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Development and validation of a sensitive liquid chromatography/electrospray tandem mass spectrometry assay for the quantification of olanzapine in human plasma

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Abstract

A simple, sensitive and rapid liquid chromatography/electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) method was developed and validated for the quantification of olanzapine, atypical antipsychotic drug, in human plasma using loratadine as internal standard (IS). Following liquid–liquid extraction, the analytes were separated using an isocratic mobile phase on a reverse phase C_{18} column and analyzed by MS in the multiple reaction monitoring mode using the respective $[M+H]^+$ ions, *m/z* 313/256 for olanzapine and *m/z* 383/337 for the IS. The assay exhibited a linear dynamic range of 0.1–30 ng/mL for olanzapine in human plasma. The lower limit of quantification was 100 pg/mL with a relative standard deviation of less than 10%. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. The average absolute recovery of olanzapine from spiked plasma samples was 85.5 ± 1.9%. A run time of 2.0 min for each sample made it possible to analyze more than 400 human plasma samples per day. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability or bioequivalence studies.

Keywords: Olanzapine; Liquid chromatography-tandem mass spectrometry; Electrospray ionization; Pharmacokinetic study

1. Introduction

Olanzapine (Fig. 1) is one of the most widely used atypical antipsychotics [1]. It is effective against both positive and negative symptoms of schizophrenia [2,3] and is often used in the treatment of patients who are non-responders to classical neuroleptics, such as butyrophenones and phenothiazines and in comparison it has the advantage of not causing extrapyramidal side effects [2] as classical neuroleptics do. Recently, the USFDA has approved the use of olanzapine also for the treatment of acute mania [4].

In order to carry out efficient clinical monitoring, reliable analytical methods are obviously needed. Since the atypical antipsychotic drugs are very active, they are usually administered at low daily dosages and consequently their levels in the plasma of patients tend to be very low (ng/mL levels). Olanzapine is usually administered at very low doses (2–20 mg/day) [5] and the plasma levels are correspondingly low, in the range of 8–47 ng/mL [6,7]. Furthermore, psychiatric patients are often subjected to polypharmacy, which can have dramatic effects on the metabolism and bioavailability of the drugs involved. Therefore, there is a need to implement new analytical methods, which can reliably quantify the analytes with good accuracy and precision even at very low concentrations and in the presence of other potentially interfering drugs.

Several papers on the analysis of olanzapine in biological fluids are found in the literature. Most of the methods are based on the use of high performance liquid chromatography (HPLC)

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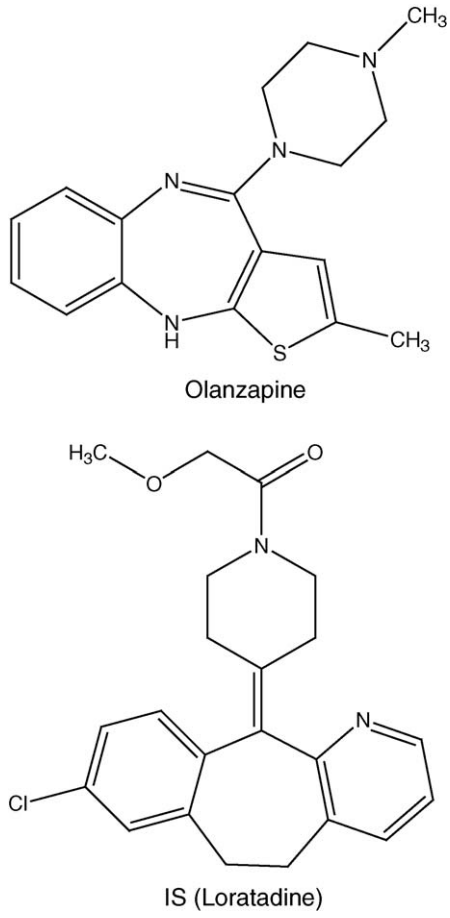

Fig. 1. Chemical structures for olanzapine and IS (loratadine).

with ultraviolet [8–14] or electrochemical detection [8,15–19]. The shortcoming of all these methods is their limited specificity. The advent of the atmospheric pressure ionization (API) source was a breakthrough that allowed the efficient coupling of LC and MS, which leads to a more specific technique. The usefulness of liquid chromatography-electrospray-tandem mass spectrometry (LC–ESI-MS/MS) has been demonstrated for a wide range of applications in the bioanalytical, environmental and pharmaceutical fields [20–24]. Several LC–MS/MS methods were reported for the quantification of olanzapine in biological fluids [25–33]. In all reported methods, chromatographic run time was longer, plasma volume requirement was high and sensitivity was inadequate for pharmacokinetic studies.

