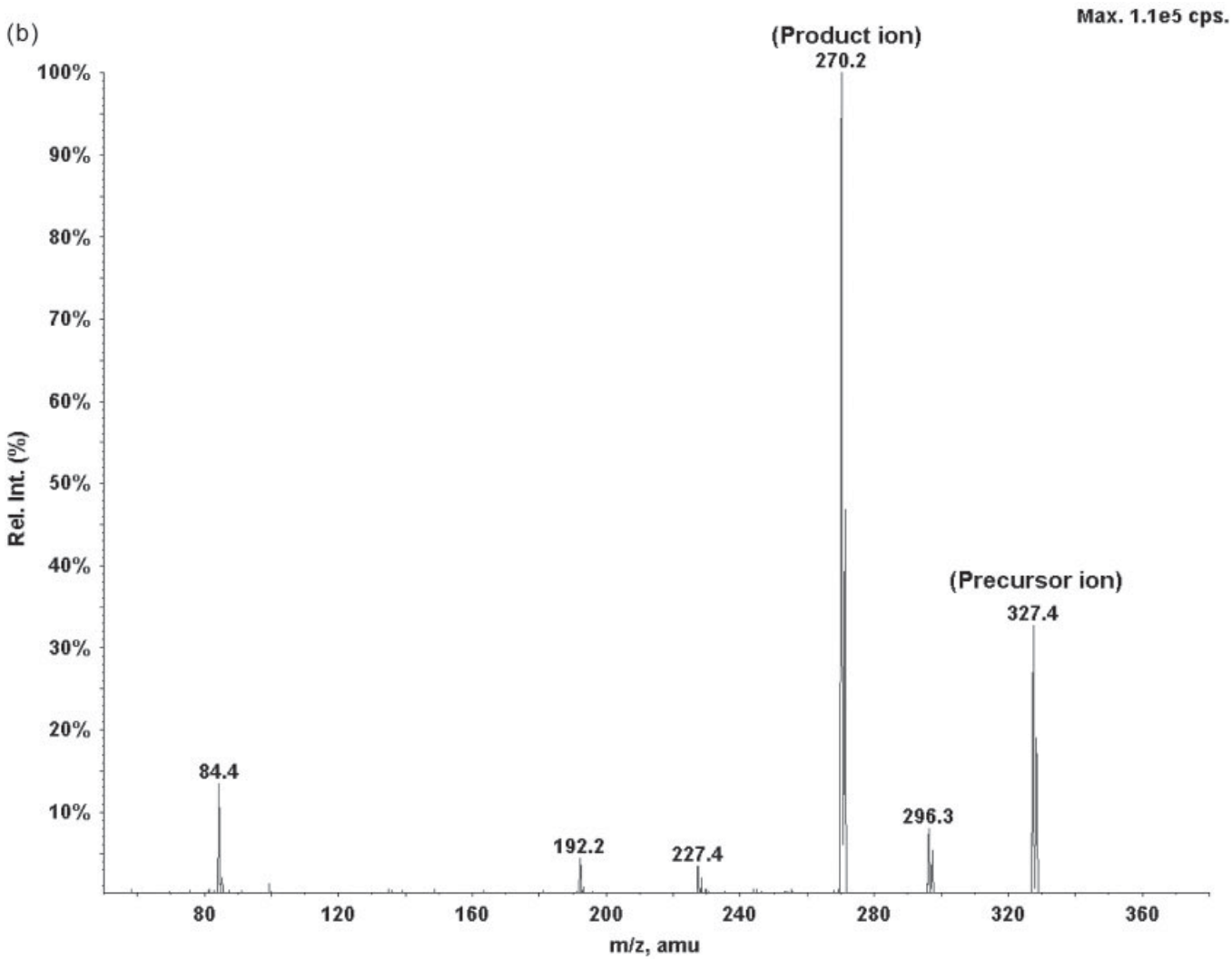


**Figure 1.** (*Continued*)

available and structurally similar compound, was found to be suitable. Clean chromatograms were obtained and no significant direct interferences in the MRM channels at the relevant retention times were observed. However, in ESI, signal suppression or enhancement may occur due to co-eluting endogenous components from the sample matrix. The importance of including the evaluation of matrix effect in any LC-MS/MS method is outlined in an excellent paper by Matuszewski *et al.* (2003). Their data strongly emphasize the need to use a blank matrix from (at least five) different sources/individuals instead of using one blank matrix pool to determine method precision and accuracy. Therefore all validation experiments in this assay were performed with matrixes obtained from different individuals. In addition, validation experiments were performed using hemolytic and strongly lipemic matrixes. As all data fall within the guidelines, we conclude that the degree of matrix effect was sufficiently low to produce acceptable data, and the method can be considered as valid.

