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A Rapid and high Sensitive LC-MS/MS Method for the Quantification of Zolpidem Tartrate in Human Plasma and its application to pharmacokinetic study

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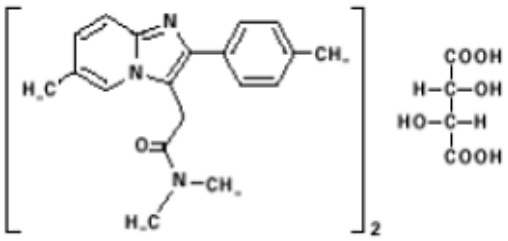
ABSTRACT

A rapid, high sensitive and selective liquid chromatography-tandem mass spectrometric (LC-MS/MS) method has been developed and validated for the quantification of Zolpidem (Zol) in human EDTA plasma using Mirtazapine (IS) as an internal standard. Analyte and Internal standard were extracted from human plasma by Solid-phase extraction (SPE) using Oasis HLB cartridges. The eluted samples were chromatographed on a C18 column by using a 20:80 v/v mixture of ammonium formate buffer (20 mM, pH 5.00) and acetonitrile as an isocratic mobile phase at a flow rate of 0.4 mL/min and analyzed by mass spectrometry in the multiple reaction monitoring mode using the respective $[M+H]^+$ ions, m/z *308.13 → 235.21 for Zolpidem and* m/z *266.35 → 195.31 for the IS. The linearity of the response/concentration curve was established in human plasma over the concentration range 0.10-149.83 ng/mL. The lower detection limit (LOD, S/N > 3) was 0.04 ng/mL and the lower limit of quantization (LOQ, S/N > 10) was 0.10 ng/mL. This LC-MS/MS method was validated with Intra-batch and Inter-batch precision of 0.67-9.82.The Intra-batch and Inter-batch accuracy was 87.70-107.53 respectively. Recovery of Zolpidem in human plasma is 87.70% and ISTD recovery is 85.78%. The main pharmacokinetic parameters were T_{max} (hr) = (1.50 ± 0.754), C_{max} (ng/mL) (115.341 ± 34.741), $AUC_{0\to t}$, = (663.614 ± 370.888) and $AUC_{0\to\infty}$, 694.020 ± 407.540 respectively. This method was successfully applied for the zolpidem 10 mg tablets bioequivalence study.*

Keywords: Zolpidem tartrate, UPLC-MS/MS, Human plasma, Bioequivalence study and Pharmacokinetic.

INTRODUCTION

Zolpidem (Ambien) is a prescription medication used for the short-term treatment of insomnia, (difficulty falling asleep or staying asleep) as well as some brain disorders. It is a short-acting no benzodiazepine hypnotic that potentiates gamma-amino butyric acid (GABA), an inhibitory neurotransmitter, by binding to $GABA_A$ receptors at the same location as benzodiazepines [1]. It works quickly (usually within 15 minutes) and has a short half-life (2–3 hours). Zolpidem has not adequately demonstrated effectiveness in maintaining sleep; however it is effective in initiating sleep [2]. Its hypnotic effects are similar to those of the benzodiazepine class of drugs, but it is molecularly distinct from the classical benzodiazepine molecule and is classified as an imidazopyridine. Flumazenil, a benzodiazepine receptor antagonist, which is used for benzodiazepine overdose, can also reverse zolpidem's sedative/hypnotic and memory impairing effects [3, 4]. Due to its selective binding, Zolpidem has very weak anxiolytic, my relaxant, and anticonvulsant properties but very strong hypnotic properties [5]. Zolpidem tartrate, chemically bis [N, N dimethyl-2[6-methyl-2-(4-methyl phenyl)] imidazo [1, 2-α] pyridine-3-yl] acetamide] (2R, 3R)-2,3dihydroxybutane dioate, is a hypnotic agent [6]. It has a molecular weight of 764.88. Fig.1. shows structure for Zolpidem tartrate.



Several analytical methods have been reported for the quantification of Zolpidem in human plasma on HPLC [7]. HPLC method with fluorescence detection [8-10] and LC-MS/MS [11-12]. The following are the advantages of the proposed method over those reported earlier: (1). Greater sensitivity is achieved (0.10 ng/mL) even with low plasma volumes and method is well suited for pharmacokinetic analysis. (2). Employing a single-step solid phase extraction procedure minimizes the chances of errors, saves considerable time and simplifies the sample preparation procedure. (3). Because of the use of less plasma volume (0.300mL), the volume of the sample to be collected for time point from subjects during the study is reduced significantly-this allows inclusion of additional points (4).The rapid sample analysis turnaround time of 3.00 minutes makes it an attractive procedure in high-throughput bioanalysis of Zolpidem in human plasma. The chromatographic conditions were optimized and the results of validation in terms of Specificity, linearity, precision, accuracy, extraction efficiency, dilution integrity, and Stabilities were provided. The devised method was used in Zolpidem tartrate bioequivalence study, which was conducted in accord with USFDA guidelines [13]. Typical bioavailability including $AUC_{0\rightarrow t}$ (the area under plasma concentration-time curve) and C_{max} (the maximum plasma concentration) $AUC_{0\rightarrow\infty}$, (Area under the concentration time-curves from time zero to infinity) parameters were compared.

