International Journal of Medical Science and Clinical Invention 9(4): 6046-6055, 2022
DOI:10.18535/ijmsci/v9i04.03
https://valleyinternational.net/index.php/ijmsci

Research Article,

Bioanalytical method Development and Validation for Estimation of Olanzapine in K3edta Human Plasma by Liquid Chromatography–Tandem Mass Spectrometry and Application to a Pharmacokinetic Study

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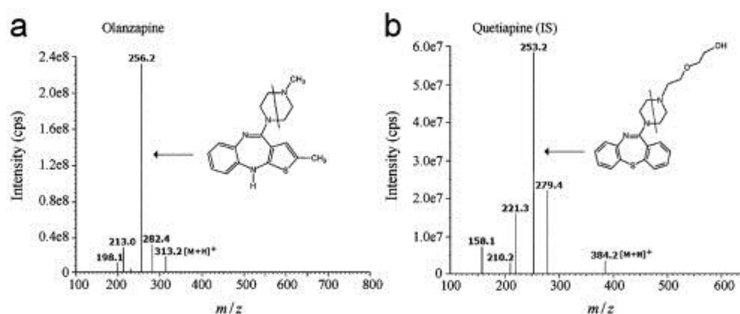
Abstract:

A rapid and sensitive LC–MS/MS method for the bioanalytical method development and validation for estimation of olanzapine in k_3EDTA human plasma using Olanzapine D_3 as internal standard has been developed and validated. The analytes and IS were extracted from plasma by solid phase extraction using Oasis HLB 1cc (30mg) Extraction Cartridge and separated on a Cosmosil, 5µm, C18 150*4. 6 mmcolumn using a 10 mM ammonium formate in Water: Acetonitrile (10: 90) at a flow rate of 1.0 mL/min. Detection involved an API-4000 LC–MS/MS with electrospray ionization in the positive ion mode and multiple-reaction monitoring for analysis. The method was validated according to FDA guidelines and shown to provide intra- and inter-day precision and accuracy within acceptable limits in a run time of only 4.0 min. The method was successfully applied to a pharmacokinetic study involving a single oral administration of a combination tablet to human male volunteers

Keywords: Olanzapine, Solid phase extraction, LC–MS/MS, Pharmacokinetics

Graphical abstract:
A selective, sensitive and rugged liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay for the determination of olanzapine in human plasma is developed and applied to a bioequivalence study of 40 healthy Indian male subjects under fasting and fed condition.



Introduction:

Olanzapine (OLZ), a thienobenzodiazepine derivative, is an antipsychotic drug which is highly effective in the treatment of schizophrenia and bipolar disorders[1,2]. It is an atypical antipsychotic with less extrapyramidal side effects and greater positive effects on cognitive deficits than typical antipsychotics[3]. This is because of its affinity for multiple receptors including dopamine D2, 5-hydroxytryptamine (5-HT) 2A and 2C, histamine H1, *α*-adrenergic and muscarinic receptors. OLZ has been approved by the US FDA for use as monotherapy or in combination with mood stabilizers for the treatment of acute mania in bipolar disorders, It is available as coated tablets in dose strengths ranging from 2 to 20 mg under the brand name Zyprexa and as an orally disintegrating tablet known as Zyprexa Zaydis. Following oral administration, OLZ is about 93% plasma protein bound[4], mainly to albumin and *α*-acid glycoprotein. It has an oral bioavailability of about 60% mainly due to hepatic first pass metabolism to the 10-*N*-glucuronide, 4′-*N*-desmethylolanzapine and olanzapine-*N*-oxide *via* uridine diphosphate glucuronyl transferase (UDPGT)[5], cytochrome P_{450} (CYP) $1A_2$ and Flavin monoxygenase (FMO), respectively. After absorption, OLZ reaches its maximum plasma concentration within 6 h and has a mean half-life of about 33 h.[5]

2. Materials and methods

2.1. Chemicals and materials

Chemicals and materials (suppliers) were as follows: Reference standard OLZ (99.60%) (Cadila Healthcare Ltd., Ahmedabad, India); HPLC grade methanol and acetonitrile, analytical grade Ammonium formate L-Ascorbic acid, ammonia and ammonium format (S.D. Fine Chemicals Ltd., Mumbai, India); Oasis HLB extraction cartridges (1 cc, 30 mg) (Waters Corporation, Milford, MA, USA); Control buffered (K_3-EDTA) human plasma stored at −20 °C (Clinical Department, BA Research India Ltd., Ahmedabad, India); Mettler Toledo AG XP26DR micro balance (Greifensee, Switzerland); Eppendorf 5810 centrifuge (Hamburg, Germany); deionized water for LC–MS/MS prepared using a Milli *Q* water purification system (Millipore, Bangalore, India).