The purpose of this investigation was to explore the high selectivity and sensitivity of a triple–quadrupole MS system 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 olanzapine in human plasma using loratadine as the internal standard. It was essential to establish an assay capable of quantifying olanzapine at concentrations down to 100 pg/mL. At the same time, it was expected that this method would be efficient in analyzing large numbers of plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies after therapeutic doses of olanzapine.

2. Experimental

2.1. Chemicals

Olanzapine drug substance was obtained from Hetero Drugs Limited (Hyderabad, India), and loratadine (internal standard, IS) was from the R&D Department of this institute (Hyderabad, India). Chemical structures are presented in Fig. 1. Drug-free human plasma was obtained from the Usha Mullapudi Cardiac Center (Hyderabad, India). HPLC-grade LiChrosolv methanol and LiChrosolv acetonitrile were purchased from Merck (Darmstadt, Germany). Diethyl ether, dichloromethane and ammonium acetate were purchased from Merck (Worli, Mumbai, India). HPLC water from a Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

2.2. LC–MS/MS instrument and conditions

The 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) is equipped with a G1312A binary pump, a G1379A degasser, a G1367A autosampler equipped with a G1330B thermostat, a G1316A thermostatted column compartment and a G1323B control module. The chromatographic separation was on an Inertsil® ODS column (3.0 μm, 100 × 3.0 mm i.d.) at 30 °C. The isocratic mobile phase composition was a mixture of 10 mM ammonium acetate buffer/acetonitrile (10/90, v/v), which was pumped at a flow rate of 0.8 mL/min with a split ratio of 20:80.

Mass spectrometric detection was performed using an API 4000 triple quadrupole instrument (MDS-SCIEX, Toronto, Canada) using MRM. A turbo-electrospray interface in positive ionization mode was used. The main working parameters of the mass spectrometer are summarized in Table 1. Data processing was performed using Analyst 1.4.1 software package (SCIEX).

2.3. Sample preparation

A plasma sample (0.5 mL) was transferred to a 15 mL glass test tube, then 25 μL of IS working solution (500 ng/mL) and

Table 1
Tandem mass spectrometer main working parameters

Parameter	Value
Source temperature (°C)	250
Dwell time per transition (ms)	200
Ion source gas 1 (psi)	10
Ion source gas 2 (psi)	20
Curtain gas (psi)	25
Collision gas (psi)	8
Ion spray voltage (V)	5000
Entrance potential (V)	10
Declustering potential (V)	70 (analyte) and 70 (IS)
Collision energy (V)	30 (analyte) and 30 (IS)
Collision cell exit potential (V)	6 (analyte) and 15 (IS)
Resolution	Unit
Mode of analysis	Positive
Ion transition for olanzapine (*m/z*)	313.4 ± 0.5/256.4 ± 0.5
Ion transition for loratadine (*m/z*)	383.2 ± 0.5/337.3 ± 0.5

50 μL of sodium hydroxide (0.1N) were added. After vortex mixing for 10 s, 4 mL aliquot of extraction mixture, diethyl ether/dichloromethane (7/3, v/v), was added using Dispensette Organic (Brand GmbH, Postfach, Germany). The sample was vortex-mixed for 3 min using Multi-Pulse Vortexer (Glas-Col, Terre Haute, USA). The organic layer (3 mL) was transferred to a 5 mL glass tube and evaporated to dryness using TurboVap LV Evaporator (Zymark, Hopkinton, MA, USA) at 40 °C under a stream of nitrogen. Then the dried extract was reconstituted in 250 μL of mobile phase and a 10 μL aliquot was injected into the chromatographic system.