MATERIALS AND METHODS

Experimental

2.1 Chemicals and materials

Reference standards of Zolpidem (Potency-97.1%) and Mirtazapine (Potency-99.5%) were procured from Aurobindo pharma Ltd. (Hyderabad, India). Methanol and Acetonitrile were of HPLC Grade purchased from J.T. Baker (Philipsburg, USA). Analytical-grade Ammonium formate was purchased from sd fine chemicals (Mumbai, India.), Oasis HLB, 1CC, 30mg cartridges were purchased from Waters (Milford, USA), and Formic acid (AR Grade) was purchased from (RFCL Chemicals New Delhi, India). Polypropylene vials (Torsens products Pvt Ltd Kolkata, India.) Water used for the LC-MS/MS analysis was prepared using a Milli Q water purification system procured from Millipore (Bangalore, India). Human plasma was procured from Cauvery Diagnostics and blood bank Hyderabad, India).and was stored at -20°C until use.

Fig.2.

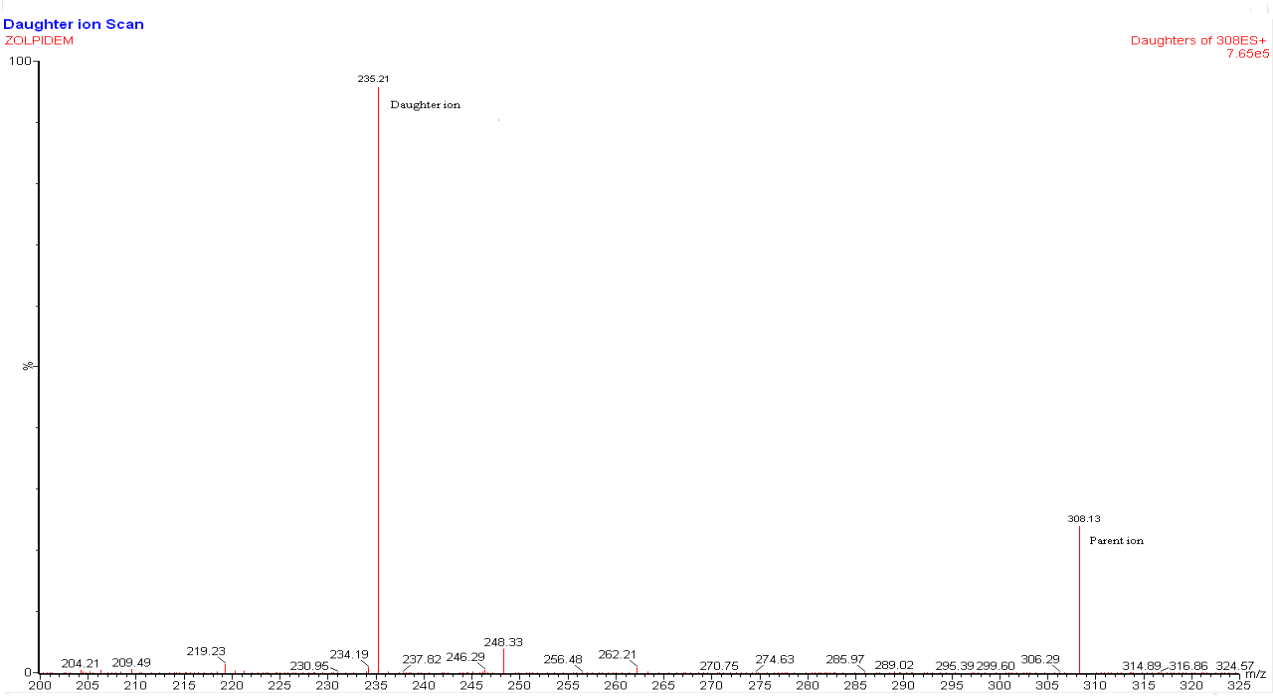


Fig. 2 (A). Product ion mass spectra of Zolpidem (m/z 308.13→235.21, scan range 200-325 amu)

2.2. Liquid chromatographic conditions

A waters Acquity UPLC system (Milford, MA, USA) consisting of binary solvent manager, sample manager and column manager was used for setting the reverse-phase liquid chromatographic conditions. The separation of Zolpidem and Mirtazapine (IS) was performed on X-terra RP8 (50mm×4.6mm (length inner diameter), with 5 µm particle size) and was maintained at 35 °C in column oven. The mobile phase consists of 20mM Ammonium formate (pH 5.00±0.05) and acetonitrile in 10:90 (v/v) ratio. For isocratic elution, the flow rate of the

mobile phase was kept at 0.40 ml/min. The total chromatographic run time was 3.0 min. The sample manager temperature was maintained at 10°C and the pressure of the system was 800 psi.

2.3. Mass spectrometric conditions

Ionization and detection of analyte and IS was carried out on a triple quadrupole mass spectrometer. WATERS, Quattro Micro (Milford, MA, USA) equipped with electro spray ionization and operating in positive ion mode. Quantization was performed using multiple reaction monitoring (MRM) mode to monitor Parent → Product ion (m/z) transitions for Zolpidem 308.13 → 235.21 and 266.35 → 195.31 for IS respectively. (Fig. 2 shows the product ion mass spectra of analyte and IS).