## Assay performance and validation

The nine-point calibration curve was linear over the concentration range 0.25–500 ng/mL. The calibration model was selected based on the analysis of the data by linear regression with/without intercepts and weighting factors ($1/x$, $1/x^2$ and none). The best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x^2$ weighting factor, giving a mean linear regression equation for the calibration curve of: $y = 0.00207\ (\pm\ 0.0023)x + 0.00008\ (\pm\ 0.00001)$, where $y$ is the peak area ratio of the analyte to the IS and $x$ is the concentration of the analyte. The mean correlation coefficient of the weighted calibration curve generated during the validation was $0.9991 \pm 0.0005$.

The selectivity of the method was examined by analyzing ($n = 6$) blank rat plasma extract [Fig. 4(a)] and an extract spiked only with the IS [Fig. 4(b)]. As shown in Fig. 4(a), no significant direct interference in the blank plasma traces was observed from endogenous



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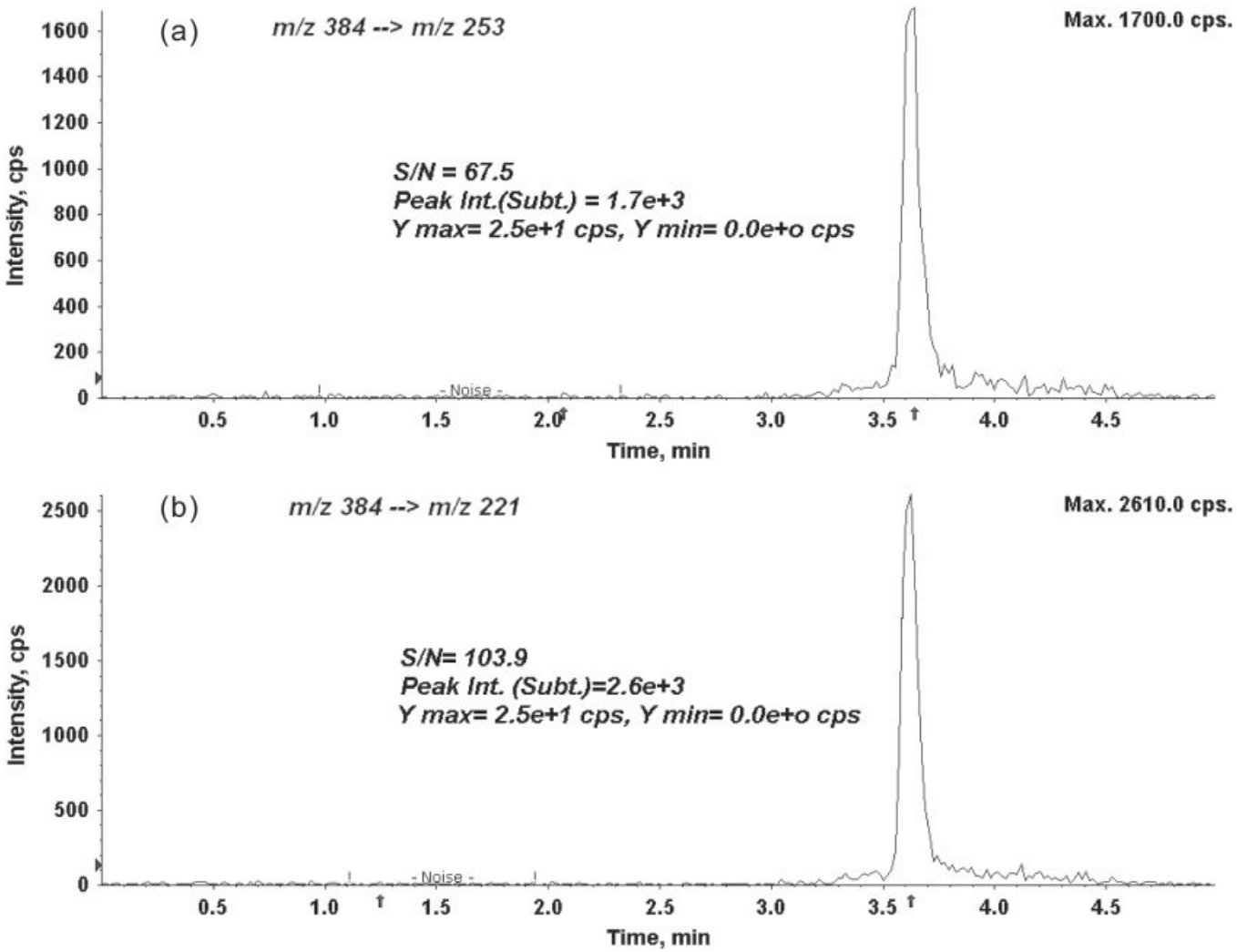


**Figure 2.** A representative chromatogram showing the sensitivity obtained for quetiapine using transition (a) from *m/z* 384 to 253 (collision energy [CE] = 32 mV) and (b) from *m/z* 384 to 221 (CE = 51 mV).

substances in drug-free rat plasma at the retention time of the analyte. Similarly, Fig. 4(b) shows the absence of direct interference from the IS to the MRM channel of the analyte. Figure 4(c) depicts a representative ion-chromatogram for the LLOQ (0.25 ng/mL). Excellent sensitivity was observed for a 10 µL injection volume; the LLOQ corresponds to *ca* 10 pg on-column. A representative MRM chromatogram resulting from analysis of a rat plasma sample after the administration of a 30 mg/kg subcutaneous dose of quetiapine is shown in Fig. 4(d).