2.4. Bioanalytical method validation

Standard stock solutions of olanzapine (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 (500 ng/mL) was prepared by diluting its stock solution with diluent. Working solutions (0.5 mL) were added to drug-free human plasma (9.5 mL) as a bulk, to obtain olanzapine concentration levels of 0.1, 0.25, 0.5, 1, 2, 5, 10, 20 and 30 ng/mL as a single batch at each concentration. Quality control (QC) samples were also prepared as a bulk on an independent weighing of standard drug, at concentrations of 0.1 (LLOQ), 0.3 (low), 12.5 (medium) and 25 ng/mL (high) as a single batch at each concentration. The calibration and control bulk samples were divided into aliquots in micro centrifuge tubes (Tarson, 2 mL) and stored in the freezer at below −50 °C until analyses.

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 0.1–30 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 five 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 five sets of QC samples in a batch. The between-batch precision and accuracy were determined by analyzing five sets of QC samples on three different batches. The QC samples were randomized daily, processed and analyzed in a position either (a) immediately following the standard curve, (b) in the middle of the batch, or (c) at the end of the batch. 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 olanzapine from the extraction procedure was determined by a comparison of the peak area of olanzapine in spiked plasma samples (five each of low, medium and high QCs) with the peak area of olanzapine in samples prepared by spiking extracted drug-free plasma samples with the same amounts of olanzapine at the step immediately prior to chromatography. Similarly, recovery of IS was determined by comparing the mean peak areas of extracted QC samples ($n = 5$) 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.

3. Results and discussion

3.1. Mass spectrometry

In order to develop a method with the desired LLOQ (100 pg/mL), it was necessary to use MS–MS detection, as MS–MS methods provide improved limit of detection for trace-mixture analysis [21]. The inherent selectivity of MS–MS detection was also expected to be beneficial in developing a selective and sensitive method. The product ion mass spectrum of olanzapine and the IS are shown in Fig. 2. $[M + H]^+$ was the predominant ion in the Q1 spectrum and was used as the precursor ion to obtain the product ion spectra. The most sensitive mass transition was from m/z 313 to 256 for the olanzapine and from m/z 383 to 337 for the IS. A proposed fragmentation pattern is also shown in Fig. 2. The collisionally activated dissociation (CAD) mass spectrum of olanzapine shows formation of characteristic product ions at m/z 84, 213, 230, 256 and 282. The product ions detected at m/z 84, 230, 256 and 282 are presumably formed from internal α-cleavages and hydrogen rearrangements from within the methyl piperazine ring [34]. The major product ion at m/z 256 for olanzapine could be explained by the loss of a CH_3–NH–CH=CH_2 fragment from the piperazine ring of the protonated precursor molecule. The CAD mass spectrum of IS shows formation of characteristic product ions at m/z 259, 267, 281, 294 and 337. The major product ion at m/z 337 arose from the loss of CH_3CH_2OH of the protonated precursor molecule.

LC-MRM is a very powerful technique for pharmacokinetic studies since it provides sensitivity and selectivity requirements for analytical methods. Thus, the MRM technique was chosen for the assay development. The MRM state file parameters were optimized to maximize the response for the analyte. The parameters presented in Table 1 are the result of this optimization.

3.2. Method development

Two ionization mechanisms commonly used in API are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). It is hypothesized that APCI is associated with a smaller degree of ion suppression because analytes are already in the gas phase when molecular reactions occur and therefore the smaller degree of suppression observed with APCI is due to analyte precipitation [35]. Therefore, in most of the reported LC–MS/MS methods [26–28,32], APCI mode was preferred over the ESI mode, for the quantification of olanzapine in biological fluids. In these LC–APCI-MS/MS methods, the LLOQ was ≥1 ng/mL except Bernal et al. method [26], in which