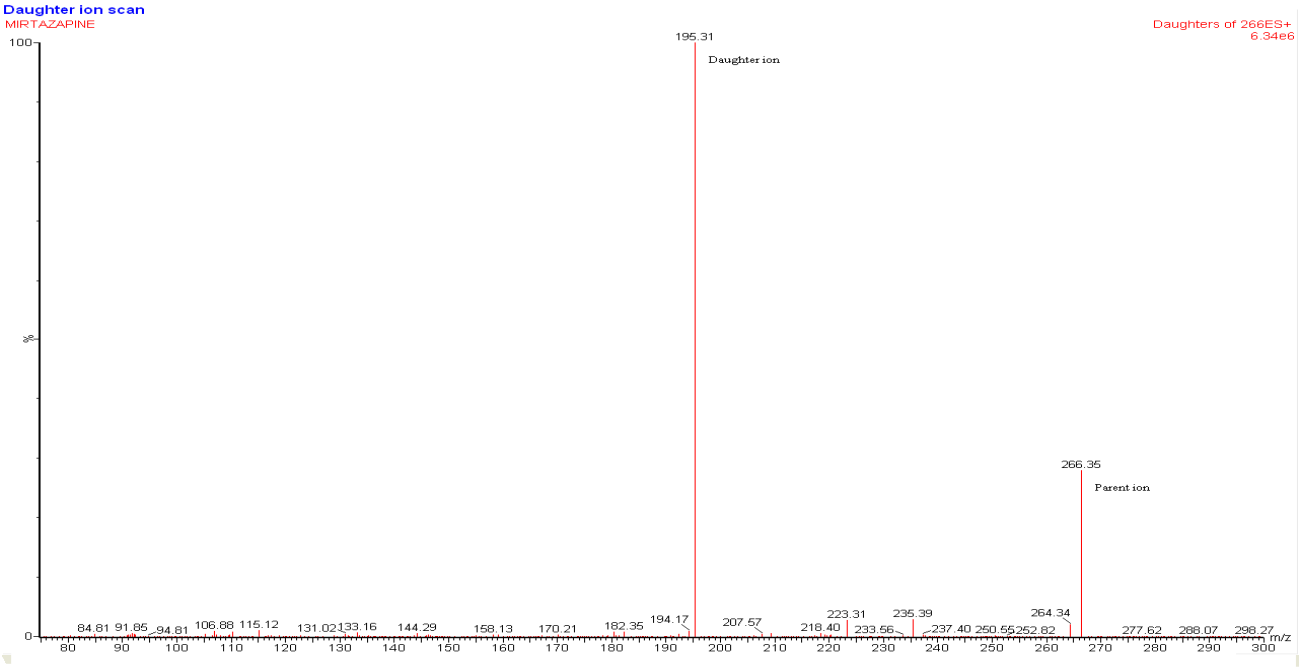


Fig. 2 (B). Product ion mass spectra of Mirtazapine (m/z 266.35→195.31 amu), scan range 80-300 amu

The source dependent parameters maintained for Zolpidem and Mirtazapine were capillary; 3.50kV; extractor; 2.0V; RF lens; 0.0V; source temperature: 100°C; desolvation temperature: 400°C;cone gas flow; 50±10L/h desolvation gas flow: 600±10L/h. The optimum values for compound dependent parameters (MRM file parameters) like cone voltage and collision energy set were 50 V and 35.0 eV for the analyte and 20.0V and 25.0eV for IS respectively. The dwell time easy set at 500ms. Mass Lynx software version 4.1 was used to control all parameters of UPLC and MS.

2.4. Standard stock, calibration standards and quality control sample preparation.

The standard stock solution of 1 mg/mL of Zolpidem and Mirtazapine was prepared by dissolving requisite amount in methanol. Calibration standards and quality control (QC) samples were prepared by spiking (2 % total volume of blank plasma) blank plasma with stock solution. Calibration curve standards were made at 0.10, 0.20, 1.00, 50.01, 100.03, 125.03 and 149.83 ng/mL respectively while quality control samples were prepared at four levels, viz. 113.85

ng/mL (HQC, high quality control), 68.88 (MQC, middle quality control), 0.30 ng/mL (LQC low quality control), 0.10 ng/mL (LLOQQC lowest level quality control). Stock solution (1 mg/mL) of the internal standard was prepared by dissolving 1 mg of Mirtazapine in 10 mL of methanol. An aliquot of 10.0 µL of this solution was further diluted to 10.0 mL in the same diluent to obtain solution of 1.0 µg/mL. All the solutions (standard stock, calibration standards and quality control samples) were store at 2-8°C until use.

2.5. Protocol for sample preparation

Prior to analysis, all frozen subjects samples, calibration standards and quality control samples were thawed and allowed to equilibrate at room temperature. To an aliquot of 250µL of spiked plasma sample, 50µL internal standard was added and vortexed for 20 s. Further, 250µL of 20 mM Ammonium formate was added and vortexed 20 s. Condition the HLB 1CC 30mg cartridges with 1.0 ml of methanol and followed by 1.00 mL of water, load the total volume of prepared sample and elute slowly and completely, was the cartridges with 1.0 mL of water twice. Dry the cartridges for two minutes under vacuum elute the cartridges with 0.250 mL of methanol twice and 20 µL was injection in the chromatographic system.

2.6. Method validation

The method validation was performed as per USFDA guidelines [14]. System suitability experiment was performed by injecting six consecutive injections using acqueous standard mixture of Zolpidem and internal standard at the start of each batch during the method validation.The carryover effect of the autosampler was evaluated by injecting a sequence of injections solutions of aqueous standard, Mobile phase, standard blank, extracted standard equivalent to highest standard in the calibration range. As per the acceptance criteria, the response in blank should not be greater than 20% of LLOQ response [15]. The linearity of the method was determined by analysis of five linear curves containing eight non-zero concentration. The ratio of area response for drug and IS was used for regression analysis. Each calibration curve was analyzed individually by using least square weighed $(1/x^{2)}$ Linear regression. The lowest standard on the calibration curve was accepted as the lower limit of quantitation (LLOQ), if the analyte response was at least five times more than that of the drug free (blank) extracted plasma. The deviation of than that of drug free (blank) extracted plasma. The deviation of standards other than LLOQ from nominal concentration should not to be more than ± 15.0%.