The LLOQ was defined as the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision and was found to be 0.25 ng/mL in rat plasma. The mean response for the analyte peak at the assay sensitivity limit (0.25 ng/mL) was >10-fold greater than the mean response for the peak in eight blank rat plasma samples at the retention time of the analyte. The between-batch precision at the LLOQ was 5.9%, and the between-batch accuracy was 104.1% (Table 1). The within-batch precision was 6.1% and the accuracy was 99.8% for quetiapine.

The lower and upper quantification levels of quetiapine ranged were 0.75 and 400 ng/mL in rat plasma. For the between-batch experiments the precision ranged from 2.2 to 5.9% and the accuracy from 99.2 to 104.1% (Table 1). For the within-batch experiments the precision and accuracy for the analyte met the acceptance criteria (<±15%).

## Stability studies

For short-term stability determination, stored plasma aliquots were thawed and kept at room temperature for a period of time exceeding that expected to be encountered during routine sample preparation (around 24 h). Samples were extracted and analyzed as described above and the results indicate reliable stability behavior under the experimental conditions of the regular analytical



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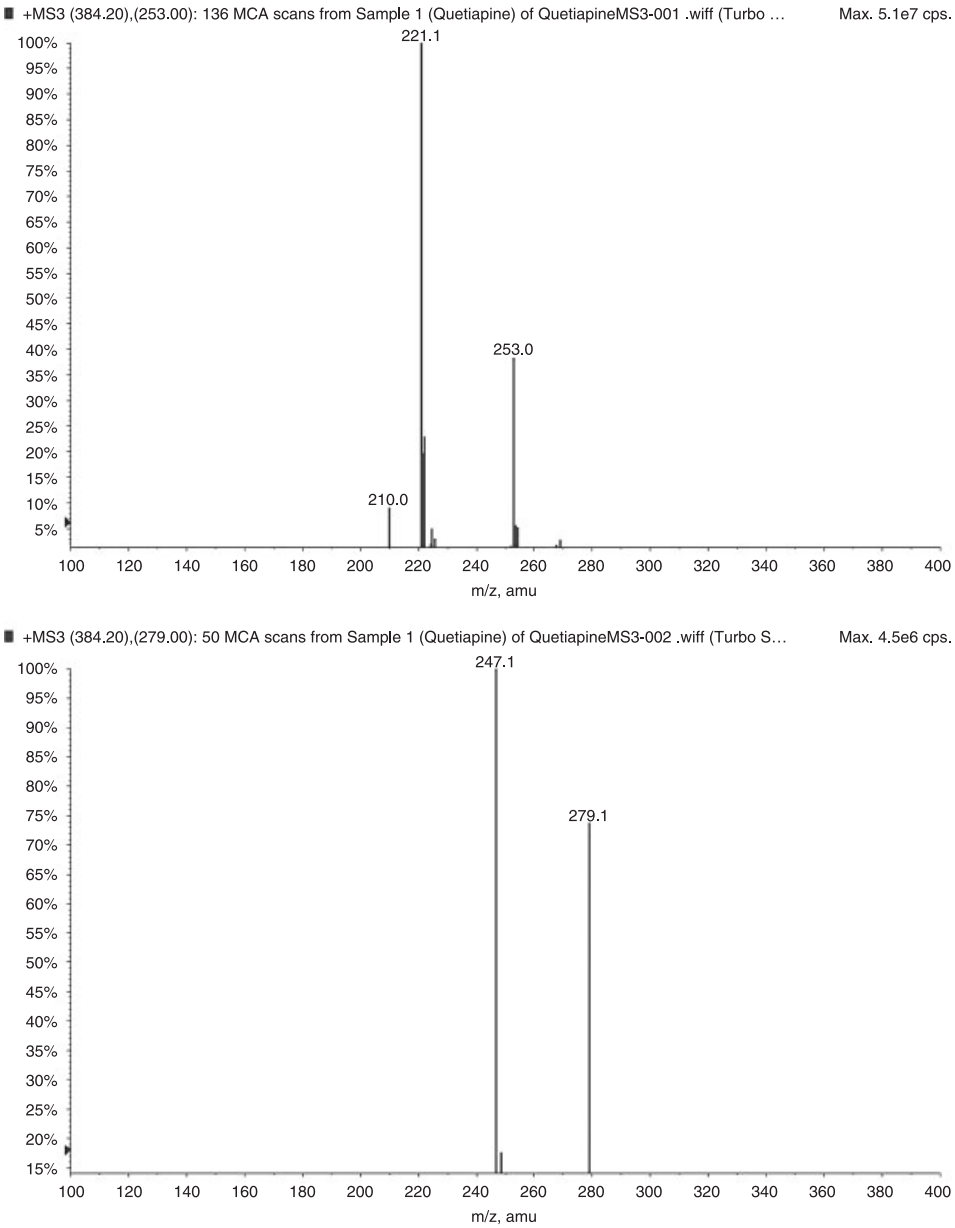