2.2. Instrumentation and conditions

The LC system (Shimadzu, Kyoto, Japan) consisted of an LC-10ADvp pump, an auto sampler (SIL-HTc) and an on-line degasser (DGU-14A). Separation was performed by isocratic elution on and Cosmosil, 5µm, C18 150*4. 6 mm column (Chromatographic peak Analytical Instrumentation (India) Pvt. Ltd., Mumbai, India) with a mobile phase consisting of 10 mM Ammonium Format in Water: Acetonitrile (10: 90) at a flow rate of 1.0 mL/min. The auto sampler was maintained at 4°C and the injection volume was 10.0 µL. Ionization and detection of analyte and IS was performed on an API-4000 triple quadrupole mass spectrometer equipped with Turbo Ion spray® (MDS SCIEX, Toronto, Canada) operating in the positive ion mode. Quantitation was performed using multiple reaction monitoring (MRM) of the protonated precursor to product ion transitions at *m/z* 313.2→256.2 for OLZ and 384.2→253.2 for IS (Figs. 1a and b). All LC and MS parameters were controlled by Analyst software version 1.4.2.

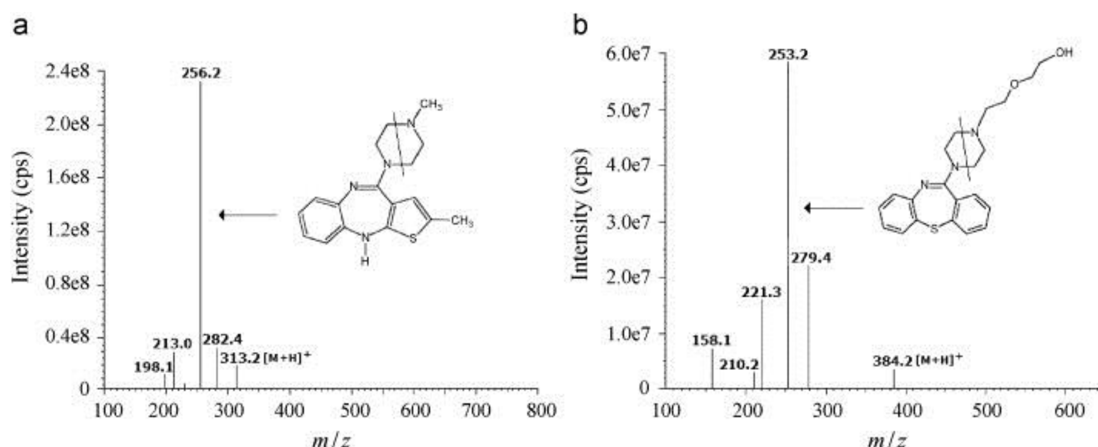


Figure 1.Product ion mass spectra of (a) olanzapine (*m/z* 313.2→256.2) and (b) quetiapine (IS, *m/z* 384.2→253.2) in positive ionization mode.

Optimized source dependant MS parameters were as follows: Gas 1 (Nebulizer), 55 psi; Gas 2 (heater), 50 psi; ion spray voltage (ISV), 5500 V; turbo heater temperature (TEM), 550 °C; entrance potential (EP), 10 V; collision activation dissociation (CAD), 6 psi; curtain gas (CUR), 30 psi. Compound dependent MS parameters viz declustering potential (DP), collision energy (CE) and cell exit potential (CXP) were, respectively 70, 35 and 15 V for OLZ and 85, 23 and 12 V for IS. Quadrupole 1 and quadrupole 3 were maintained at unit resolution and a dwell time of 600 ms was set for both OLZ and IS.

2.3. Calibration standards and quality control (QC) samples

An OLZ stock solution (100 μg/mL) in methanol was diluted with methanol: water (80:20, *v/v*) to produce an intermediate stock solution (800 ng/mL). This was further diluted to prepare standard and (independently) QC solutions. An IS stock solution (100 μg/mL) was also prepared in methanol and diluted with water to give an IS working solution (40.0 ng/mL). All solutions were stored at 4°C when not in use. OLZ calibration standards (CS-1 to CS-8, 0.10, 0.20, 0.60, 1.00, 3.00, 6.00, 15.0, and 30.0 ng/mL), low (LQC, 0.30 ng/mL), medium (MQC-1, 12.0 ng/mL; MQC-2, 4.00 ng/mL) and high (HQC, 24.0 ng/mL) QC samples and QC samples at the lower limit of quantitation (LLOQ QC, 0.10 ng/mL) and upper limit of quantitation (ULOQ QC, 30.0 ng/mL) were prepared by spiking blank plasma[6] with respective standard solutions (at 2% of the total volume of plasma). All CS, QC and study samples were stored at −70 °C pending analysis.

