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Research Article

DEVELOPMENT AND VALIDATION OF A BIOANALYTICAL METHOD FOR DETERMINATION OF QUETIAPINE FROM HUMAN PLASMA

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ABSTRACT
A simple high-performance liquid chromatographic (HPLC) method for the analysis of the antipsychotic drug quetiapine fumarate in human plasma has been developed. Zolpidem tartrate was employed as the internal standard (IS). Biological samples were pretreated by liquid-liquid extraction (LLE) technique using tert-butyl methyl ether (TBME). Separation was performed on a HiQSil C18HS (250x4.6 mm, 5µm) column. The mobile phase used was Acetonitrile-ammonium acetate buffer (pH 3.5, 10 mM) (40:60 v/v) pumped at a flow rate of 1 mL/min. Samples were injected by means of an autosampler via a variable loop and detected using UV detector at a wavelength of 254 nm. A good linearity was found in the concentration range of 100-2000 ng/mL. Within and between batch precision and accuracy of the proposed method were evaluated by percent relative standard deviation (% R.S.D.) and percent relative error (% RE) respectively; both being within the acceptable limits. The method was also validated for recovery, carry-over and stability. Freeze and thaw stability, short term stability and long term stability were evaluated for the developed bioanalytical method. The described method can be applied for quantitation of quetiapine in real clinical samples.
Keywords: Quetiapine, human plasma, HPLC, bioanalytical method validation.

INTRODUCTION

The emergence of the field of bioanalysis as a critical tool during the process of drug discovery and development is well understood and globally accepted. Over the past few decades, a plethora of bioassays has been continuously developed for NCEs to support various stages of discovery and development, including assays for important metabolites[1]. The need for clinical monitoring of patients undergoing therapy of antipsychotic drugs is still evident because the onset of side effects is often related to high plasma concentrations of these drugs. Clinical monitoring of patients can significantly improve the knowledge of pharmacological interactions among different antipsychotic drugs, as well as enhance the compliance of the patients, thus leading to higher treatment efficacy[2]. Plasma levels of psychoactive drugs resulting from a given dose are highly variable between individual patients. This is primarily due to inter-individual variations in compliance and in the activities of the various drug metabolizing enzymes which leads to poor predictability of drug concentrations at a given dose[3]. Therefore reliable bioanalytical methods are needed to carry out efficient clinical therapeutic drug monitoring and drug metabolism studies.

Quetiapine fumarate, chemically 2-[2-(4-dibenzo(b,f)(1,4)thiazepine-11yl-1-piperazinyl) ethoxy] ethanol (E)- 2-butanedioate is a dibenzothiazepine atypical antipsychotic agent (**Figure 1**) bearing structural similarity to clozapine and olanzapine[4]. It is a serotonin and dopamine receptor antagonist used in the management of psychotic disorders like schizophrenia, bipolar disorder and as an add-on to treat depression. Quetiapine has a wide therapeutic dose range of 150 to 750 mg per day, in which it is effective in improving the positive and negative symptoms of schizophrenia. It is well absorbed following oral administration. Peak plasma concentrations are reached in about 1.5 hours. Quetiapine is widely distributed throughout the body with an apparent volume of distribution of 10+4 L/kg. It is about 83% bound to plasma proteins. It is extensively metabolized in the liver by P450 isoenzyme CYP3A4 and by oxidation. The elimination half-life has been reported to be about 6 to 7 hours[5].

Several HPLC methods for the determination of quetiapine from biological fluids have been reported in the literature. With regards to the analytical technique used for determination from human plasma, some of the methods have utilized HPLC with UV and electrochemical detection[6], UV detection[7-10] and mass detection[11-15]. The reported methods are however time-consuming, required complex sample pretreatment and had long run times of analysis. Gas chromatography–mass spectrometry (GC–MS) methods have also been employed in which quetiapine needs to be derivatized before analysis[16-18].