**Figure 3.** ESI/MS$^3$ spectrum of (a) *m/z* 253 and (b) *m/z* 279 derived from protonated quetiapine (*m/z* 384).

**Table 1. Precision and accuracy of the method for determining quetiapine concentrations in plasma samples**

| | Within-batch ($n$ = 3) | | | Between-batch ($n$ = 5) | | |
|---|---|---|---|---|---|---|
| Concentration added (ng/mL) | Concentration found (mean ± SD; ng/mL) | Precision (%) | Accuracy (%) | Concentration found (mean ± SD; ng/mL) | Precision (%) | Accuracy (%) |
| 0.25 | 0.25 ± 0.05 | 6.1 | 99.8 | 0.25 ± 0.08 | 5.9 | 104.1 |
| 0.75 | 0.75 ± 0.08 | 4.8 | 98.9 | 0.75 ± 0.07 | 4.6 | 102.5 |
| 200 | 201.5 ± 3.8 | 3.8 | 102.9 | 202.3 ± 3.4 | 2.5 | 102.5 |
| 400 | 400.5 ± 5.6 | 2.8 | 100.2 | 395.6 ± 1.9 | 2.2 | 99.2 |



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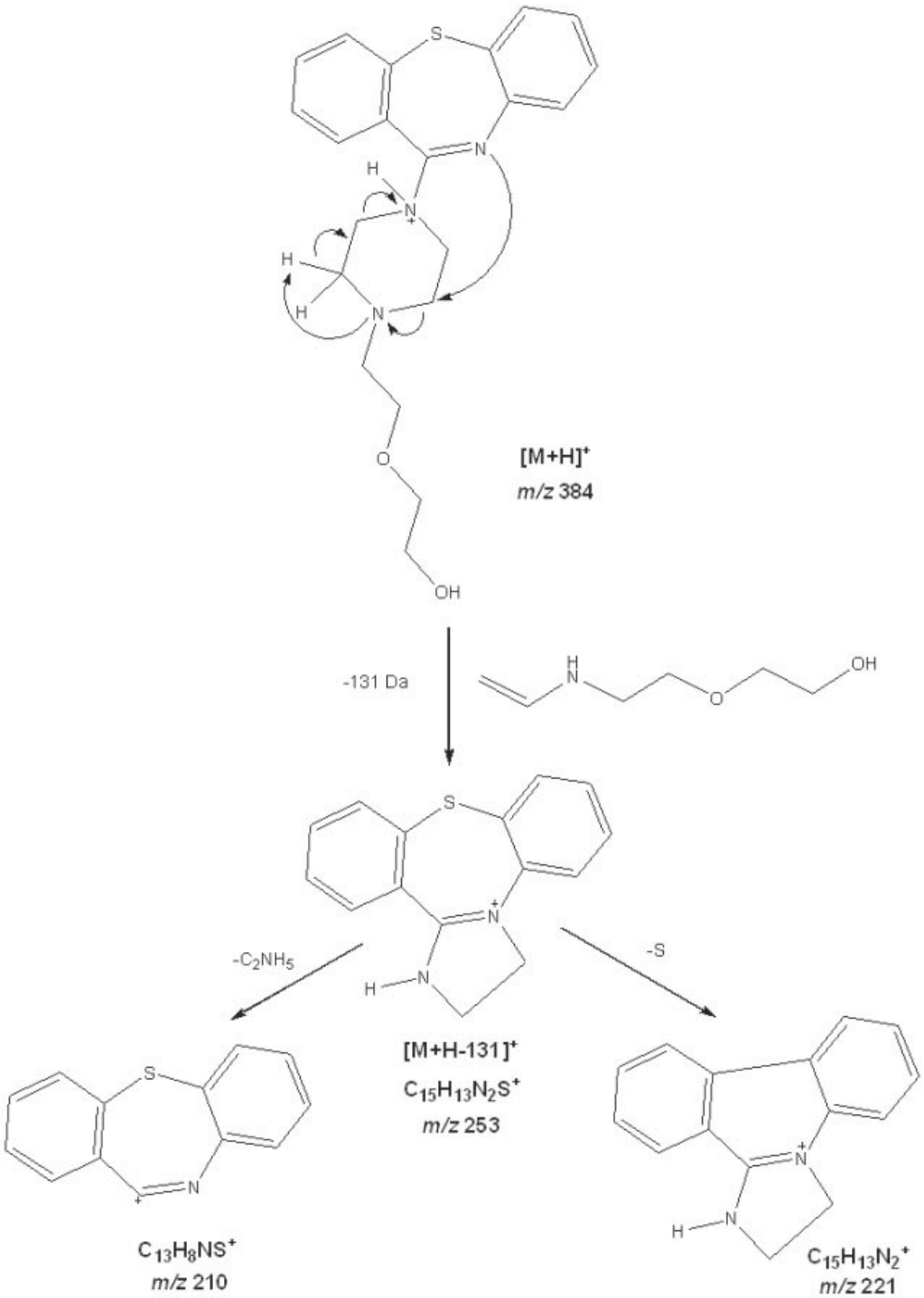