2.4. Sample preparation

Aliquot 0.300 ml of sample into pre-labelled tubes. Add 50 µl of ISTD dilution (60.000 ng/ml) to all the samples except STD BL and vortex for about 30 seconds. Add 50 µl Methanol to STD BL sample. Add 300 µl of Extraction Buffer (5% Ortho Phosphoric Acid in water) to all samples and vortex to mix. Arrange the required number of pre-labelled Oasis HLB 1cc 30mg/1ml extraction cartridges on EZYPRESS 48-48 Position Positive Pressure Processor. Condition the cartridges with 1.0ml Methanol followed by 1.0 ml Water. Load the prepared samples on conditioned cartridges carefully. Wash the cartridges with 1.0 ml of Water, dry the cartridges for about 5 minutes by applying positive pressure at maximum flow rate or by applying full vacuum. Elute the contents from the cartridges with 1.000 ml Acetonitrile into pre- labelled tubes. Then Reconstitute the sample 100µl Mobile Phase. Transfer appropriate volume of each sample into separate pre-labelled Autosampler vials, arrange them in the Autosampler and inject by using LC-ESI-MS/MS.

2.5. Assay validation

Assay validation was carried out according to US FDA guidelines Validation included evaluation of selectivity, interference, carryover, linearity, precision and accuracy, reinjection reproducibility, recovery, ion suppression/enhancement, matrix effects, stability and dilution integrity.

Selectivity was assessed by assay of 9 different lots of blank human plasma (including haemolysed, lipemic and sodium heparin samples) collected with K_3-EDTA as anticoagulant[7]. For each lot, two replicates (294 µL) were spiked with 6 µL methanol: water (50:50, *v/v*) in one case containing IS. (Total 18 samples). In addition, a system suitability sample (SSS) with the same concentration as CS-2 and two replicates of CS-1 were prepared. The blank human plasma used for spiking these samples was chosen from one of the 9 lots. The acceptance criterion was that at least 90% of samples[7] should be free from any interference at the retention times of analyte and IS.

Analytical recovery (precision and accuracy) and chromatographic interference at the MRM transitions of analyte and IS. Their stock solutions (100 μg/mL) were prepared in methanol and diluted in methanol: water (50:50, *v/v*) to 20.0 μg/mL working solutions. They were then analysed in triplicate under the same conditions as the LQC and HQC samples along with freshly prepared CS and two sets (8 samples) of HQC, MQC-1, MQC-2 and LQC. The acceptance criteria were that the accuracy should be in the range 85–115%. MRM transitions (positive ionization mode) for clozapine (327.1/270.2), was studied[8].

Carryover was assessed by injecting the following sequence of samples; double blank plasma, two LLOQ samples, double blank plasma, an ULOQ sample, double blank plasma, an ULOQ sample and finally double blank plasma.

Linearity was determined by construction of six calibration curves based on peak area ratios at ten non-zero concentrations. Each calibration curve was analysed individually by least squares weighted ($1/x^2$) linear regression. A correlation coefficient (r^2)>0.99 was desirable for all calibration curves. The lowest standard on the calibration curve was accepted as the LLOQ if the analyte response was at least 10 times more than that of blank plasma.

To determine intra-day accuracy and precision, a calibration curve and six replicates of LLOQ QC, LQC, MQC-2, MQC-1, HQC and ULOQ QC were analysed on the same day. Inter-day accuracy and precision were assessed by analyzing three batches of samples on three consecutive days[8]. The deviation from the nominal concentration is required to be <±15% except at the LLOQ where ±20% is allowed. Similarly, accuracy should be within ±15% except at the LLOQ where it can be within ±20% of nominal concentrations. Reinjection reproducibility was performed by reinjection one complete validation batch.