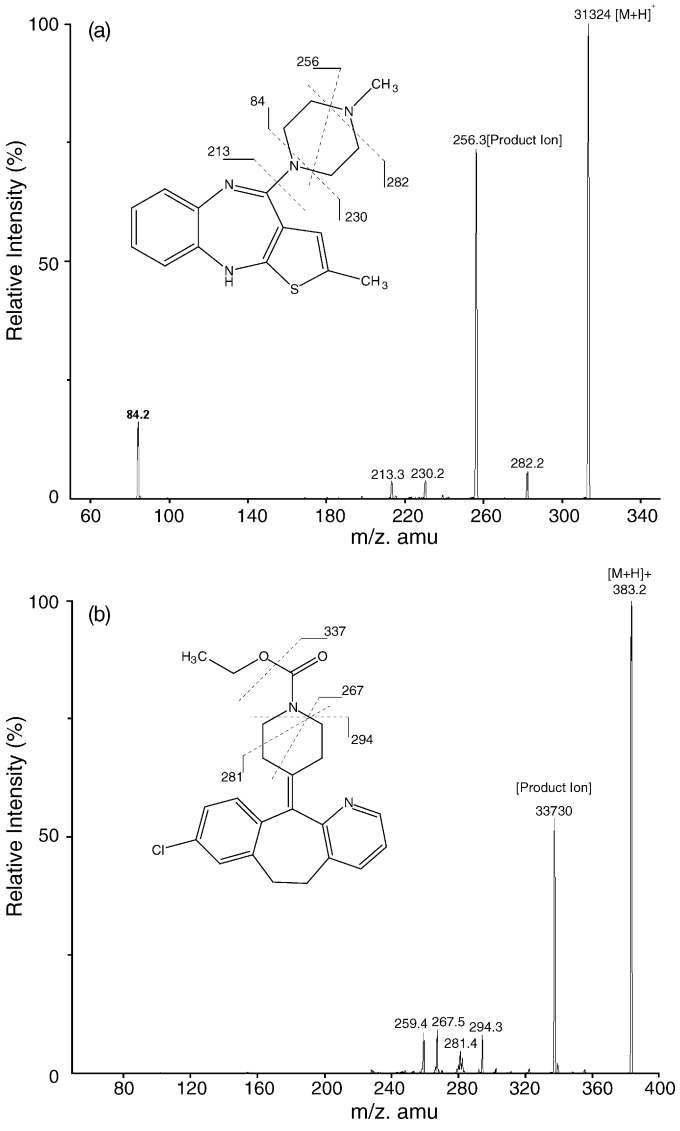

Fig. 2. Full scan positive ion turboIonspray product ion mass spectra and the proposed patterns of fragmentation of (a) olanzapine and (b) loratadine (internal standard). The protonated molecules were used as precursor ions for MS/MS.

LLOQ was 0.25 ng/mL reported with 96-well solid phase extraction.

A problematic issue in ESI is that of ion suppression matrix effects. These matrix effects are the result of co-eluting components from the matrix that can cause a variable reduction in response. There are several possible mechanisms which occur during desolvation and ionization which could be responsible for a loss of analyte response [35]. In the positive mode, if ionized analyte is transferred to the gas phase, gas phase proton transfer reactions may cause neutralization if another neutral species is present in the gas phase with a higher proton affinity than the analyte. Other ionic species, such as salts, in biological samples with high ionization efficiency or surface activity may compete with analytes during ion evaporation. High levels of non-volatile substances may affect the transfer of ionized analyte into the gas phase by preventing the radius and surface charge of the droplets from reaching the levels necessary for ion emission [35,36].

Recently, Chin et al. [29] reported that the analyte response with APCI was five times less than with ESI. The LLOQ, 0.05 and 0.1 ng/mL for olanzapine and desmethyl olanzapine could not be detected with APCI. The purpose of Chin et al. research was to investigate potential matrix effects of anticoagulant and lipemia on the response of olanzapine and desmethyl olanzapine in an LC–MS/MS assay. In this method the analytes were separated using a gradient mobile phase on a phenyl hexyl column following solid phase extraction. Kollroser et al. [25] reported a direct-injection LC–ESI-MS/MS method for the quantitative determination of olanzapine, clozapine and *N*-desmethylclozapine in human plasma. The LLOQ was 5 ng/mL for olanzapine and the total analysis time was 6 min per sample. Recently, Zhou et al. [31] reported an HPLC–ESI/MS method with a LLOQ of 1 ng/mL using a double liquid–liquid extraction procedure.

In the present investigation, a method incorporating HPLC with positive ESI-MS/MS is developed for the quantification of olanzapine concentrations down to 100 pg/mL with simple liquid–liquid extraction from human plasma with a run time of 2 min per sample.