**Scheme 1.** Proposed pathways of quetiapine dissociation product ions at *m/z* 279, 253, 247, 221 and 210.

procedure. The stability of QC samples kept in the autosampler for 22 h was also assessed. The results indicate that solutions of the analyte and the IS can remain in the autosampler for at least 22 h without showing significant loss in the quantified values, indicating that samples should be processed within this period of time.

The stability data of the analyte in plasma over three freeze–thaw cycles indicates that the analyte is stable in rat plasma for three freeze–thaw cycles, when stored at below –50°C and thawed to room temperature.

The long-term stability data of the analyte in rat plasma stored for a period of 30 days at below –50°C showed reliable stability behavior, as the means of the results of



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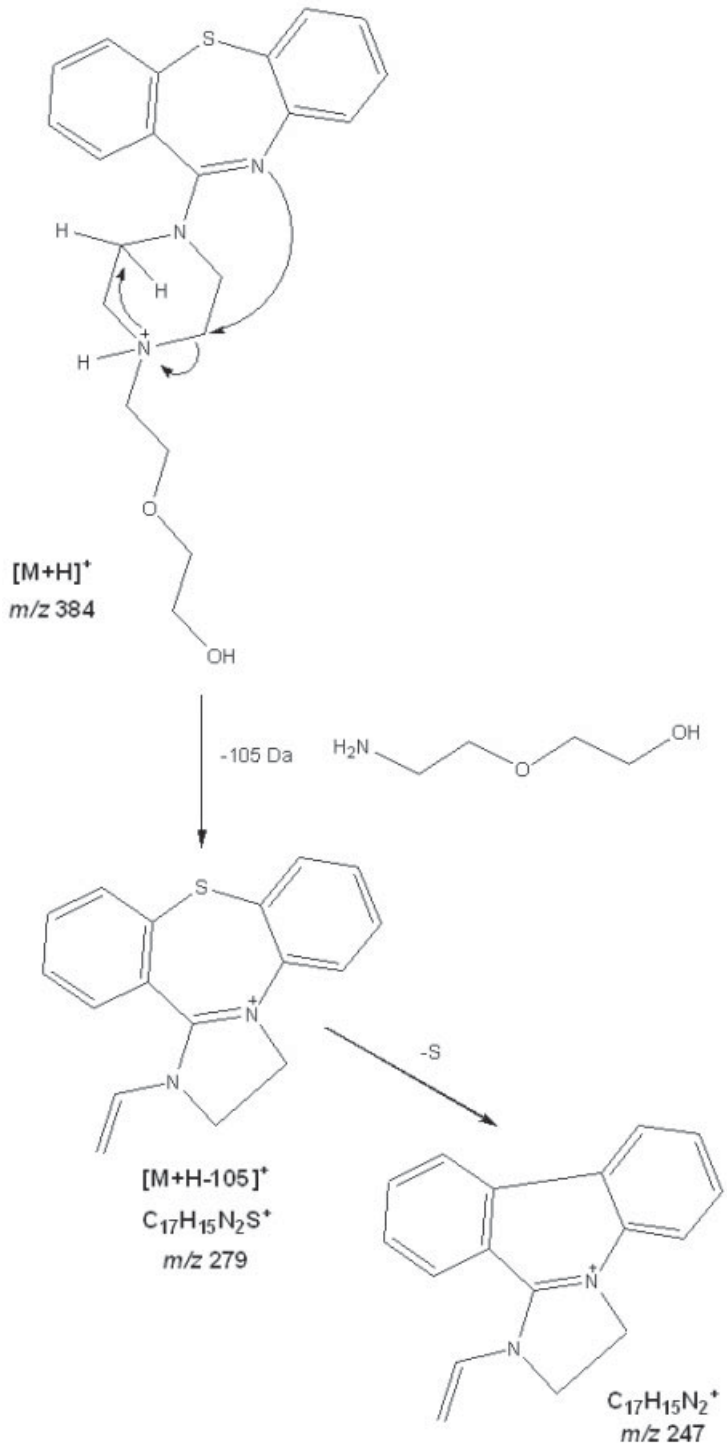