The selectivity of the method towards endogenous plasma matrix components was assessed in twelve batches (7 normal of K2 EDTA plasma, 2 haemolysed, 2 lipidemic and and 1 heparinised) of blank human plasma. This was done to estimate the extent to which endogenous plasma components contribute towards interference at the retention time of analytes and IS. The cross talk of MRM for analytes and IS was checked using highest standard on calibration curve and working solution of IS.

For determining the intra-day accuracy and precision, replicate analysis of plasma samples of Zolpidem was performed on the same day. The run consisted of a calibration curve and six replicates of LLOQ, LQC, MQC and HQC samples. The inter-day accuracy and precision were assessed by analysis of three precision and accuracy batches on three consecutive validation days. The precision of the method was determined by calculating the percent coefficient of variation (%CV) for each level. The deviation at each concentration level from the nominal concentration

was expected to be within ± 15.0% except LLOQ, for which it should be within ± 20.0%.

The relative recovery, matrix effect and process efficiency were assessed as recommended by [16]. (Matuszewski.) All three parameters were evaluated at Std-1, Std-3, Std-5, Std-6 and Std-8 levels in six replicates. Relative recovery (RE) was calculated by comparing the mean area response of extracted samples (spiked before extraction) to that of unextracted samples (spiked after extraction) at each CC level. The recovery of IS was similarly estimated. Absolute matrix effect (ME) was assessed by comparing the mean area response of unextracted samples (spiked after extraction) with mean area of neat standard solutions. The overall 'process efficiency' (%PE) was calculated by comparing the mean area response of extracted samples (spiked before extraction) to that with mean area of neat standard solutions at each CC level.The assessment of relative matrix effect was based on direct comparison of the MS/MS responses (peak areas) of the analytes spiked into extacts originating from different lots of plasma. The variability in these responses, expressed as %CV was considered as the measure of relative matrix effect.

Stability experiments were carried out to examine the analyte stability in stock solutions and in plasma samples under different conditions. Short term stability at room temperature and long term stability of spiked solution stored at – 70ºC was assessed by comparing the area response of stability sample of analyte and IS with the area response of sample prepared from fresh stock solutions. The solutions were considered stable if the deviation from nominal value was within ±10%. Autosampler,wet extract stability, bench top stability, dry extract stability and freeze-thaw stability were performed at LQC and HQC, using six replicates at each level. The samples were considered stable if the deviation from the mean calculated concentration of freshly thawed quality control samples was within ±15%.

To authenticate the ruggedness of the proposed method, it was done on two precision and accuracy batches. The first batch was analysed by different analysts while the second batch was analysed on different column and different LC-MS/MS. Dilution integrity experiment was conducted by diluting the stock solution prepared as spiked standard at concentration of 299.66 ng/mL for Zolpidem. The precision and accuracy for dilution integrity standards at 1/5th and $1/10^{th}$ determined by analyzing the samples against calibration curve standards.

2.7. Bioequivalence study design

A pharmacokinetic study was conducted on 12 healthy, adult, male, human subjects under fed conditions. (n = 12) following oral administration of Zolpidem Tartrate 10 mg tablets. Each volunteer was judged to be in good health through medical history, physical examination and routine laboratory tests. Written consent was taken from all the volunteers after informing them about the objectives and possible risks involved in the study. An independent ethics committee constituted as per Indian council of Medical Research (ICMR) approved the study protocol. The study was conducted strictly in accordance with guidelines laid down by international conference on Harmonization and USFDA [17]. A single oral dose of 10 mg drug was given to the volunteers with 240 mL of water. Blood samples were collected at 0.0 (pre-dose), 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.501, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00 and 24.00 h after oral administration of the dose for test formulation in labeled K2 EDTA- vaccuettes. Plasma was separated by centrifugation (3200 × g, 10 º c, 10 min) and kept

frozen at – 70 º c until analysis. During study, volunteers had a standard diet while water intake was free.

RESULTS AND DISCUSSION

3.1. Method development

Chromatographic resolution of Zolpidem and IS was initiated under isocratic conditions to obtain adequate response, sharp peak shape and a short analysis time. Thus, separation was tried using various combinations of methanol/acetonitrile, acidic buffers and additives like formic acid on different reversed-phase columns with 5µm particle size viz. Chromolith, Hypersil, X-terra, Kromasil, Intertsil and Grace ACE Cyano (150 mm and 250mm × 4.6 mm), Chromolith RP-18 (50 mm × 4.6 mm), Kromasil (50 mm and 100 mm × 4.6 mm), and Gemini C-18 (50 mm × 4.6 mm) to find the optimal column that produced the best sensitivity, efficiency and peak shape. The analytes showed poor separation and reproducibility for proposed linear range except for X-terra RP-8 column that offered superior peak shape, baseline separation, desired linearity and reproducibility. The mobile phase consisting of 20mM ammonium formate adjusted the pH 5.00 ± 0.05 with formic acid and acetonitrile (20:80, v/v) ratio and having 20mM ammonium formate pH around 5.0-5.5 were found most suitable for eluting Zolpidem and IS at 1.37 and 1.46 min respectively. Also, the reproducibility of retention times for the analytes, expressed as %CV was ≤2% for 100 injections on the same column.