3.3. Method optimization

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes for the analyte and IS, as well as a short run time. It was found that a mixture of 10 mM ammonium acetate buffer/acetonitrile (10:90, v/v) could achieve this purpose and was finally adopted as the mobile phase. Moreover, it was necessary to reconstitute the residues with the mobile phase to produce the expected peak shapes of the analyte. The high proportion of organic solvent eluted the analyte and the IS at retention times of 1.0 and 1.3 min, respectively. A flow rate of 0.8 mL/min produced good peak shapes and permitted a run time of 2.0 min.

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. Six organic solvents, diethyl ether, ethyl acetate, hexane, dichloromethane, chloroform and butyl tert-methyl ether, and their mixtures in different combinations and ratios were evaluated. Finally, a mixture of diethyl ether and dichloromethane (7:3, v/v) was found to be optimal, which can produce a clean chromatogram for a blank plasma sample and yield the highest recovery for the analyte from the plasma.

For an LC–MS/MS analysis, utilization of stable isotope-labeled drugs as internal standards proves to be helpful when a significant matrix effect is possible. Isotope labeled analyte was not obtainable to serve as IS, so, in the initial stages of this work, several compounds were investigated to find a suitable IS, and finally loratadine was found to be suitable for the present purpose. Clean chromatograms were obtained and no significant direct interferences in the MRM channels at the relevant reten-

tion times were observed. However, in ESI, signal suppression or enhancement may occur due to co-eluting endogenous components of the sample matrix. These potential matrix effects were evaluated by spiking blank plasma extracts (after LLE treatment as described above) at the low and high QC levels. The resulting chromatograms were compared with those obtained for clean standard solutions at the same concentrations. Five independent plasma lots were used, with five samples from each lot. The results (data not shown) showed that there was no significant difference between peak responses for spiked plasma extracts and clean solutions. This result most likely reflects the efficacy of the sample clean-up with LLE. In any event, the use of matrix-matched calibration standards would have minimized any such effects on the quantification.

3.4. Assay performance and validation

The nine-point calibration curve was linear over the concentration range 0.1–30 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 $1/\sqrt{x}$). The best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x^2$ weighing factor, giving a mean linear regression equation for the calibration curve of:

$$y = 0.1070(\pm 0.0211)x - 0.0057(\pm 0.0007)$$

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 0.998; Table 2 summarizes the calibration curve results.

The selectivity of the method was examined by analyzing ($n = 5$) blank human plasma extract (Fig. 3(A)) and an extract spiked only with the IS (Fig. 3(B)). As shown in Fig. 3(A), no significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free human plasma at the retention time of the analyte. Similarly, Fig. 3(B) shows the absence of direct interference from the IS to the MRM channel of the analyte. Fig. 3(C) depicts a representative ion-chromatogram for the LLOQ (100 pg/mL). Excellent sensitivity was observed for a 10 μL injection volume; the LLOQ corresponds to ca. 1 pg on-column.

The MRM chromatograms obtained for an extracted plasma sample of a healthy subject who participated in a bioequivalence study conducted on 18 subjects are depicted in Fig. 4. Olanzapine was identified and was quantified as 2.3 ng/mL.

3.5. Validation parameters at the LLOQ

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 100 pg/mL in human plasma. The mean response for the analyte peak at the assay sensitivity limit (100 pg/mL) was ~15-fold greater than the mean response for the peak in five blank human plasma samples at the retention time of the analyte. The between-batch precision at the LLOQ was 10.1%, and the between-batch accuracy was 111.3% (Table 3). The within-batch precision was 7.8% and the accuracy was 109.4% for olanzapine.

3.6. Validation parameters at the middle and upper concentrations

The middle and upper quantification levels of olanzapine ranged from 0.3 to 25 ng/mL in human plasma. For the between-

Table 2
Precision and accuracy data for back-calculated concentrations of calibration samples for olanzapine in human plasma

Concentration added (ng/mL)	Concentration found (mean ± S.D., $n = 5$) (ng/mL)	Precision (%)	Accuracy (%)
0.10	0.11 ± 0.01	9.8	105.4
0.25	0.27 ± 0.02	5.5	109.8
0.50	0.51 ± 0.03	6.3	101.8
1	1.00 ± 0.04	4.4	99.5
2	1.99 ± 0.13	6.6	99.4
5	5.14 ± 0.17	3.2	102.8
10	9.98 ± 0.64	6.4	99.8
20	20.23 ± 1.16	5.7	101.2
30	31.20 ± 1.09	3.5	104.0