Relative recovery (RE), matrix effects (ME) and process efficiency were assessed as recommended by Matuszewski et al. All three parameters were evaluated by assay of six replicates of HQC, MQC-1, MQC-2 and LQC samples. RE was calculated by comparing the mean area responses of samples spiked before extraction with those of samples spiked after extraction at each QC level. The recovery of IS was estimated in the same way. Absolute ME were assessed by comparing the mean area responses of samples spiked after extraction with those of standard solutions in mobile phase. Overall "process efficiency" (PE, %) was calculated as (ME×RE)/100. In addition, the effect of the plasma matrix was checked using eight different lots of K_2-EDTA anticoagulated plasma including haemolysed and lipemic samples. For each lot, four samples at LQC and HQC levels were spiked after extraction and checked for accuracy and precision. The effect of the matrix onion suppression was evaluated by post-column infusion at 5.0 μL/min of a standard solution containing 16.0 ng/mL OLZ and 30.0 ng/mL IS in mobile phase *via* a 'T' connector employing a Harvard infusion pump (Harvard Bioscience, USA). Aliquots (5.0 μL) of extracted blank plasma (without OLZ and IS) were then injected and chromatograms acquired. Any reduction in the baseline on injection of the blank plasma was taken to indicate ion suppression whereas a peak at the retention time of either OLZ or IS was taken to indicate ion enhancement.

Stability was evaluated by comparing concentrations of LQC and HQC samples subjected to various conditions with those of freshly prepared samples. Samples were considered stable if deviation from nominal values was <±10.0%. Stock solutions of OLZ and IS were checked for short term stability at room temperature and long term stability at 4 °C. Bench top stability, processed sample stability at room temperature and at 4 °C, freeze–thaw stability and long term stability at −20 °C were performed by assay of six replicates[9]. To meet acceptance criteria, concentrations in at least 2/3 of QC samples should remain within ±15% with accuracy in the range 85–115%.

Dilution integrity was assessed by assay of five samples with analyte concentrations above the ULOQ i.e., at 200 ng/mL and at the HQC level[10]. Six replicates of samples after ten-fold dilution (20.0 and 3.0 ng/mL) were prepared and their OLZ concentrations calculated by applying the dilution factor of 10 to concentrations obtained from the calibration curve.

2.6. Bioequivalence study design

The design of the study comprised an open label, randomized, two periods, two treatments, two sequence, crossover, balanced, single dose, evaluation of the relative oral bioavailability of a 5 mg OLZ disintegrating tablet from a sponsor company (test) and a 5 mg OLZ disintegrating tablet (ZYPREXA® ZYDIS®) from Eli Lilly and Co., Indianapolis, Indiana 46285, USA (reference) in 40 healthy Indian males under fasting and fed conditions. The study also included evaluation of a 10 mg OLZ disintegrating tablet from a Sponsor company (test) and a 10 mg OLZ disintegrating tablet (ZYPREXA® VELOTAB™) from Eli Lilly and Co., Netherlands (reference) in 40 healthy Indian males under fasting conditions. All participants were informed of the aims and risks of the study and gave written consent. Inclusion criteria were; age 18–45 years, body mass index 18.5–30.0 kg/height^2, and no abnormalities on general physical examination, electrocardiogram and laboratory tests (hematology, blood and urine chemistry and immunological tests). Exclusion criteria were; allergy to OLZ, alcoholism, smoking, diabetes, psychosis, and any disease which could compromise the haemopoietic, gastrointestinal, renal, hepatic, cardiovascular, respiratory or central nervous systems. The

protocol was approved and subject to review by the relevant Institutional Ethics Committee. All procedures in dealing with human subjects were based on the International Conference on Harmonization, E6 Good Clinical Practice (ICH, E6 GCP) guidelines.

Subjects in fasting studies were required to fast for 10 h before administration of drug. Subjects in fed studies were given a high fat, high (969) calorie breakfast (consisting of 200 mL milk with 16 g sugar, 80 g black gram, two slices bread and butter and two cheese cutlets) 30 min prior to drug administration. Blood samples were collected in vacutainers containing K_3-EDTA anticoagulant before and at 1–10, 12, 16, 24, 36, 48, 72, 96, 120, 144 and 168 h after administration of drug. Plasma obtained by centrifugation at 1811×*g* and 4 °C for 15 min was stored at −20 °C until assayed. An incurred sample reanalysis (ISR) was also conducted for all three studies by computerized random selection of 442 samples (10% of the total study sample) with concentrations near the C_{max} or during the elimination phase. The results were compared with corresponding values obtained earlier for the same sample using the same procedure. The percent change should not be more than ±20% where (1) Change (%)=[(Repeatvalue−Initialvalue)/Meanofrepeatandinitialvalues]×100%