In this paper, we present a simple, selective and cost effective HPLC method for determination of Quetiapine from human plasma.

MATERIALS AND METHODS

Chemicals

Quetiapine fumarate and zolpidem tartrate (internal standard) (**Figure 2**) were procured from Shreeji Pharma International (Gujrat, India). Acetonitrile (HPLC Grade), tert-butyl methyl ether (TBME) (HPLC Grade), dichloromethane (AR grade), ethyl acetate (AR grade), methanol (AR grade), ammonium acetate (AR) and acetic acid (AR grade) were purchased from S. D. Fine Chemicals, (Mumbai, India). Ultrapure water was obtained from PURELAB Flex (ELGA). All the solvents to be used for HPLC analysis were first filtered through 0.45µm filter and then sonicated to remove entrapped air bubbles.

Blank human plasma was purchased from the blood bank of K. E. M. Hospital, Mumbai. Plasma from six sources was obtained which was stored in deep freezer at -20^{0}C until used.

Apparatus and chromatographic condition

HPLC analysis was performed on Agilent 1200 series HPLC system. It consists of a degasser, a quadratic pump compartment, an autosampler with a variable injection loop (10µL to 100 µL), a column compartment and variable wavelength UV detector (VWD). The instrument was operated using computer via EZChrom Elite software version 3.2.1.

Quetiapine was subjected to chromatographic analysis using HiQSil C18HS (250x4.6 mm, 5µm) column. The mobile phase consisting of Acetonitrile-ammonium acetate buffer (pH 3.5, 10 mM) (40:60 v/v) was pumped at a flow rate of 1 mL/min. The column oven temperature was kept at room temperature (25ºC). The volume of the sample injected for each analysis was 50 µL and UV detector was set at 254 nm.

Preparation of stock and working solutions

Stock solutions of quetiapine and IS having concentration of 100 µg/mL (100 ppm) were prepared in methanol. The standard stock solution of quetiapine (100 µg/mL) was diluted to obtain working standard solutions with concentrations 1, 2, 4, 8, 10, 15, 20 µg/mL. The IS working solution of 10 µg/mL was prepared by diluting stock solution with methanol. All stock and working solutions were stored at 8°C until use.

Preparation of calibration standards and QC samples

Working solutions were used to spike the plasma to yield calibration curve standards corresponding to the concentration range of 100-2000 ng/mL. Quality control (QC) solutions were spiked in plasma to obtain concentrations of 300 (Low Quality Control i.e. LQC), 900 (Middle Quality Control i.e. MQC) and 1800 ng/mL (High Quality Control i.e. HQC).

Sample preparation

Liquid-liquid extraction technique was used to extract the drug from spiked plasma samples. 225 µL blank human plasma was spiked with 25 µL of appropriate concentration of drug solution and 25 µL IS solution in an eppendorf tube. After vortexing for 30 seconds, 1.5 mL extracting solvent was added and was mixed again for 1 minute on the vortex mixer. The eppendorf tubes were than centrifuged at 5000 rpm for 5 minutes at -15ºC. 1.3 mL aliquot was transferred to test tubes and evaporated to dryness under a steady stream of nitrogen gas at 50ºC for 2 minutes. The residue thus obtained was reconstituted with 250 µL mobile phase and 50 µL was injected into HPLC system for chromatographic analysis.

Bioanalytical method validation

The fundamental validation parameters to be studied for a bioanalytical method are described in various guidelines such as US-FDA guidance for industry[19] and EMEA guidelines[20]. The parameters selected for validation of the developed method were selectivity, linearity, accuracy, precision, recovery, carryover and stability.

Selectivity: Selectivity was ensured at the lower limit of quantification (LLOQ) of the drug as well as for the internal standard. The experiment was performed using plasma from six different sources. Absence of interfering components is accepted where the response for interferent is less than 20% of LLOQ for the analyte and less than 5% for IS.

