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Development & Validation of Liquid Chromatography Tandem Mass Spectrometry Assay for Quantification of Quetiapine Fumarate in Human Plasma

KEYWORDS: Quetiapine Fumarate, LC-MS/MS, Human Plasma

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ABSTRACT *A simple, rapid, sensitive and specific Liquid Chromatography tandem mass spectrometric (LC-MS/MS) method was developed and validated for the quantification of Quetiapine Fumarate in human plasma. Analyte was chromatographed on a peerless basic C18 column (50×4.6 mm) 3µm [Chromatopack] with mobile phase composition of [Acetonitrile: 5mM Ammonium Formate buffer (70:30)] at a flow rate of 0.700 mL/minute and Quetiapine- D4 was used as the internal standard. The assay involves a simple solid-phase extraction procedure of 0.100mL human plasma and the analysis was performed on a triple-quadrupole tandem mass spectrometer by MRM mode via electrospray ionization (ESI). The method was linear in the concentration range of 1004.176ng/mL to 5.038ng/mL. The lower limit of quantification (LLOQ) was 5.038ng/mL.*

Introduction:
Quetiapine, A atypical antipsychotic drug having unique receptor binding profile belonging to dibenzothiazepine derivatives. In the treatment of schizophrenia or bipolar manic episodes, Quetiapine is very effective. The graph of concentration vs Time of Quetiapine Fumarate linear and there is no difference in men and women Pharmacokinetic profile [1-3]. The main advantage of therapeutic profile of Quetiapine being used as atypical antipsychotic drug led to increasing use of drug day by day, which makes pharmaceutical company to prepare a innovative formulations. As a result, it may increase in demand of new analytical method for determination of Pharmacokinetic and Pharmacodynamic parameters.

For the quantification of Quetiapine, many HPLC methods used and have been reported; most of them were ultraviolet detection [6-8]. The outcome also sensitive for quantification of expected amount of concentration of drug in formulation, but these methods was very time consuming, so that it require higher run time for analysis. It compares HPLC methods with ultraviolet and MS/MS detection, the result shows, run time was 35 minutes, due to longer run time method allows only 40 sample quantification per day [9]. Gas chromatography-Mass spectrometry method for Quetiapine Fumarate was also carried out [10-11].

Simple and rapid detection of drugs and their metabolites are easily quantifiable. Liquid Chromatography tandem Mass spectrometry (LC-MS/MS) is the best for the detection of drug in biological fluids. Till date, three LC-MS/MS method reported for the detection of Quetiapine Fumarate [9,12,13].

The aim of our bioanalysis was to develop LC-MS/MS method for quantification of Quetiapine Fumarate in human plasma obtained by Pharmacokinetic study to detect different Pharmacokinetic parameters. As per the literature review, concentration of Quetiapine Fumarate found out between 1.5 to 350 ng/mL in human plasma [4-5].

2. Experimental
2.1 Chemical and Reagents
Quetiapine Hemifumarate obtained from Tyche Industries Ltd (Hyderabad, India) and Quetiapine- D_4 fumarate obtained from Deviz (Mumbai, India). Methanol was from (Rankem, India) [HPLC grade], Ammonium formate was purchased from (Rankem, India) [GR grade], Acetonitrile also purchased from (Rankem, India) [HPLC grade], water used for HPLC was Milli-Q-Water (Millipore,U.S.A), Orochem Cartridge (Reverse phase- 30mg, 1mL DVB-LP). 5mM Ammonium formate Buffer prepared by Weighed approximately 0.3153gm of ammonium formate and dissolved in 1000mL of HPLC grade/Milli-Q-Water and pH 5 was adjust with help of formic acid. The mobile phase was 70:30 of Acetonitrile:5mM Ammonium formate.

2.2 Mass Spectrometry
Mass spectrometry was carried out by a triple quadrupole Quattro premier and Quattro Premier XE mass spectrometer detector from Waters corp. (USA). equipped with an ESI source. The multiple reaction monitoring (MRM) mode used for the determination of Quetiapine due to its high selectivity. The dwell times used were 0.1 and 0.1 s, respectively. The collision energy was 27 eV for both compounds. The cone voltage was set at an optimized value (28 kV) in the positive-ion mode. The capillary voltage was 2.0 kV and the entrance and exit. Nitrogen was used as desolvation. The source and desolvation temperatures were optimized and kept at 100 and 400 ◦C, respectively. For data acquisition, Mass lynx V 4.0 software, Waters (Manchester, UK).

2.3 Liquid Chromatography
An Acquity Ultra Pressure Liquid Chromatrography system (waters corp, Milford, MA, USA) with column of Peerless Basic C_{18} 3µm [Chromatopack] (50×4.6 mm, Thermo Scientific, USA). The column temperature was maintained at 45^0 C. The mobile phase consisted of composition of [Acetonitrile: 5mM Ammonium Formate buffer (70:30)]. The injection volume was 20 µl, and the analysis time was 2.0 min per sample.

