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A NEW RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR ESTIMATION OF QUETIAPINE FUMARATE IN HUMAN PLASMA

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ABSTRACT

A new reverse phase high performance liquid chromatography (RP-HPLC) method for the quantitative determination of Quetiapine Fumarate in human plasma was developed and validated as per US-FDA guidelines. The drug was spiked in the plasma and extracted with mobile phase by Precipitation method. The extracted analyte was injected into Symmetry C18 (4.6 x 150mm, 5 µm, Make: Thermo) or equivalent, maintained at ambient temperature and effluent was monitored at 290 nm. The mobile phase consisting of Sodium Dihydrogen Phosphate: Methanol (35:65 v/v). The pH of the mobile phase was adjusted to 4.0 by using O-Phosphoric Acid. The flow rate was maintained at 1.0 mL/min. The developed method shows high specificity for Quetiapine. The calibration curve for Quetiapine was linear from 5 to30 µg/ml. The inter-day and intra-day precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility, and specificity for the determination of Quetiapine in Human Plasma. The Lower limit of quantification (LLOQ) for Quetiapine was found to be 0.05µg/ml. The

Average % recovery was of the drug was found to be 99.74-100.32% (r^2= 0.999) and reproducibility was found to be satisfactory. The proposed method is simple, fast, accurate, and precise for the quantification of Quetiapine Fumarate in the human plasma as well as for routine analysis in quality control. The method was validated for parameters like Selectivity, Sensitivity, Precision, Intermediate Precision, Accuracy, Linearity, Recovery & Stability. This RP -HPLC method is suitable for determining the concentration of Quetiapine in human plasma and it can applied for routine analysis for determination of the Quetiapine from dosage form during pharmacokinetic study.

Key-Words: Quetiapine Fumarate, RP-HPLC, ICH, Validation, Human Plasma, US-FDA guideline.

INTRODUCTION

Quetiapine Fumarate (Fig. no. 1) is a white to off-white crystalline powder. Drug having efficacy in the treatment of schizophrenia and bipolar disorder is mediated through a combination of dopamine type 2 (D2) and serotonin type 2 (5HT2) antagonisms. An atypical antipsychotic, Quetiapine fumarate (2-[2-(4-dibenzo [*b*, *f*] [1, 4] thiazepin-11-yl-1-piperazinyl) ethoxy] ethanol fumarate (2:1 salt)) which has a unique receptor- binding profile belonging to a new chemical class, the dibenzothiazepine derivatives [1-2]. Quetiapine is an antagonist at a broad range of neurotransmitter receptors. Quetiapine is used in the treatment of schizophrenia or manic episodes associated with bipolar disorder. As a consequence, there is an increasing demand for new analytical methods for determination of same drug in most economical way. Several HPLC methods for the determination of Quetiapine have been reported, most of these require ultraviolet detection [3-7] as Quetiapine is not electro active, some stability indicating [8], impurity characterizing [9]. Some HPLC–MS methods have been published for determination of Quetiapine [9]. A HPTLC method has been developed [10]. However none of these methods is sensitive enough for determination of the expected drug levels and some of them are time consuming and require complex sample pretreatment or long run times. Some Gas Chromatography–mass spectrometry (GC–MS) methods have also been employed, however here Quetiapine needs to be derivatized before analysis [11].

The goal of our work was to develop a new RP HPLC method for determination of Quetiapine in Human Plasma and to use the results for analysis of drug in pharmaceuticals in most economic way, rapid and effective way. The RP HPLC method is desirable, as such that

not much analytical papers are available as per our knowledge for the determination of the drug in plasma.