**Scheme 1.** (*Continued*)

the tested samples were within the acceptance criteria of ±15% of the initial values of the controls. These findings indicate that storage of the analyte in plasma samples at below –50°C is adequate, and no stability-related problems are expected during routine analyses for pharmacokinetic studies.

The stability of the stock solutions was tested and established at room temperature for 6 and 26 h, and under refrigeration (~4°C) for 30 days (data not shown). The results revealed optimum stability for the prepared stock solutions throughout the period intended for their daily use.

## Application

The method was applied to determine the plasma concentration of quetiapine following a single 30 mg/kg



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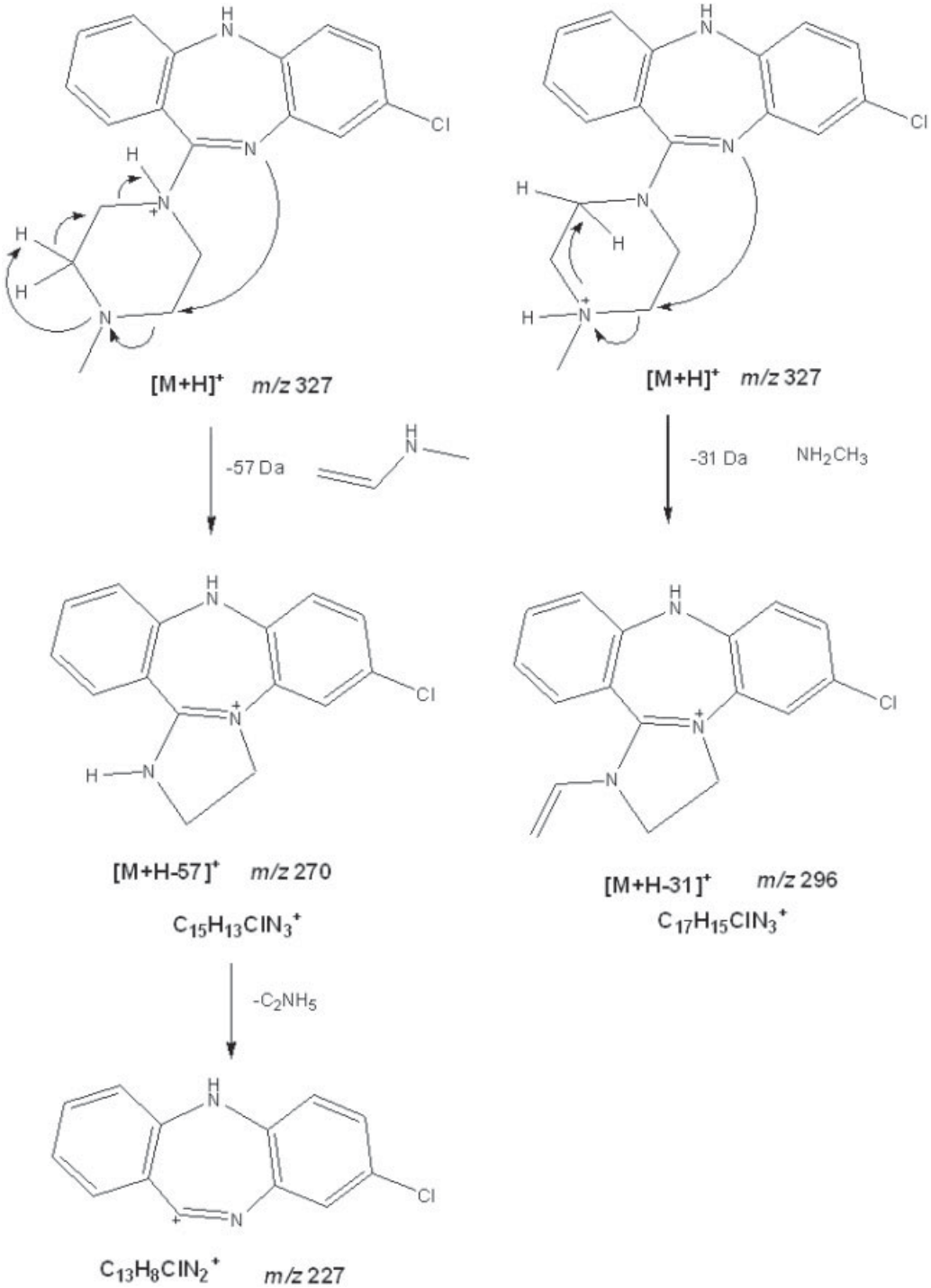


**Scheme 2.** Proposed pathways of clozapine (IS) dissociation product ions at *m/z* 296, 270, 227 and 192.

subcutaneous dose administration to Wistar rats. A representative MRM chromatogram resulting from analysis of a rat plasma sample after the administration of a 30 mg/kg subcutaneous dose of quetiapine is shown in Fig. 4(d).