The inherent selectivity of MS/MS detection was also expected to be beneficial in developing a selective and sensitive method. The present study was conducted using ESI as the ionization source as it gave high intensity for drug and IS as they have similar sites for protonation. Initially, the extraction of Zolpidem and IS was carried out via protein precipitation with common solvents like acetonitrile, methanol and acetone, but the sensitivity and reproducibility were poor, in all the solvents with frequent clogging of the column, which required online flushing of the column. Liquid-liquid extraction technique was also tested to isolate the drugs from plasma using diethyl ether, dichromethane, ethyl acetate, methyl tert butyl ether and isopropyl alcohol (alone and in combination) as extracting solvents. However, the recovery was inconsistent with some ion suppression (greater than 15% CV) in most of these solvent systems. Hence solid-phase extraction with waters HLB-1CC, 30mg cartridges solved the problem, sample clean up and matrix effect. Trying with different ion exchange resigns and elution solvents, HLB cartridges with methanol elution proved to give consistent recoveries for the analyte and IS, especially at the LLOQ level with minimum matrix interference. Mirtazapine was used as an internal standard (IS) in the present study, which had similar chromatographic behavior and was quantitatively extracted with the proposed extraction procedure. Also, there was no effect of IS on analyte recovery, sensitivity or ion suppression.

3.2. System suitability and auto sampler carryover

Throughout the method validation, the % CV of system suitability was observed below 4.0 at the retention time of Zolpidem and the IS. Carryover evaluation was performed in each analytical run so as to ensure that it does not affect the accuracy and the precision of the proposed method. There was negligible carryover (≤4% of the LLOQ response) observed during autosampler carryover experiment, No enhancement in the response was observed in double blank after

subsequent injection of highest calibration standard (aqueous and extracted) at the retention time of analytes and IS.

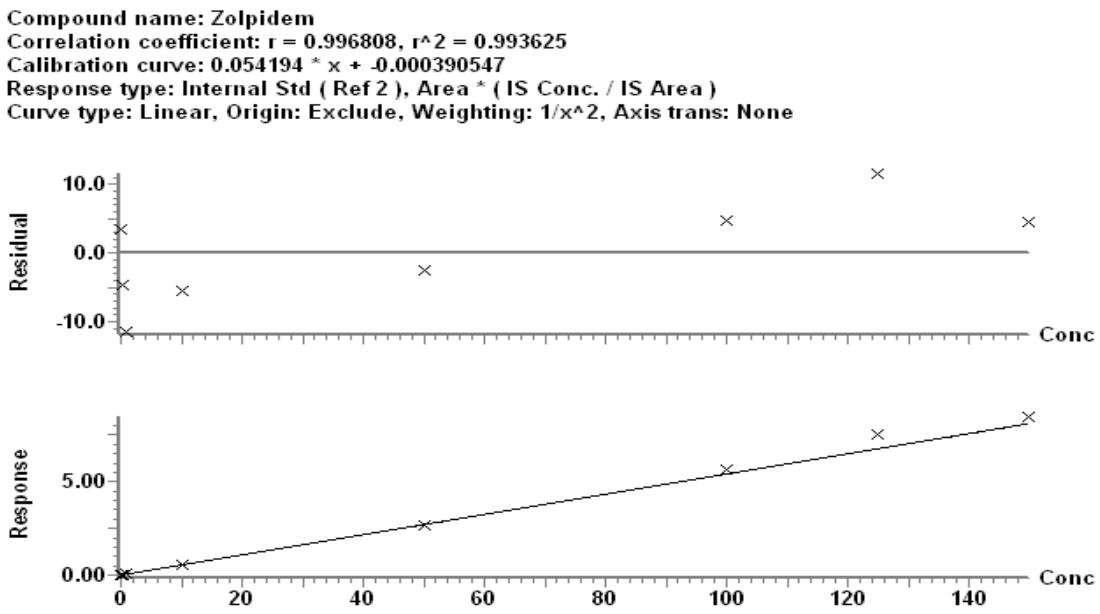
3.3. Linearity and lower limit of quantification (LLOQ)

The calibration curves were linear over the concentration range of 0.10 to 149.83 ng/mL with correlation coefficient r2 ≥ 0.9939 for Zolpidem respectively. The equations for means (n=5) of five calibration curves for Zolpidem. The standard deviation value for slope, intercept observed were 0.0446 and 0.00086. The equations for means (n=5) of five calibration curves for Zolpidem respectively. The accuracy and precision (%CV) observed for the calibration curve standards ranged from 92.70 to 99.71% and 0.67 to 7.32% respectively. The lowest concentration (LLOQ) in the standard curve for both the isomers was measured at a signal-to-noise ratio (S/N) of ≥ 100.