2.4 Preparation of standard and quality control solutions
Individual stock solutions of Quetiapine Hemifumarate (1mg/mL) & IS (1mg/mL) were prepared accurately by weighing the required amounts into separate volumetric

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flasks & dissolving in methanol. The secondary solution of Quetiapine was prepared in methanol: water (1:1) to produce 1000.000µg/mL of concentration solution which was further used for preparing dilutions by methanol: water (1:1). All the standard stock & spiking solution were prepared & stored at 2-8°C.

The plasma calibration standards (S9–S1) were prepared from S1 Concentration solutoin with blank plasma directly for the S2-S9. The final concentrations of plasma calibration standards were 5.03, 10.07, 30.12, 50.20, 105.52, 501.76, 752.83, 903.75, 1004.17 ng/mL. The quality control samples (HQC, MQC, and LQC) were obtained from the most concentrated quality control sample QC by sequential dilution with blank plasma to get the final concentrations of QCs 823.56, 502.37, and 15.07 ng/mL, respectively.

2.5 Preparation of Plasma samples

Solid phase extraction method, used for extraction Quetiapine Fumarate by using mobile phase as a solvent gave consistent recovery of analyte from plasma. Subject samples & spiked plasma samples were retrieved from deep freezer and thawed at room temperature. The thawed samples were vortexed using vortexer. To 0.100ml of subject plasma & spiked plasma, 25µl of IS solution was added & vortexed. Following the addition of 0.500ml of 0.1% formic acid, the sample was vortex-mixed for 1 min and was ready for extraction. Orochem-Reversed phase- 30mg cartridge was conditioned with 1mL of methanol followed by 1mL of milli-Q- water. After conditioning, samples were loaded & washed with 2mL of milli-Q-water. The samples were then eluted with 1mL of mobile phase and transfer to HPLC vials, and inject 10µl of the sample was into LCMS/MS.

2.6 Pharmacokinetic study

A open, randomized, bioequivalence study on Quetiapine Fumarate 100 mg in healthy volunteers was performed. A doses of 25 mg for two times preceded the administration of the 100 mg dose.

Plasma samples were obtained from 64 volunteers in different 24 various time intervals within drug administration. The analytical batch consisted of blank, blank with internal standard (S0), seven calibration standards (S9, S8,S7, S6, S5, S4, S3, S2, S1) and plasma samples gained from two volunteers involved in the study with nine quality control (QC) samples interspersed (Three series of HQC, MQC, and LQC).

2.7 Method Validation

The method was validated in terms of precision, accuracy, recovery, selectivity, linearity, sensitivity and stability according to the guidelines issued by the food and drug administration (FDA) for the validation of bioanalytical methods [12].

2.7.1 Accuracy and Precision

Coefficient of variance (CV),used to determined Intra and Inter day assay precision and intra- and inter-day assay accuracies were expressed as percentages of the theoretical concentration, as accuracy (%) = (found concentration/theoretical concentration)×100. Intra-day assays were performed using five replicates during 1 day and inter-day assays were performed on four separate days. FDA Recommended that, acceptance criterion for each back-calculated standard concentration was a 15% deviation from the normal value except at the LLOQ, which was set at 20% [12].

2.7.2 Recovery and Selectivity

Specificity was evaluated by using 8 batches of blank Human plasma including Hemolytic and lipimic plasma. It was tested for the presence of endogenous compounds that might interfere with analyte, using the Solid Phase Extraction procedure and chromatographic conditions, and results were then compared with those obtained with a solution of the analyte at a concentration near the LLOQ. The Absolute recovery and Absolute matrix effect were calculated by using pre extraction and post extraction peak level. The recovery calculated in amount of percentage.

2.7.3 Linearity and sensitivity

A calibration curve was prepared using a double-blank sample (a plasma sample without Quetiapine and Intenal Standard) and nine calibration samples covering the whole range (0.1–25 µg/mL) by the peak area ratio of Quetiapine against Intenal standard. Concentrations of Quetiapine Fumarate were calculated from these area ratios using the calibration curve. The linearity was calculated as a correlation coefficient (r^2) of 0.99 or better was deemed satisfactory.

3. Result and Discussion

3.1 Mass spectrometry

The major instrumental parameters of the mass spectrometry were summarized. Full scan mass spectra and product ion scan spectra of Quetiapine and IS were obtained by the mass spectrometer via MRM mode (Multiple Reaction Monitoring) at a flow rate of 0.700 mL/min. The unique ion transitions monitored were m/z 384.3→263.4 for Quetiapine and m/z 388.2→267.5 for Quetiapine-D4. which were chosen as precursor ions. The limits of quantification (LLOQ) of Quetiapine were obtained 5.038 ng/mL.

3.2 Method validation

3.2.1 System suitability

The system suitability was evaluated by performing six replicate injections from a highest standard aqueous solution vial. The %CV of area ratio with ISTD for Quetiapine was 0.9%, which is within the acceptance range of %CV ≤ 0%. The %CV of retention time for Quetiapine and ISTD were 0.0% and 0.3% respectively, which is within the acceptance range of ≤ 2%. The details are given in Table 1.