## CONCLUSIONS

In summary, a method is described for the quantification of quetiapine in rat plasma by LC-MS/MS in positive electrospray ionization mode using multiple reaction



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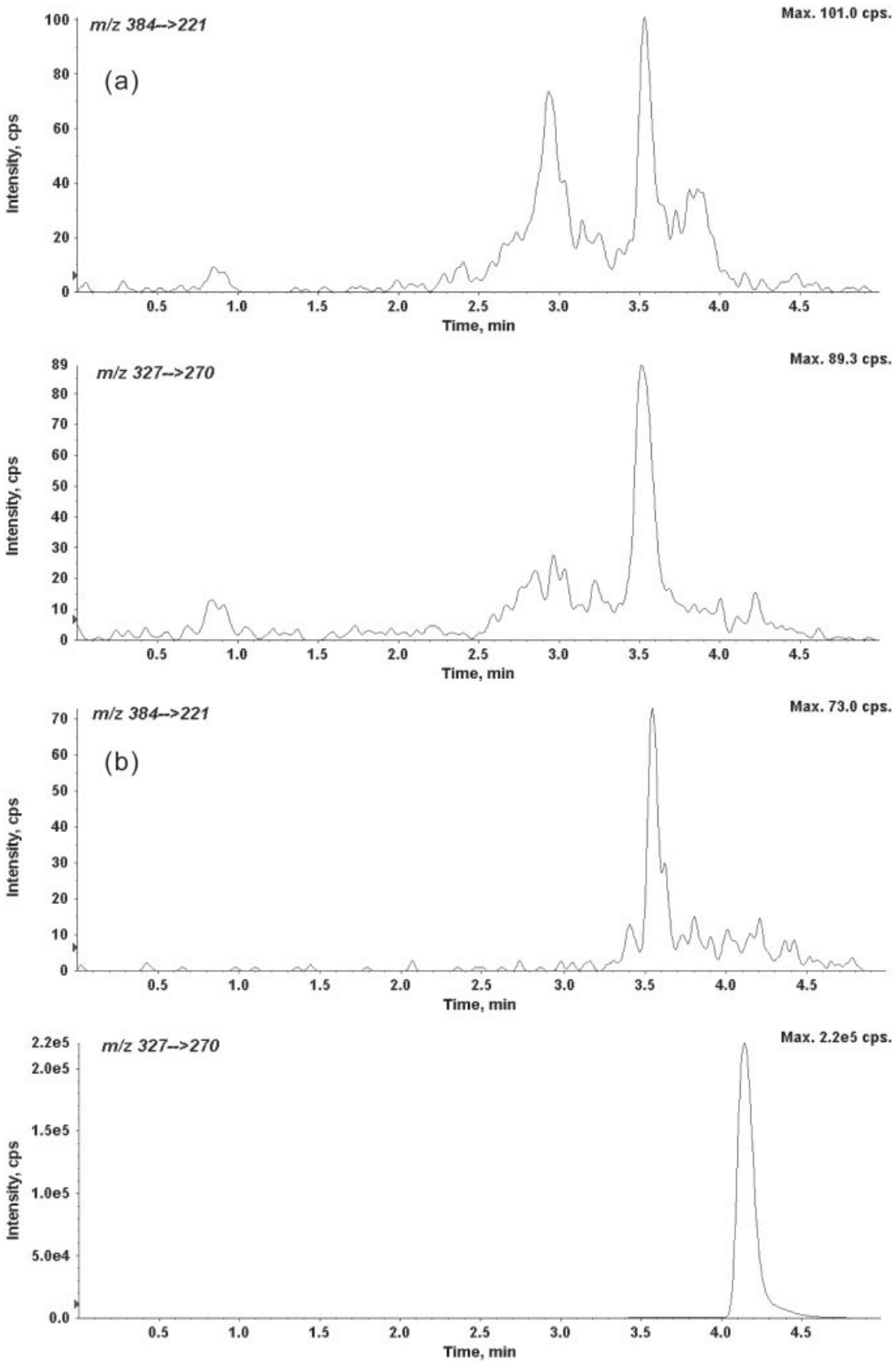


**Figure 4.** MRM Chromatograms for quetiapine and IS resulting from analysis of (a) blank (drug- and IS-free) rat plasma; (b) zero sample (drug-free spiked with IS) rat plasma; (c) 0.25 ng/mL (LLOQ) of quetiapine spiked with IS; and (d) MRM chromatograms resulting from analysis of a rat plasma sample after the administration of a 30 mg/kg subcutaneous dose of quetiapine.