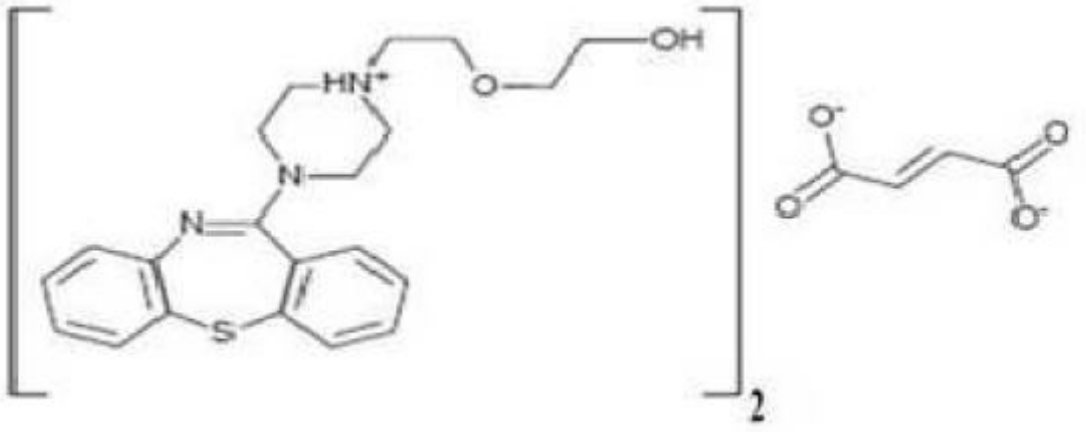

Fig. no.1Chemical Structure of Quetiapine fumarate

MATERIALS & METHOD [11-12]

Chemical and Reagent Used: The following chemicals have been procured for the process Water [HPLC Grade], Quetiapine **[**Working Standards], Methanol [HPLC Grade], Ortho phosphoric acid all the chemicals are procured from STANDARD SOLUTIONS and the tablets [25mg Label Claim] was collected from the Local market and the manufacturer was Lupin company and the brand name of the Tablet was Placidine-25mg.

Apparatus and Chromatographic Conditions

Equipment	: High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector. (Waters, Alliance 2695 Separation Module with 2487 UV Vis Detector)
Column	: Symmetry C18 (4.6 x 150mm, 5 µm Make: Thermo)
Flow rate	: 1.0mL per min
Wavelength	: 290 nm
Injection volume	: 20 µl
Temperature	: Ambient
Run time	: 6.0 min

Preparation of Sodium Dihydrogen Phosphate buffer [13]: The Buffer Solution was prepared by weighing accurately and transferred 2.5 grams of sodium dihydrogen phosphate

into a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water. The pH of the buffer was adjusted to 4.0 by using Orthophosphoric acid.

Preparation of mobile phase: The mobile phase was prepared by mixing the above buffer 350mL (35%) and 650 mL of Methanol [HPLC grade] (65%) and degassed in ultrasonic water bath for 5 minutes. Then the solution was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as diluent.

Preparation of the Quetiapine Standard & Sample Solution

Standard Solution Preparation: The Standard Stock Solution was prepared by weighing accurately and transferred 100mg of Quetiapine [Working standard] into a 70 mL volumetric flask. Initially about 70 mL of diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above Stock Solution pipette out 0.2 ml solution into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed thoroughly and then it was filtered through 0.45µm filter.

Spiking of Quetiapine to Plasma and Extraction of Quetiapine from plasma: The serial dilutions of analyte were prepared in mobile phase and 0.5ml of each dilution was spiked into 0.5ml of plasma in a polypropylene tubes. Then all the tubes were cyclo mix for 5 min. Then 1ml of acetonitrile was added and centrifuged for 20 min at 3000 rpm. Further the supernatant liquids were collected in another eppendorf tube and 20µL supernatant was injected into the analytical column.

VALIDATION DEVELOPMENT [14-17]

1. **Selectivity:** Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample.

Preparation of the solution: An aqueous mixture of Quetiapine [20ppm concentration] was prepared and injected in the column and the retention time was checked. As per the respective sample processing procedure all the blank samples were injected & evaluated the interference at the retention time of Quetiapine by comparing the response in the blank. There were no interferences found in the retention of drug extracted from plasma. The method was found to be precised and specific.

2. **Sensitivity:** To determine the sensitivity in terms of LLOQ, 'Lower Limit of Quantification' where the response of LLOQ must be at least five times greater than the response of interference in blank matrix at the retention time or mass transitions of the analyte(s).

Preparation of 20µg/ml solution: The solution was prepared by weighing accurately and transferred 10mg of Quetiapine [Working Standard] into a 100 mL volumetric flask and added about 70 mL of the diluent and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. From the above prepared solutions pipette out 2.0 ml into a 10ml volumetric flask and the volume was upto the mark with the diluent. The resultant solution was mixed well and filtered through 0.45µm filter.

Preparation of 0.5%solution At Specification level (0.05µg/ml solution): From the above prepared solution pipette out 0.5 mL of solution into a 10 ml of volumetric flask and the volume was made upto the mark with the diluent. Further from the above solution pipette out 0.5 mL into a 10 ml of volumetric flask and the volume was made upto the mark with the diluent. As in the standard preparation, the above concentration sample was spiked to the plasma and it was extracted and collected in vial and injected into the HPLC system.