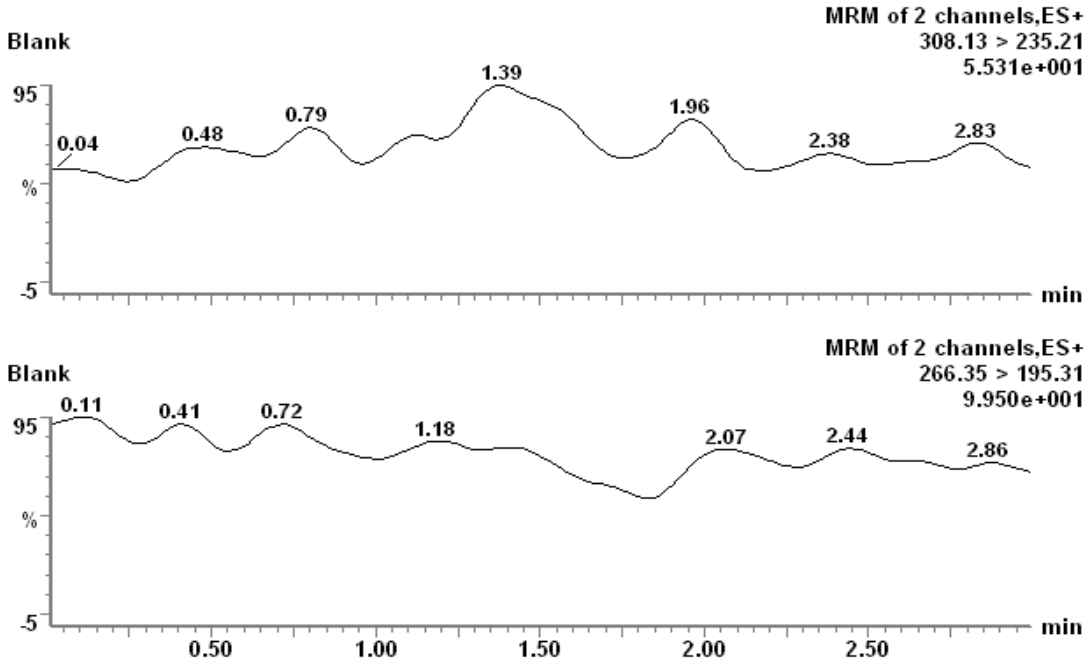
Selectivity, accuracy and precision

To establish the selectivity of the method for interference due to endogenous plasma components from haemolysed, lipidemic, heparinised and K2 EDTA blank plasmas, the % change in the area ratio (analyte/IS) at LLOQ level was within 4-8%, while the precision (%CV) in their measurement varied from 2.7 to 4.3%. The extraction procedure together with mass detection gave very good selectivity for the analysis of both the drug and IS in the blank plasma. No endogenous interferences were found at the retention times of analytes and IS. Figure.3. Representative MRM ion chromatograms extracted (A) Calibration curve of Zolpidem. (B). Blank human plasma (double blank), (C) blank plasma fortified with IS (m/z 266.35→195.31), (D). Zolpidem at LLOQ (m/z 308.13→235.21) the selectivity of the method.

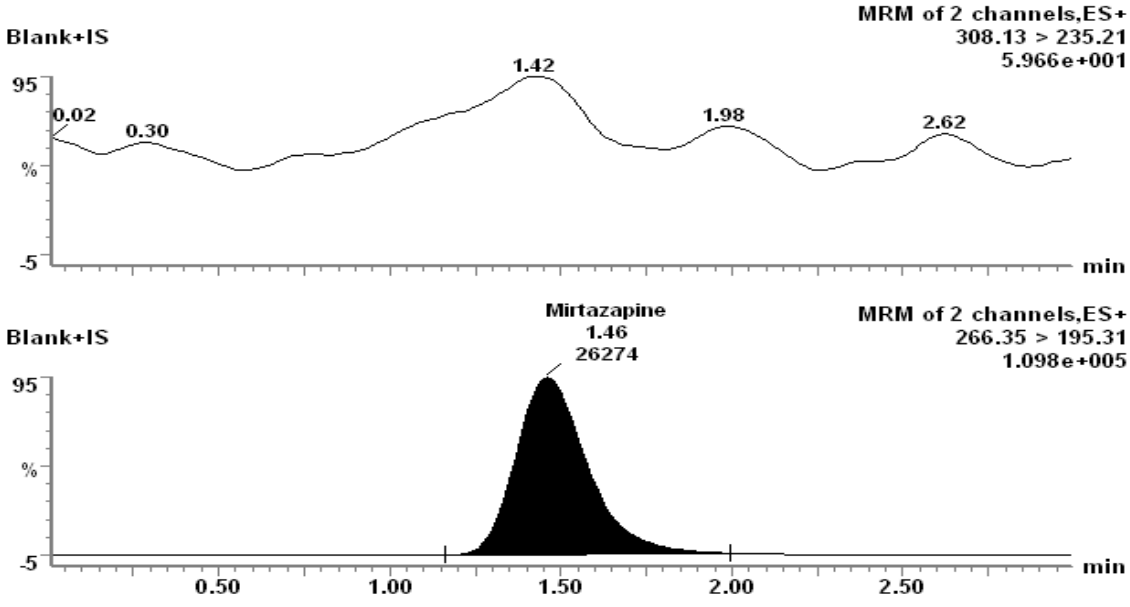
Fig. 3.
(A) Calibration curve of Zolpidem



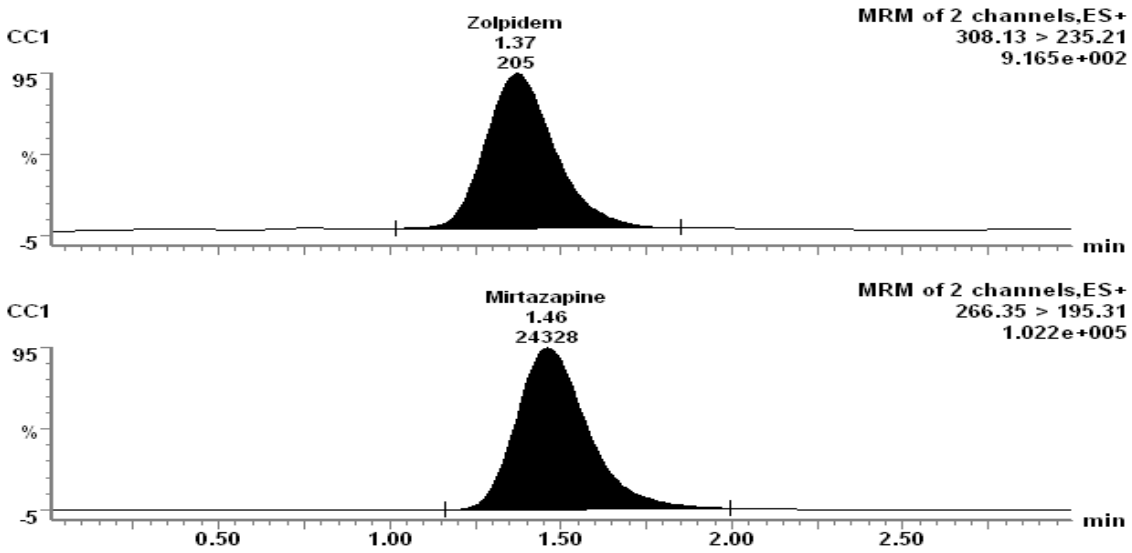
(B) Double blank plasma (without IS)