2.7. Statistical analysis

Pharmacokinetic parameters for OLZ were estimated by non-compartmental analysis using WinNonlin software version 5.2.1 (Pharsight Corporation, Sunnyvale, CA, USA). The C_{max} values and the time to reach maximum plasma concentration (T_{max}) were determined directly from plasma concentration-time curves. Area under plasma concentration-time curves from time 0 to 168 h ($AUC_{0–168}$) was calculated using the linear trapezoidal rule. $AUC_{0–\infty}$ was calculated as $AUC_{0–168}+C_t/k_{el}$, where C_t is the last plasma concentration measured and k_{el} is the elimination rate constant determined by linear regression of the logarithm linear part of the plasma concentration-time curve. The $T_{1/2}$ of OLZ was calculated as ln $2/k_{el}$. To determine whether test and reference formulations were pharmacokinetic ally bioequivalent, the mean and 90% confidence intervals (CI) of log transformed C_{max}, $AUC_{0–168}$, $AUC_{0–\infty}$ and their ratios (test/reference) were determined using SAS® software version 9.1.3 (SAS Institute Inc., Cary, NC, USA)[11]. The formulations were considered bioequivalent if the differences between the compared parameters were not significantly different ($P \geq 0.05$) and the 90% CIs for these parameters fell within the range 0.8–1.25.

3. Results and discussion

3.1. Method development

The objective of the present work was to develop and fully validate an LC-MS/MS method for the determination of OLZ in human plasma with sensitivity adequate to monitor the concentration of OLZ for least five half-lives after a therapeutic dose. To realize this aim, the extraction procedure, mass spectrometry and chromatographic conditions were optimized. The ESI was operated in the positive ion mode as both OLZ and IS are basic in nature. OLZ and IS gave predominant, singly charged protonated precursor $[M+H]^+$ ions at *m/z* 313.2 and 384.2, respectively in Q1 full scan spectra. Furthermore, the most abundant ion in the product ion mass spectra of OLZ was at *m/z* 256.2, resulting from cleavage of the piperazine ring to the neutral fragment, $CH_3NHCH=CH_2$. As previously reported[43], other characteristic fragments were observed at *m/z* 282.4 and 213.0 attributed to formation of CH_3NH_2 and elimination of the piperazine ring, respectively. The proposed fragmentation pathway of OLZ is depicted in Fig. 2a.