Linearity: Standard curves were generated on each validation day. The linearity was confirmed by plotting the peak area ratio of quetiapine (y) to the internal standard versus quetiapine concentration (x). Regression analysis was performed by ordinary least square linear regression.

Accuracy and precision: Accuracy and precision were studied by analyzing a minimum of three bioanalysed batches over three different days. Each run consisted of one blank sample, one zero sample, one standard curve containing all CC standards along with five replicates of LLOQ, LQC, MQC and HQC samples.

Recovery: Recovery experiment was performed by comparing the analytical results for extracted drug samples at three concentrations (LQC, MQC and HQC) with unextracted standards that represent 100% recovery.

Carry-over: Carry-over is the appearance of an analyte signal in blank sample after the analysis of samples with a high analyte concentration. As the HPLC system was equipped with autosampler injection mode, the carryover has been minimised. It was performed by injecting blank plasma sample immediately after the injection of ULOQ sample of the standard curve and studying the chromatogram of blank sample obtained.

Stability: Stability studies were carried out by analysing three replicates each of LQC (300 ng/mL) and HQC (1800 ng/mL). Freeze and thaw stability for quetiapine in plasma samples was studied by freezing at -20^{0}C and thawing unassisted at room temperature. Such 3 cycles were performed. Short term stability was assessed by keeping the samples at room temperature for 6 hours prior to analysis. For long term stability, three replicates of LQC and HQC were kept at -20ºC in deep freezer for 20 days prior to analysis. The samples obtained were then spiked with IS, subjected to LLE and chromatographic analysis.

TABLE 1: ACCURACY AND PRECISION RUNS OF QUETIAPINE.

Concentration (ng/mL)	Mean	% RSD	% Relative error
100	98.58	9.71	-1.42
300	281.95	6.06	-6.02
900	920.61	5.69	2.29
1800	1809.40	4.57	0.52

Table 2: RESULTS OF RECOVERY STUDIES.

Samples	Mean peak area of extracted samples	Mean peak area of un-extracted solutions	Mean % recovery of quetiapine
LQC	24366	33290	73.19
MQC	58996.2	82583.8	71.44
HQC	130108.6	173885.2	74.82

Table 3: RESULTS OF STABILITY STUDIES OF QUETIAPINE IN PLASMA.

Parameter	Short term stability		Freeze-thaw stability		Long-term stability	
	LQC	HQC	LQC	HQC	LQC	HQC
Mean	331.34	1780.41	307.01	1806.57	293.32	1711.91
SD	1.47	72.81	15.72	80.52	3.87	10.79
%RSD	0.44	4.09	5.12	4.46	1.32	0.63
% Nominal	110.45	98.91	102.34	100.37	97.78	95.11

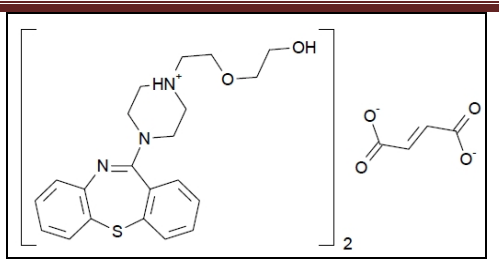

Figure 1: Structure of quetiapine fumarate.

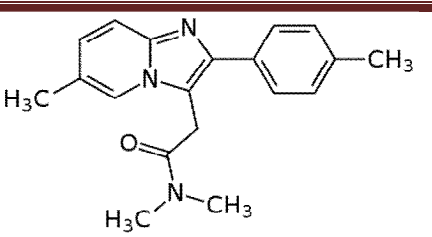

Figure 2: Structure of zolpidem.