(C) Blank plasma with IS



(D) Zolpidem and Mirtazapine at LLOQ (m/z) 308.13→235.21



The intra- and inter batch precession and accuracy were established from validation runs performed at HQC, MQC, LQC and LLOQ QC levels. The intra- and inter batch precision ranged from 4.00to 9.82% for Zolpidem.The accuracy values were within 87.70-107.53% for both the analytes in intra- and inter batches. The precision and accuracy values for intra- and inter day experiments in plasma are shown in Table 1.

Table 1. Comparison of intra- and inter-batch precision and accuracy for Zolpidem

QC ID	Nominal concentration (ng/mL)	Intrabatch			
		n	Mean concentration observed (ng/mL)	% CV	% Accuracy
LLOQQC	0.10	6	0.10	9.82	96.33
LQC	0.30		0.29	9.35	95.06
MQC	68.88		60.41	7.63	87.70
HQC	113.85		122.42	4.00	107.53

QC ID	Nominal concentration (ng/mL)	Inter batch			
		n	Mean concentration observed (ng/mL)	% CV	% Accuracy
LLOQQC	0.10	24	0.10	6.80	99.71
LQC	0.30		0.29	7.32	97.97
MQC	68.88		63.86	0.67	92.70
HQC	113.85		107.37	2.21	94.31

3.4. Recovery and stability results

The relative recovery, absolute matrix effect and process efficiency data at LQC, MQC and HQC levels is presented. The recovery for drug and IS in human plasma was 87.94% and 85.78%. Further, the extent of matrix effect in different lots of plasma (spiked after extraction) was within the acceptable limit as evident from the precision (%CV) values in Table 2.

Table 2 . Stability of Zolpidem under various conditions (n=6)

Storage conditions	Nominal concentration (ng/mL)	Mean calculated conc. (ng/mL) ± SD	% Mean accuracy
Bench top stability (After 5.67 h at ~ at 25°C			
LQC	0.30	0.29 ± 0.027	98.49
HQC	113.85	121.91 ± 7.638	107.08
Freeze thaw stability (3 Cycles)			
LQC	0.30	0.32 ± 0.013	106.86
HQC	113.85	103.73 ± 2.791	91.11
Dry extract stability (25.82 h below 10°C)			
LQC	0.30	0.29 ± 0.021	96.88
HQC	113.85	117.63 ± 11.212	103.32
Wet extract stability (26.43 h below 10°C)			
LQC	0.30	0.30 ± 0.021	101.28
HQC	113.85	114.33 ± 8.374	100.42
Auto sampler stability (21.88 h 10°C)			
LQC	0.30	0.29 ± 0.024	97.05
HQC	113.85	105.68 ± 6.159	92.82
Long term stability in plasma at -70°C (23.66 days a 70°C)			
LQC	0.30	0.32 ± 0.0026	108.25
HQC	113.85	120.17 ± 1.261	105.55

Table 3 Absolute matrix effect, relative recovery and process efficiency for Zolpidem

Analyte ISTD	(%CV) [a]	(%CV) [b]	(%CV) [c]	Absolute matrix effect (%ME) [d]	Relative recovery (%RE) [e]	Process efficiency (%PE) [f]
STD 1 Zolpidem Mirtazapine	135 (3.87) 26233 (0.70)	124 (2.42) 25723 (0.67)	116 (2.22) 22752 (0.39)	91.87 98.06	93.57 88.45	85.96 86.73
STD 3 Zolpidem Mirtazapine	1217 (1.19) 26366 (0.55)	1255 (2.47) 26532 (0.75)	1135 (2.19) 23258 (0.22)	103.05 100.63	90.45 87.66	93.21 88.21
STD 5 Zolpidem Mirtazapine	70076 (0.66) 25385 (0.52)	68461 (0.81) 25385 (0.52)	61355 (3.55) 22131 (1.70)	97.70 100.00	89.62 87.18	87.56 87.18
STD 6 Zolpidem Mirtazapine	121740 (3.66) 22604 (2.18)	108577 (2.82) 21287 (1.75)	87661 (2.54) 17488 (1.51)	89.19 94.17	80.74 82.15	72.01 77.36
STD 8 Zolpidem Mirtazapine	132191 (2.23) 17910 (1.42)	121904 (1.36) 17385 (1.28)	104021 (1.49) 14508 (1.16)	92.22 97.07	85.33 83.45	83.48 84.10