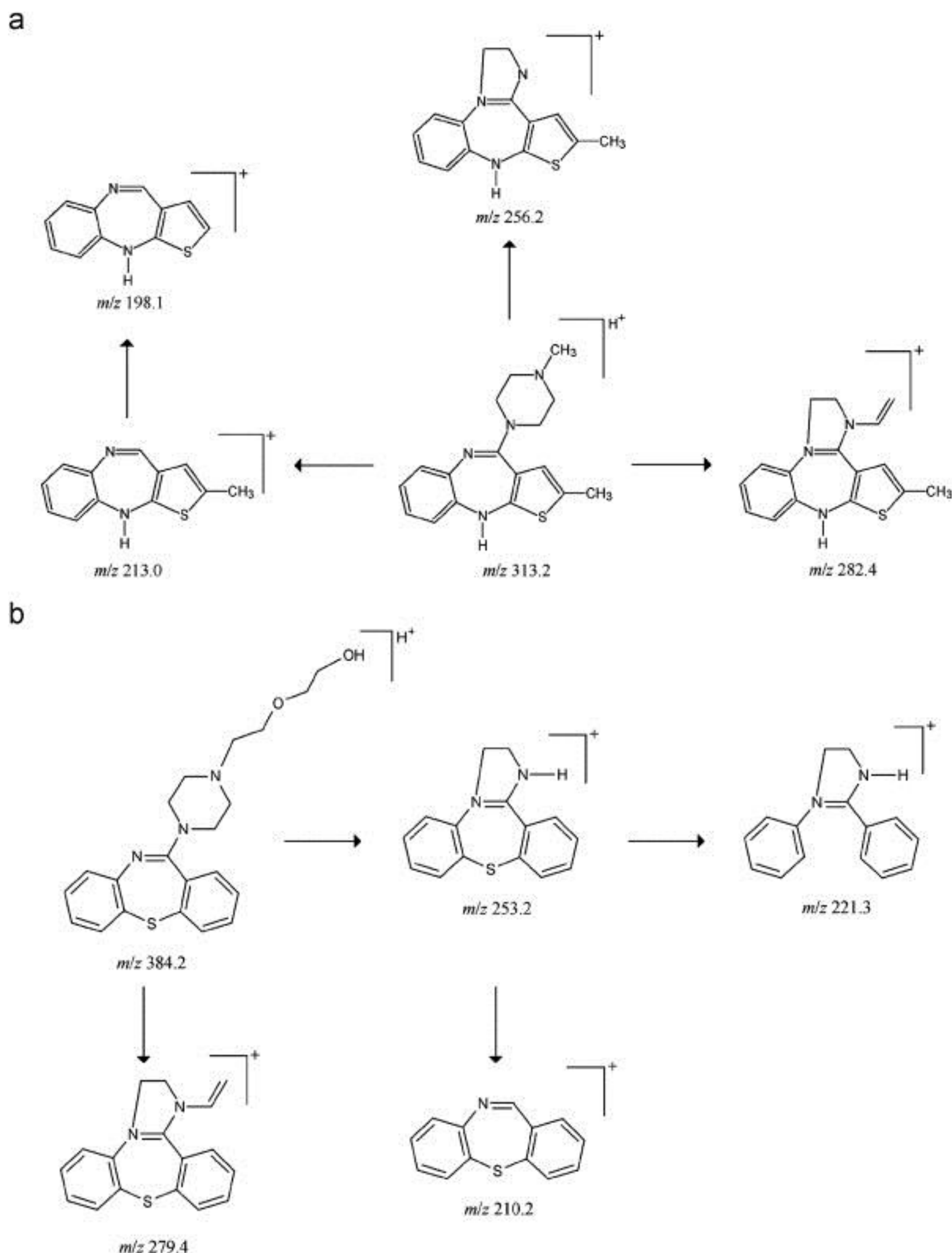


Figure 2. Proposed fragmentation pathways for (a) olanzapine and (b) quetiapine.

For quetiapine, the most stable and reproducible product ion at *m/z* 253.2 arises from cleavage of the piperazine ring. The other product ion at *m/z* 279.4 is due to breaking of two C–N bonds from the precursor ion to eject the fragment, ($HOCH_2CH_2OCH_2CH_2NH_2$) *m/z* 105, as shown in Fig. 2b. To attain an ideal Taylor cone and a better impact on spectral response, the nebulizer gas (gas 1) pressure was optimized at 55 psi. Fine tuning of the nebulizer and CAD gas was carried out to obtain a consistent and stable response. Ion spray voltage and temperature did not affect analyte response and were maintained at 5500 V and

550 °C, respectively. A dwell time of 600 ms was found adequate for OLZ and IS and no cross talk was observed between the MRMs of analyte and IS.

Chromatographic conditions including mobile phase selection, flow rate, column type and injection volume were optimized. Mobile phases containing different ratios (5:95, 10:90, 15:85, 20:80 and 30:70, *v*/*v*) of water–methanol/acetonitrile together with either formic acid, ammonia (0.01–0.005%), ammonium trifluoroacetate, ammonium acetate or ammonium formate buffers in varying strengths (2–20 mM) at flow rates of 0.5–1.0 mL/min were evaluated. A number of columns including Hypurity C8 (50 mm×4.6 mm, 5 μm), Hypurity cyano (50 mm×4.6 mm, 5 μm), Beta basic cyano (100 mm×2.1 mm, 5 μm), BDS Hypersil C18 (50 mm×4.6 mm, 5 μm) and ACE 5C18-300 (100 mm×4.6 mm, 5 μm) were also evaluated. Finally it was found that the ACE 5C18-300 column with a mobile phase consisting of acetonitrile : 0.01% ammonia in 2 mM ammonium formate solution (pH 6.6) (85:15, *v*/*v*) at a flow rate of 0.9 mL/min provided the best combination of efficiency, peak shape and resolution within 3.0 min. Under these conditions, the retention times for OLZ and IS were 2.36 and 2.05 min, respectively with a reproducibility for the OLZ retention time (as CV %) of ≤1.3% for 100 injections on the same column. Capacity factors for OLZ and IS based on a solvent front of 1.1 min were 0.86 and 1.15, respectively with a selectivity factor (α) of 0.74. The number of theoretical plates for OLZ and IS were 660 and 620, respectively with a resolution factor of 0.9.

In terms of the choice of IS, a deuterated analogue is preferable but was not available. Therefore an atypical antipsychotic belonging to the same class of dibenzothiazepines was selected in the present work. Quetiapine showed similar chromatographic behavior and did not affect analyte recovery, sensitivity or ion suppression.

As part of the investigation into the extraction efficiency of OLZ from plasma, the effect of the anticoagulants K_3EDTA was evaluated. Chin et al. extensively studied the potential matrix effects of anticoagulants and lipemia on an LC-MS/MS assay for OLZ and its metabolite. As a result, they suggested that K_3EDTA and Na-heparin should be avoided, especially for lipemic samples. Consistent with their observation, the best result was obtained using K_3EDTA as anticoagulant.