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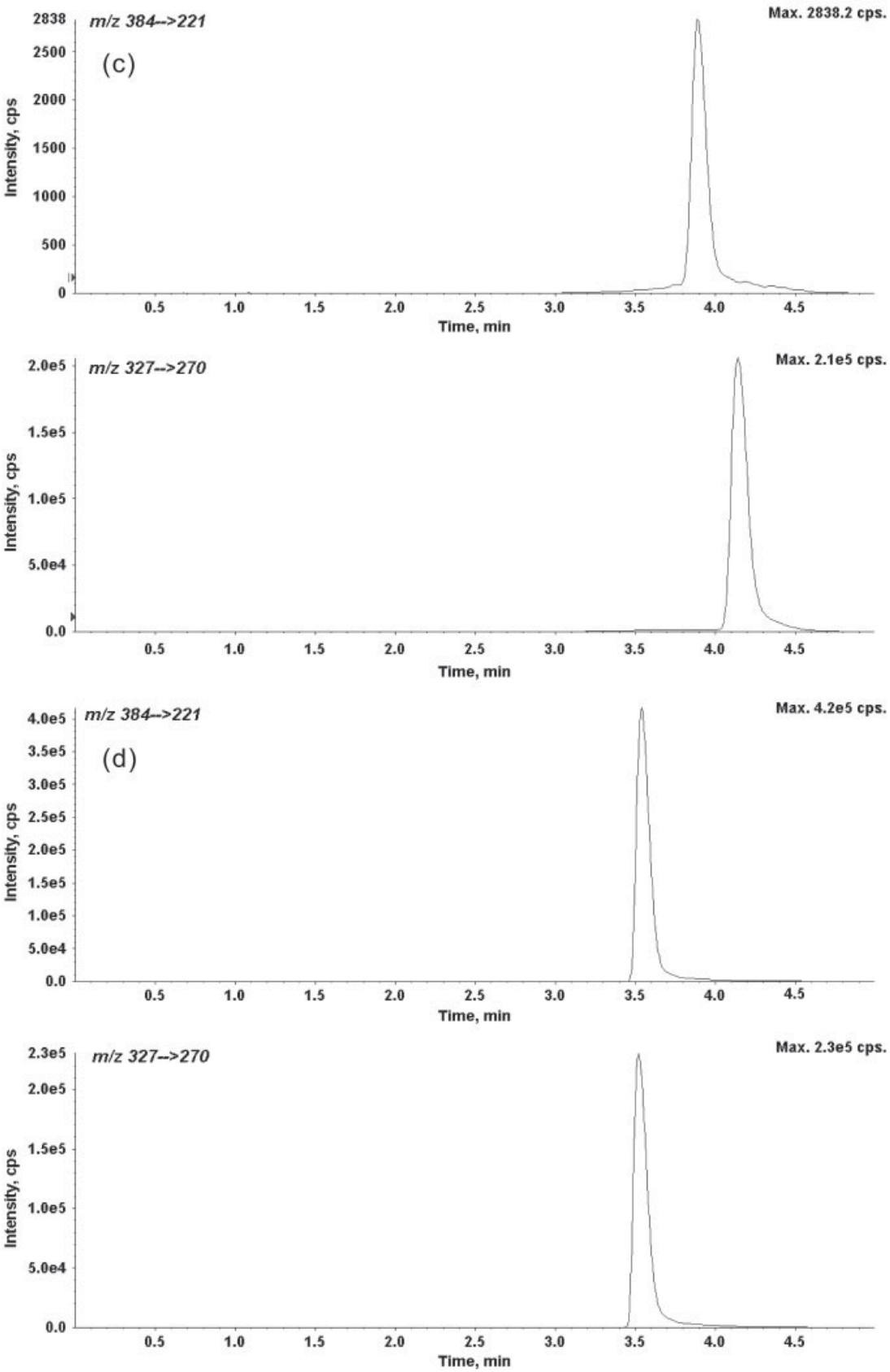


**Figure 4.** (*Continued*)



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monitoring and fully validated according to commonly accepted criteria. The current method has shown acceptable precision and adequate sensitivity for the quantification of quetiapine in rat plasma samples obtained for pharmacokinetic studies. The sensitivity of quetiapine was achieved with an LLOQ of 0.25 ng/mL, and a within- and between-batch CV of 6.1 and 5.9% respectively. Many variables related to the electrospray reproducibility were optimized for both precision and sensitivity to obtain these results. The method was successfully applied to quantify the concentrations of quetiapine in pharmacokinetic studies.

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