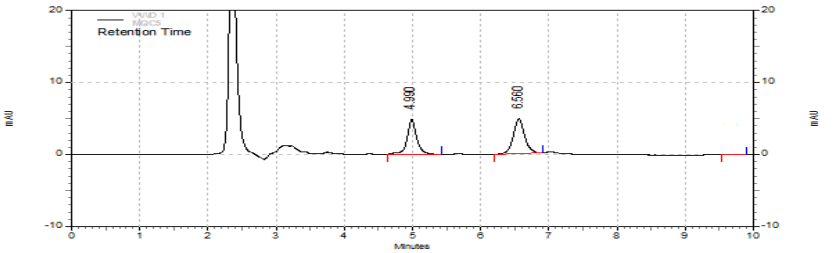

Figure 3: Representative chromatogram of MQC sample in quetiapine bioanalysis.

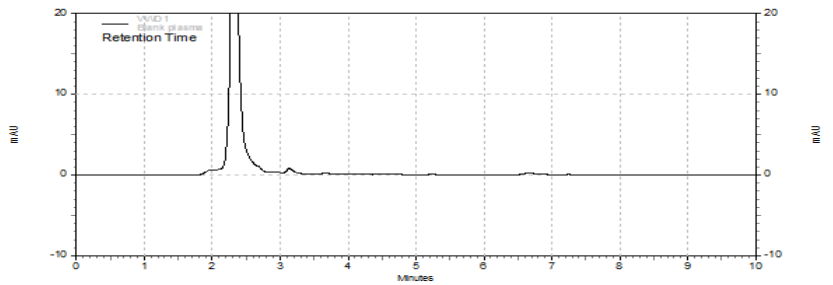

Figure 4: Representative chromatogram of blank plasma sample.

RESULTS

Chromatographic behaviour of quetiapine

Standard quetiapine solution of 10 µg/mL prepared in methanol was scanned in the range of 200-400 nm and wavelength of 254 nm was selected for detection. A trial and error approach was used in the bioanalytical method development. The drug and IS were subjected to varying chromatographic conditions. Their chromatographic behaviour in various mobile phases was studied. Chromatographic conditions were first optimized for the standard solutions of drug and IS and then applied to the processed plasma samples.

The final chromatographic conditions that were used for validation purpose are as given below:

- Injection volume: 50 µL
- Column: HiQSil C18HS (250 × 4.6 mm, 5µm)
- Column oven 25ºC temperature:
- Mobile phase: Acetonitrile: 0.01M Ammonium acetate buffer pH 3.5 (40:60 v/v)
- Detection : 254 nm
- Flow rate: 1 mL/min
- Run time: 10 min

Under these conditions, quetiapine and IS were separated at retention time of 6.5 min and 5 min respectively. (**Figure 3**)

Optimization of LLE experiments

Various solvents like ethyl acetate, tert-butyl methyl ether (TBME), Dichloromethane (DCM) and methanol used for extraction. Extracts obtained with TBME were cleaner and the chromatograms also showed no significant interference at the retention times of the drug as well as IS. The recoveries were also moderate. Hence, TBME was chosen as extraction solvent for sample preparation.

During the repeated analysis of spiked samples, the extraction procedure was modified to optimize the parameters for the development of a simple and efficient method of extraction. The modifications were:

1. Temperature of centrifugation was changed to -15ºC from 4ºC.
2. Evaporation time was reduced to 2 min from 3 min.
3. Evaporation temperature was increased to 50ºC from 40ºC.
4. Supernatant Aliquot changed to 1.3 mL from 1 mL.

Bioanalytical method validation

Selectivity: Blank plasma from six different sources and six replicates of LLOQ i.e. spiked sample containing 100 ng/mL of quetiapine were analysed for interference at retention time of drug. Chromatograms of blank plasma did not show any interfering peaks at the retention time of drug and IS (**Figure 4**). Thus, the method was selective for LLOQ of 100 ng/mL.

Linearity: The standard curve was generated in the concentration range of 100-2000 ng/mL for quetiapine on days of analysis. Peak area ratios of quetiapine/I.S. versus analyte concentrations were plotted on the graph. The linearity of the method was determined by unweighted least-squares linear regression analysis of standard plots associated with seven point standard calibration curves.