As is clear from Table 1, both liquid–liquid extraction (LLE) and solid phase extraction (SPE) have been used to extract OLZ. In fact, SPE has been successfully carried out on Varian C_8 BondElut[12], StepBio C_8, Oasis HLB and Oasis MCX columns. Similarly, LLE using heptane–isoamyl alcohol, hexane–dichloromethane, ethyl acetate-*n*-hexane–isopropanol and diethyl ether–dichloromethane has provided quantitative recoveries. Nevertheless, LLE sometimes required an additional back extraction step under acidic conditions to produce clean extracts. In the present work, protein precipitation (PP) using methanol and acetonitrile was first evaluated but gave poor recovery and considerable ion suppression. LLE under alkaline conditions using different solvents (diethyl ether, *n*-hexane, dichloromethane, methyl *tert*-butyl ether and ethyl acetate) alone and in combination was then evaluated but, although diethyl ether–dichloromethane gave promising results, the recovery was not consistent at all QC levels. As regards the previous use of SPE, Oasis MCX columns under acidic conditions (citric acid) gave quantitative recovery of psychotropic drugs and their metabolites and Raggi et al. obtained a recovery of OLZ of 97.9% using Oasis HLB under neutral conditions. Accordingly, SPE on Oasis columns with hydrophilic–lipophilic balance (HLB) were evaluated under both acidic and neutral conditions and shown to provide quantitative recovery with minimum matrix effects in both cases. However, given that a slightly higher recovery was obtained using an acidic media (100 μL 25% orthophosphoric acid), the latter conditions were used in the present work.

3.2. System suitability and carryover

During assay validation, the precision (CV, %) of a system suitability test was found to be in the range 0.11–0.54% for retention time and 1.6–2.2% for the area response of OLZ and IS. The signal-to-noise ratio for system performance was ≥25 for both analyte and IS. Carryover was shown to be negligible (≤0.51%) in that no enhancement in response was observed in extracted blank plasma (without IS and analyte) after subsequent injection of the ULOQ standard at the retention times of OLZ or IS.

3.3. Selectivity and interference

All samples were found to be free of interference from endogenous substances in plasma as shown in a–c. Similarly, in addition, the three antipsychotic drugs (clozapine, risperidone and aripiprazole) did not interfere in the determination of OLZ. This was because, although their retention times were 2.72, 2.60 and 3.21 min, respectively, their MRM transitions were different from that of OLZ. Accuracy (%) for OLZ at all QC levels was in the range 93.4–101.4%.

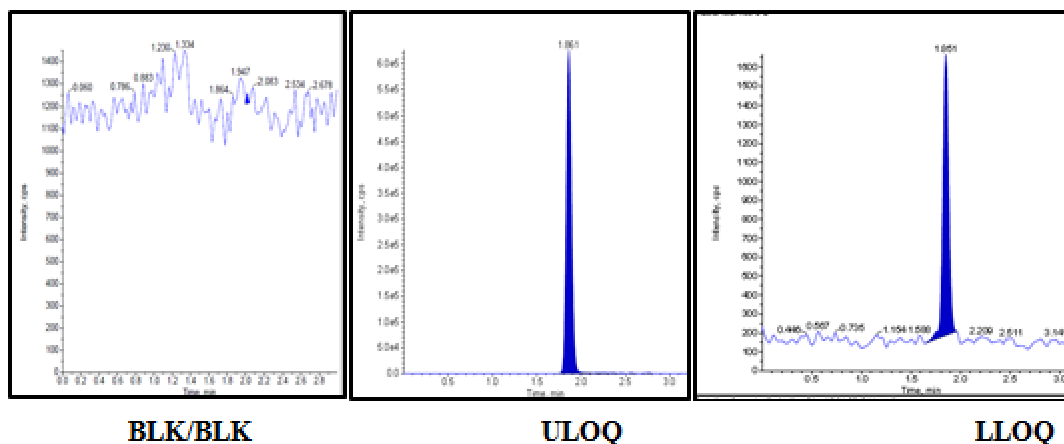


Figure3. MRM ion-chromatograms of olanzapine (m/z 313.2→256.2) and quetiapine (IS, m/z 384.2→253.2) in (a) blank plasma without analyte and IS, (b) blank plasma with ULOQ, (c) blank plasma with olanzapine at the LLOQ with IS with study sample at C_{max} after administration of a 5 mg dose of olanzapine.

3.4. Linearity, sensitivity, accuracy and precision

All six calibration curves were linear over the concentration range 0.10–40.0 ng/mL with correlation coefficients $r \geq 0.9996$. The equation of the calibration curve was y = (0.1196±0.0106) x−(0.0007±0.0001) where y is analyte/IS peak area ratio and x is analyte concentration. Accuracy (%) and precision (CV, %) of calibration standards were 97.0–103.1% and 1.2–3.9%, respectively. The LLOQ was 0.10 ng/mL at a signal-to-noise ratio ≥25 and the limit of detection (LOD) was 0.012 ng/mL.