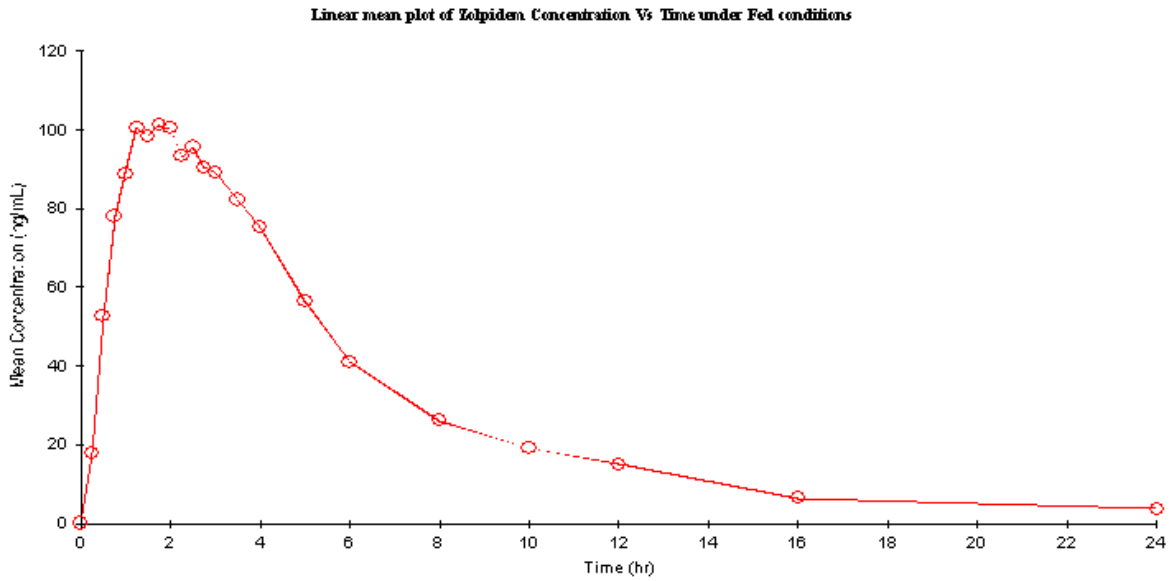
a Mean area response of six replicate samples prepared in Mobile phase (neat samples)
b Mean area response of six replicate samples prepared by spiking in post extracted blank
c Mean area response of six replicate samples prepared by spiking in plasma before extraction
d % Matrix effect: Post extracted mean response/Aqueous (Neat) mean response x 100
e %Recovery: Extracted mean response / Post extracted mean response x 100
f %Process efficiency: Extracted mean response / Aqueous Mean response x 100

Stock solutions for short term stability of Zolpidem and IS were stable at room temperature for minimum period of about 6 h and between 2 and 8 °C for about 7 days. Zolpidem in control human plasma (bench top) at room temperature was stable at least for 5.08 h at ambient temperature and for minimum of three freeze and thaw cycles. Auto sampler stability of the spiked quality control samples maintained at 10 °C was maintained up to 21.55 h. Long-term stability of the spiked quality control samples stored at -70 °C was determined up to 18.12 days. The accuracy values for different stability experiment in plasma are shown in Table 3.

5. Ruggedness and dilution integrity

The results of ruggedness study for Zolpidem was well within the acceptance limit of 15% in Precision and 85.0-115.0. % in mean accuracy. The precision and accuracy values for both experiment at LLOQ, LQC, MQC and HQC levels for Zolpidem ranged from 2.2 to 8.6% and 95.3 to 106.5% respectively. The dilution integrity experiment was performed with an aim to validate the dilution test to be carried out on higher analyte concentration above the upper limit of quantification (ULOQ), which maybe encountered during real subject sample analysis. The precision and accuracy values for 1/5th and 1/10th dilution ranged from 2.5 to 4.9% and 95.1 to 108.6 for Zolpidem.

Fig. 4 Mean plasma concentration-time profile of Zolpidem tartrate 10 mg tablets formulation to 12 healthy volunteers



Parameter	Zolpidem
C_{max} (ng/mL)	115.341 ± 34.741
T_{max} (hr)	1.50 ± 0.754
$AUC_{0\rightarrow t}$	663.614 ± 70.888
$AUC_{0\rightarrow \infty}$	694.020 ± 407.540

3.6.Application of the method in healthy human subjects

The validated method was successfully applied for the assay of Zolpidem in healthy male Indian volunteers in the age group of 18-45 years. Fig. 4 shows the plasma concentration vs. time profile of Zolpidem human subjects under fating condition. The method was sensitive enough to monitor the Zolpidem plasma concentration up to 24 h. Approximately 700 samples including the calibration and QC samples were within the acceptable limits. The pharmacokinetic parameters viz. Cmax, Tmax, AUC0→t, and AUC0→∞ were calculated for Zolpidem in test formulations. (Figure 4). Shows the data of Mean plasma concentration-time profile of Zolpidem tartrate 10 mg tablets formulation to 12 healthy volunteers

CONCLUSION

In summary, we have developed and validated a selective, reproducible and high-throughput LC-MS/MS method to quantify Zolpidem using Mirtazapine as IS. To the best of knowledge, the cost effectiveness, simplicity of the assay of solid-phase extraction and sample turnover rate of less than 3.00 min per sample made it possible to analyze more than 250 plasma samples per day in high-throughput bioanalysis of Zolpidem. As discussed being a highly rugged method, it can be used in HPLC system. The method is proved to be reproducible with a good recovery and minimum Matrix effect and three freeze-thaw cycles, stock solutions were stable at room temperature for at least 15 days. In LC/MS-MS analytical module and from the results of the validation parameters, we can conclude that the developed method can be useful for BA/BE studies and routine therapeutic drug monitoring (TDM) with desired precision and accuracy.

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