Accuracy and precision: The within batch precision and accuracy were calculated at the LLOQ (100 ng/ mL), LQC (300 ng/mL), MQC 900 ng/mL and HQC (1800 ng/mL) levels for five replicates each of the same analytical run. The between batch precision and accuracy were calculated after

replicate analysis in three different analytical runs. The results are given in **Table** Precision was measured as percent relative standard deviation. The within batch RSDs were below 14.77% at all concentration levels and the between RSDs were below 9.71% at all concentration levels. Accuracy was determined as percent relative error. The within batch relative error varied between -10.14% and +6.22% at all concentration levels and the between batch relative error values were between -6.02% and +2.29%. The results obtained are within the acceptable limits.

Recovery: Recovery of quetiapine was calculated by comparing the peak area of the analyte from an extracted plasma standard with that obtained from an unextracted standard at the same concentration for quality-control samples containing 300 ng/mL, 900 ng/mL and 1800 ng/mL. IS recovery was tested at 1000 ng/mL by comparing five extracted and unextracted samples at each concentration. The results of recovery studies of quetiapine are represented in **Table 2**.

The recoveries ranged from 71-74% as observed from the mean recovery data.

Percent recovery of IS was 64.69% as determined by separate analysis.

Carry-over: The calculated value of carryover for both drug and IS was 0%. Blank sample injected after the ULOQ sample complied with the acceptance criteria for carryover.

f) Stability: The results of stability studies are summarized in **Table 3**.

From the data above, it was evident that, all the stability samples met the criteria laid down for accuracy and precision. There was no significant decrease in the concentration obtained in the stability samples when back calculated from the freshly spiked calibration curve standards. Hence, the drug was stable for-

- six hours at room temperature (bench top stability)
- three freeze-thaw cycles
- 20 days at -20^{0}C (long term stability).

DISCUSSION

Chromatographic behaviour of quetiapine

The spectrum of quetiapine showed one absorbance peak with a maximum at 210 nm and two shoulders at λ = 254 and 290 nm. Since the detection at 210 nm would have been difficult because of the many potentially interfering compounds, we chose to carry out the analyses at 254 nm (this wavelength grants higher sensitivity than detection at 290 nm).

Chromatographic analysis was done using different mobile phases and the HPLC method mentioned above was selected which gave good separation between drug and IS.

Optimization of LLE experiments

The parameters that were changed during LLE optimization were temperature of cooling centrifuge, time and temperature of evaporation and volume of aliquot. By further decreasing the temperature of cooling centrifuge, it was possible to get a more distinct separation between the frozen plasma and TBME layer. It was useful in reducing the contamination of supernatant aliquots with plasma. To increase the extraction efficiency, the temperature of evaporation was increased from 40ºC to 50ºC which did not show any degradation of the drug or IS. This resulted in reduction of time for evaporation to 2 min from 3 min. As very clean extracts were obtained, it was thought to increase the volume of aliquots of the supernatant solvent layer. Removal of 1.3 mL aliquot was done without disturbing the centrifuged plasma layer.

Bioanalytical method validation

The validation parameters such as selectivity, linearity, accuracy, precision, stability etc. were studied as per US-FDA guidance for industry and EMA guidelines.

CONCLUSION

The proposed method for the determination of quetiapine in human plasma based on the use of liquid chromatography with spectrophotometric detection covering a concentration range of 100-2000 ng/mL resulted to be simple, accurate and precise. A simple liquid-liquid extraction procedure was employed using TBME as the extracting solvent. The method successfully resolved quetiapine and zolpidem (IS) from each other and also from the endogenous/exogenous plasma constituents. The method met the validation criteria laid down by the bioanalytical validation guidelines. Thus, a simple and economical bioanalytical method was successfully developed and validated for the determination of quetiapine from human plasma in real patient samples.

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