Intra-day precision and accuracy were 0.6–4.3% and 93.3–102.3%, respectively. Inter-day precision and accuracy were 1.9–4.6% and 96.7–102.8%, respectively.

Table 1. Intra-batch and inter-batch accuracy and precision for olanzapine

QC	Conc. added (ng/mL)	Intra-batch				Inter-batch			
		n	Mean conc. found (μg/mL)[a]	Accuracy (%)	CV (%)	*n*	Mean conc. found (μg/mL)[b]	Accuracy (%)	CV (%)
LLOQ	0.10	6	0.10	100.0	4.3	18	0.10	100.0	4.6
LQC	0.30	6	0.28	93.3	2.3	18	0.29	96.7	2.1
MQC-2	4.00	6	3.89	96.3	3.2	18	3.00	100.0	3.2
MQC-1	12.0	6	11.9	99.4	1.1	18	15.7	98.1	2.1
HQC	24.0	6	23.9	97.7	0.6	18	29.8	99.3	1.9
ULOQ	30.0	6	30.8	102.3	1.9	18	41.1	102.8	2.6

n: total number of observations.

CV: coefficient of variation.

a=Mean of six replicate observations at each concentration.

b=Mean of 18 replicate observations over three different analytical runs

3.4. Recovery and matrix effects

The relative recoveries, absolute matrix effects and process efficiencies for OLZ and IS are presented in Table 2. The relative recovery of analyte is the "true recovery" since it is calculated by comparing the response (analyte/IS) of samples spiked before extraction with the response of samples spiked after extraction. The process efficiency/absolute recovery obtained for OLZ and IS was ≥88% at all QC levels. Furthermore, relative matrix effects which compares the precision (CV, %) between different lots of plasma samples spiked after extraction varied from 0.3 to 3.6% for OLZ at the LOQ and HQC levels (Table 2). Post-column analyte infusion chromatograms indicate there was no ion suppression or enhancement at the retention times of OLZ and IS. The average matrix factor value calculated as the response of post spiked samples divided by the response of neat solutions in mobile phase at the LLOQ level was 0.98 indicating suppression was only 2%.

Table 2. Absolute matrix effect, relative recovery and process efficiency for olanzapine

.QC	A[a] (CV, %)	B[b] (CV, %)	C[c] (CV, %)	Absolute matrix effect (ME, %)[d]	Relative recovery (RE, %)[e]	Process efficiency (PE, %)[f]
LQC	0.0324 (2.00)	0.0318 (0.44)	0.0290 (2.09)	98.2 (101.3)[g]	91.1 (97.5)[g]	89.5 (98.8)[g]
MQC-2	0.3389 (0.65)	0.3317 (2.54)	0.3081 (1.47)	97.9 (101.7)[g]	92.9 (96.4)[g]	90.9 (98.0)[g]
MQC-1	1.9075 (1.23)	1.8637 (3.09)	1.6792 (1.46)	97.7 (101.8)[g]	90.1 (96.9)[g]	88.0 (98.7)[g]
HQC	3.4498 (1.24)	3.4185 (2.29)	3.3059 (1.71)	99.1 (100.4)[g]	96.7 (94.1)[g]	95.8 (94.5)[g]

4. Conclusion:

Method for the bioanalytical method development and validation for estimation of olanzapine in k_3EDTA human plasma using Olanzapine D_3 as internal standard has been developed and validated. The analytes and IS were extracted from plasma by solid phase extraction using Oasis HLB 1cc (30mg) Extraction Cartridge and separated on a Cosmosil, 5µm, C18 150*4. 6 mm column using a 10 mM ammonium formate in Water : Acetonitrile (10 : 90) at a flow rate of 1.0 mL/min. OLZ and IS gave predominant, singly charged protonated precursor $[M+H]^+$ ions at *m/z* 313.2 and 384.2, respectively in Q1 full scan spectra. Furthermore, the most abundant ion in the product ion mass spectra of OLZ was at *m/z* 256.2, resulting from cleavage of the piperazine ring to the neutral fragment, $CH_3NHCH{=}CH_2$. During assay validation, the precision (CV, %) of a system suitability test was found to be in the range 0.11–0.54% for retention time and 1.6–2.2% for the area response of OLZ and IS. their MRM transitions were different from that of OLZ. Accuracy (%) for OLZ at all QC levels was in the range 93.4–101.4%. All six calibration curves were linear over the concentration range 0.10–40.0 ng/mL with correlation coefficients $r \geq 0.9996$.

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