Table 3
Precision and accuracy of the method for determining olanzapine concentrations in plasma samples

Concentration added (ng/mL)	Within-batch precision ($n = 5$)			Between-batch precision ($n = 3$)		
	Concentration found (mean ± S.D.) (ng/mL)	Precision (%)	Accuracy (%)	Concentration found (mean ± S.D.) (ng/mL)	Precision (%)	Accuracy (%)
0.1	0.11 ± 0.01	7.8	109.4	0.11 ± 0.01	10.1	111.3
0.3	0.32 ± 0.01	3.3	105.1	0.30 ± 0.01	4.4	101.1
12.5	12.98 ± 0.82	6.3	103.8	12.80 ± 0.75	5.9	102.4
25	26.35 ± 1.67	6.3	105.4	25.51 ± 1.04	4.1	102.1
100.0[a]	108.36 ± 3.27	3.0	108.36	104.68 ± 5.83	5.6	104.7

[a]The sample was processed with 5-fold dilution.

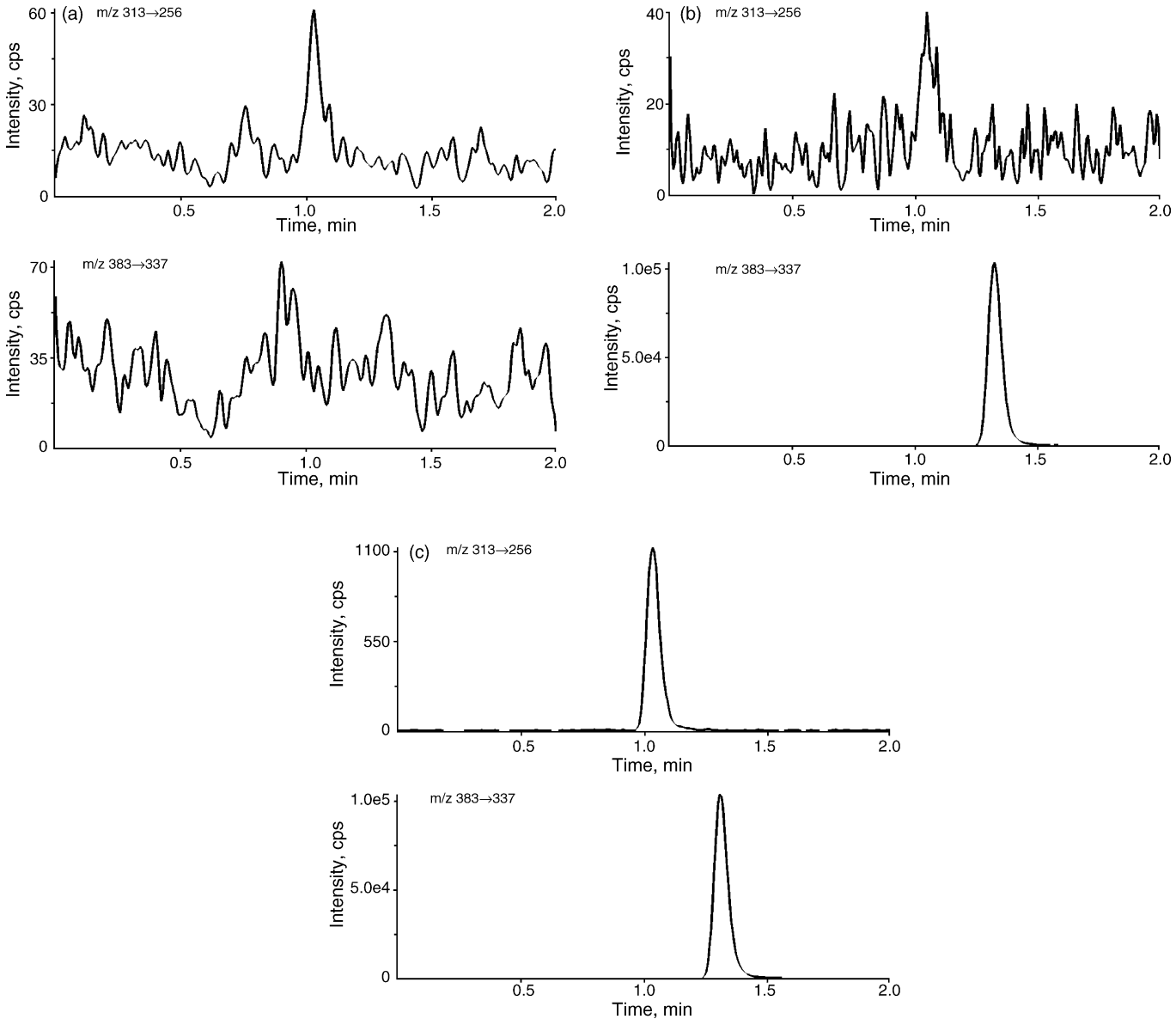

Fig. 3. MRM chromatograms for olanzapine and the IS resulting from analysis of: (a) blank (drug and IS free) human plasma; (b) blank (drug-free spiked with IS) human plasma; (c) 100 pg/mL (LLOQ) of olanzapine spiked with the IS.

batch experiments the precision ranged from 4.1 to 5.9% and the accuracy from 101.1 to 102.4% (Table 3). For the within-batch experiments the precision and accuracy for the analyte met the acceptance criteria (<±15%).

The upper concentration limits can be extended with acceptable precision and accuracy to 100 ng/mL by a 5-fold dilution with control human plasma. These results suggested that samples with concentrations greater than the upper limit of the calibration curve can in this way be assayed to obtain acceptable data (Table 3).

The average absolute recoveries for olanzapine at three different concentrations (low, medium and high QC samples) are shown in Table 4. The recovery of the analyte was high (85.5 ± 1.9%). The recovery of the IS was 49.8 ± 1.6% at the concentration used in the assay (500 ng/mL). Recovery of IS was medium, but it was consistent and reproducible. Therefore, the assay has proved to be robust in high-throughput bioanalysis.

3.7. Stability studies

The stock solutions were stable for at least 4 months when stored at 4 °C. All the stability studies were conducted at two