Table 1: System Suitability for Quetiapine and ISTD

Sr. No.	RT	Area	RT	Area	Area ratio
1	1.39	202689.766	1.35	19168.484	10.574
2	1.39	199655.719	1.36	18839.826	10.598
3	1.39	203131.031	1.36	18972.385	10.707
4	1.39	203029.125	1.36	19493.016	10.415
5	1.39	202776.672	1.36	19168.428	10.579
6	1.39	205221.969	1.36	19452.873	10.550
Mean	1.390	202750.714	1.358	19182.502	10.571
± SD	0.000	1783.964	0.004	257.484	0.094
%CV	0.0	0.9	0.3	1.3	0.9
Acceptance criteria: % CV for Retention time (RT) should be < 2%					
% CV for Area ratio should be < 5%					

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3.2.1 Selectivity and Specificity

Selectivity method was found by using 8 batches of plasma. The fig 1 blank human plasma extract; fig 2 Aqueous standard ; fig 3 Specificity of Quetiapine and IS. As shown in Fig. 1, no endogenous peaks were observed at the retention times of Quetiapine or IS. Fig. 3 shows MRM chromatograms at the LLOQ of the calibration curve. They did not show any interfering peak at the retention time of Quetiapine and ISTD. Figure 3 includes a representative chromatogram of specificity. Selectivity was established by injecting lower limit of quantification sample using the same lot of specificity. The aim of performing selectivity with different types of plasma samples was to ensure the quality of result for study sample analysis.

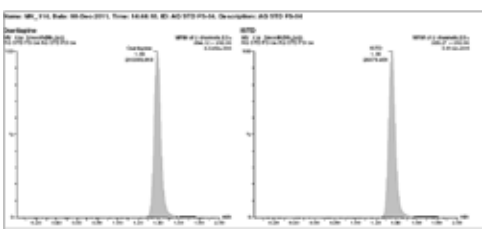

Fig 1. A Representative Chromatogram of Aqueous Standard

Fig 2. A Representative Chromatogram of Blank

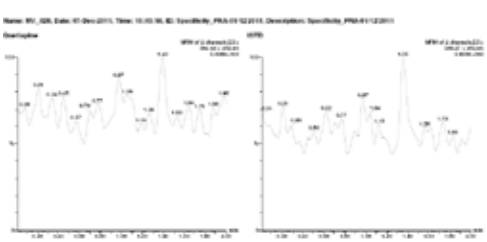

Fig 3. A Representative Chromatogram of Specificity

3.2.3 Accuracy and Precision

The intra day accuracy High, Medium and Low QC ranged from 98.1% to 102.7% which is within the acceptance range of % accuracy ±15%. The accuracy of LLOQ QC ranged from 100.3% to 108.7%, which is within the acceptance range of % accuracy ±20%. The inter-day accuracy of High, Medium and Low QC ranged from 99.0% to 102.1%, which is within the acceptance range of % accuracy ± 15%. The inter-day accuracy of LLOQ QC is 103.4%, which is within the acceptance of % accuracy ± 20%. The intra day precision of LLOQ QC ranged from 5.2% to 8.1%, which is within the acceptance range of %CV ≤ 20%. The inter-day precision of LLOQ QC is 7.8%, which is within the acceptance of %CV ≤ 20%.

3.2.4 Recovery and Reproducibility

% Recovery for Quetiapine in High, Medium and Low QC samples were 98.580%, 98.738%, 98.599% respectively. %Recovery for ISTD was 101.733%. Reinjection Reproducibility for Quetiapine was evaluated by comparing low and high quality control samples followed by reinjecting the same samples.The mean ratio of the concentration of Quetiapine for the Low and High QCs were 101.331% and 100.229% respectively. This was within the acceptance range of 90% - 110%.

3.2.5 Stability

Studies were checked to determine drug activity with stabilty in plasma and in the mobile phase used for making solution which used in analysis, and no degradation were seen (data not shown). These results indicate that Quetiapine was stable under bench (room temperature) and freeze–thaw conditions Table 2, and importantly no stability-related problems were encountered during routine sample analysis.

Table 2 : Freeze Thaw Stability for Quetiapine in Human Plasma

Sr. No.	Freshly Spiked		After 3 Cycles	
	L QC	H QC	L QC	H QC
	Nominal Concentration (ng/mL)		Nominal Concentration (ng/mL)	
	15.071	823.567	15.071	823.567
1	15.526	848.599	15.255	845.764
2	15.275	837.665	15.401	834.953
3	15.255	773.879	15.083	760.300
4	14.949	799.701	15.517	814.238
Mean	15.251	814.961	15.314	813.814
± SD	0.236	34.485	0.188	37.998
% CV	1.5	4.2	1.2	4.7
% Mean ratio			100.411	99.859
Acceptance Criteria: % mean ratio should be within 85% - 115%				

4. Conclusion

The method development of Quetiapine Fumarate in human K_2EDTA plasma limit quantification concentration range 1004.176ng/mL to 5.038ng/mL, using 0.1mL of plasma was proposed and validated. The assay showed good precision, accuracy and recovery. A simple and rapid analysis method and short retention time could allow determination of more samples per day. Liquid chromatography tandem mass spectrometry method for Quetiapine, based on solid phase extraction method, prove to rapid and sensitive in determination of Quetiapine in human plasma.

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