Table 4
Absolute recoveries of olanzapine from human plasma

Sample concentration (ng/mL)	Absolute recovery (%) (mean ± S.D., $n = 5$)
0.3	83.5 ± 5.1
12.5	87.3 ± 2.8
25.0	85.6 ± 2.6

Table 5
Stability of olanzapine in human plasma

Sample concentration (ng/mL) ($n = 5$)	Concentration found (ng/mL)	Precision (%)	Accuracy (%)
Short-term stability for 24 h in plasma			
0.3	0.31	3.8	103.1
25	24.97	4.6	99.9
Three freeze–thaw cycles			
0.3	0.31	4.1	102.9
25	27.08	4.6	108.3
Autosampler stability for 24 h			
0.3	0.30	5.8	99.9
25	24.02	6.0	96.1
Stability for 21 days at <−50 °C			
0.3	0.29	4.6	97.4
25	25.59	2.7	102.3

concentration levels (0.3 and 25 ng/mL as low and high values) with five determinations for each. QC samples were subjected to short-term room temperature conditions, to long-term storage conditions (−50 °C), and to freeze–thaw stability studies. There was no significant difference between the responses of spiked standards at time zero and after 24 h for olanzapine, indicating the stability of analyte at room temperature over 24 h. The stability of QC samples kept in the autosampler for 24 h was also assessed. The results indicate that solutions of olanzapine and the IS can remain in the autosampler for at least 24 h without showing significant loss in the quantified values, indicating that samples should be processed within this period of time (Table 5). The freeze–thaw stability tests indicate that the analyte is stable in human plasma for three freeze/thaw cycles, when stored at below −50 °C and thawed to room temperature. The long-term storage stability study of olanzapine in human plasma showed reliable stability behavior, as the mean of the results of the tested samples were within the acceptance criteria of ±15% of the initial values of the controls. These findings indicate that storage of olanzapine in plasma samples at below −50 °C is adequate, and no stability-related problems would be expected during routine analyses for pharmacokinetic, bioavailability or bioequivalence studies.

3.8. Application

The validated method has been successfully used to quantify olanzapine concentrations in human plasma samples after the administration of a single 10 mg oral dose of olanzapine in a bioequivalence study conducted in healthy subjects. The method has been successfully applied for quantification of olanzapine in more than 1000 samples in the bioequivalence study conducted on 18 subjects. The representative of MRM chromatogram resulting from the analysis of a subject plasma sample after the administration of a 10 mg oral single dose of olanzapine has been presented in Fig. 4. The sample concentration was determined to be 2.3 ng/mL. The representative concentration versus time profiles for three subjects, each receiving a single dose, is presented in Fig. 5.

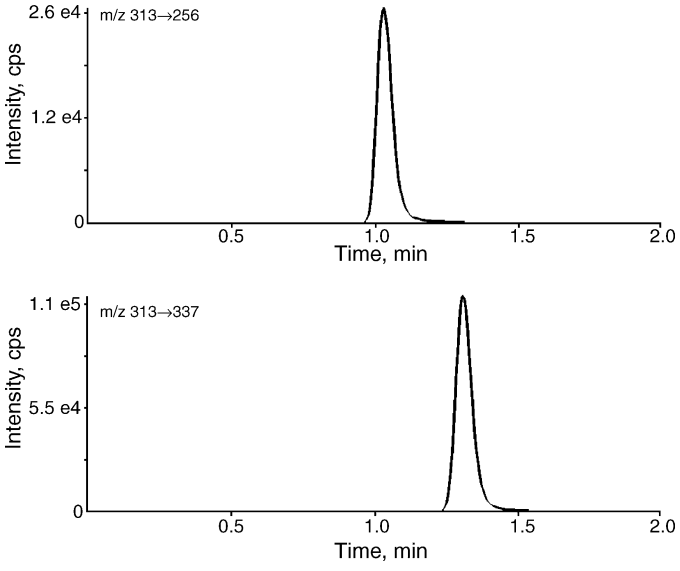

Fig. 4. MRM chromatograms resulting from the analysis of a subject plasma sample after the administration of a 10 mg oral single dose of olanzapine. The sample concentration was determined to be 2.3 ng/mL.

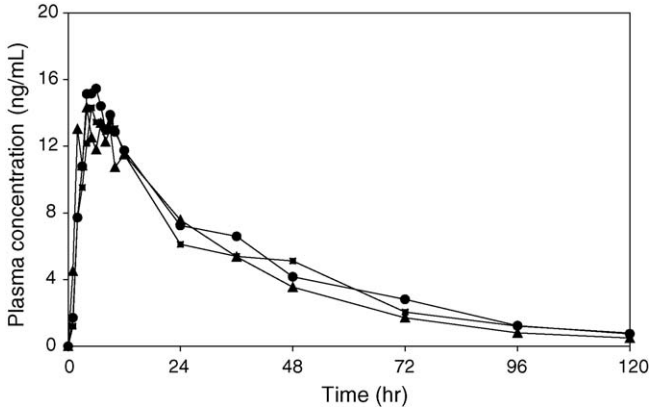

Fig. 5. Representative data showing plasma concentration–time profiles of three healthy subjects after the administration of an oral single dose of 10 mg of olanzapine.

4. Conclusions

In summary, the method is described for the quantification of olanzapine in human plasma by LC–MS/MS in positive electrospray ionization mode using multiple reaction monitoring, and fully validated as per FDA guidelines [37]. This method offers significant advantages over those previously reported, in terms of improved sensitivity and selectivity, faster run time (2.0 min) and rapid extraction. With dilution integrity up to 5-fold, we have established that the upper limit of quantification is extendable up to 100 ng/mL. Hence, this method is useful for single and multiple ascending dose studies in human subjects. The current method has shown acceptable precision and adequate sensitivity for the quantification of olanzapine in human plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies. The desired sensitivity of olanzapine was achieved with an LLOQ of 100 pg/mL, which has a within- and between-batch CV of 7.8 and 10.1%, respectively. Many variables related to the electrospray reproducibility were optimized for both precision and sensitivity to obtain these results.

Olanzapine was shown to be stable in routine analysis conditions and in human plasma for up to 21 days when stored at below −50 °C. The cost-effectiveness, simplicity of the assay, using rapid liquid–liquid extraction and sample turnover rate of 2.0 min per sample, make it an attractive procedure in high-throughput bioanalysis of olanzapine. The validated method allows quantification of olanzapine in the 0.1–30 ng/mL range.

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