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank: 46µV

Signal Obtained from LOQ solution (0.5% of target assay concentration) : 358 µV

S/N = 358 / 46 = 7.78

Acceptance Criteria: The S/N Ratio value should be ≥ 5 for LOQ solution.

3. **Precision:** The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. The precision of the analytical method was determined by analyzing the homogeneous samples of drug concentration of 20pppm of Quetiapine were spiked in plasma in six replicates. The %RSD for the area and retention time of six replicate injections was found to be within the specified limits (Table no.1).

Table no.1The precision result for the drug Quetiapine

Injection	Retention Time	Peak area
Injection-1	2.867	925864
Injection-2	2.871	925476
Injection-3	2.864	924758
Injection-4	2.821	932586
Injection-5	2.887	947582
Injection-6	2.875	932158
Average	2.864	931404
Standard Deviation	0.023	8645.177
%RSD	0.79	0.93

Acceptance Criteria: The % RSD for the area and retention time of standard injection results should not be more than 2%.

4. Intermediate Precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by using same conditions. The %RSD for the area and retention time of six replicate injections was found to be within the specified limits (Table no. 2).

Table no.2The Intermediate Precision result for the drug Quetiapine

Injection	Retention Time	Peak area
Injection-1	2.867	925864
Injection-2	2.871	925476
Injection-3	2.864	924758
Injection-4	2.821	932586
Injection-5	2.887	947582
Injection-6	2.875	932158
Average	2.864	931404
Standard Deviation	0.023	8645.177
%RSD	0.79	0.93

Acceptance Criteria: The % RSD for the area and retention time of standard injection results should not be more than 2%.

5. Accuracy: The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Precision and accuracy is determined by replicate analysis of samples containing known amounts of the analyte.

Preparation of stock solution: The Stock Solution was prepared by weighing accurately and transferred 10 mg of Quetiapine [Working standard] into a 10 mL volumetric flask and added about 70 mL of the diluent and sonicated to dissolve it completely and the volume was made upto the mark with the diluent.

Preparation of 20 µg/ml solution: From the above prepared Stock Solution pipette out 0.2 ml into a 10ml volumetric flask and the volume was made upto the mark with the diluent. The resultant solution was mixed thoroughly and was filtered through 0.45µm filter.

Preparation Sample solutions

For preparation of 50% solution (With respect to target Assay concentration): The solution was prepared by weighing accurately and transferred 5.0 mg of Quetiapine API sample into a 10 mL volumetric flask and added about 7 mL of the diluent and sonicated to dissolve it completely and the volume was made upto the mark with the same solvent. Further from the above prepared solution pipette out 0.2ml into a 10ml volumetric flask and the volume was made upto the mark with diluent. The resultant solution was mixed thoroughly and filtered through 0.45µm filter.

For preparation of 100% solution (With respect to target Assay concentration): The solution was prepared by weighing accurately and transferred 10.0mg of Quetiapine API sample into a 10 mL volumetric flask and added about 7 mL of the diluent and sonicated to dissolve it completely and the volume was made upto the mark with the same solvent. Further from the above prepared solution pipette out 0.2 ml into a 10ml volumetric flask and the volume was made upto the mark with diluent. The resultant solution was mixed thoroughly and filtered through 0.45µm filter.

For preparation of 150% solution (With respect to target Assay concentration): The solution was prepared by weighing accurately and transferred 15.0mg of Quetiapine API sample into a 10 mL volumetric flask and added about 7 mL of the diluent and sonicated to dissolve it completely and the volume was made upto the mark with the same solvent. Further from the above prepared solution pipette out 0.2 ml into a 10ml volumetric flask and the volume was made upto the mark with diluent. The resultant solution was mixed thoroughly and filtered through 0.45µm filter.

Procedure: As in the standard preparation, the samples were spiked to the plasma and it was extracted and collected in vials and injected into HPLC system. The amount of the drug Quetiapine to be added was calculated the amount found was calculated. Further the individual recovery and mean recovery values were also calculated (Table no. 3).

Table no.3The Accuracy result for the drug Quetiapine

Accuracy level	% Recovery	Avg. %Recovery	Amount Recovered	SD	%RSD
80%	99.5 99.08 100.63	99.74	7.96 7.93 8.05	0.80	0.80
100%	100.3 98.59 99.07	99.32	10.03 9.86 9.91	0.88	0.89
120%	100.84 99.82 100.31	100.32	12.1 11.98 12.04	0.51	0.51

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

6. Linearity: A calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte. A calibration curve should be generated for each analyte in the sample. A calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte. A calibration curve should consist of a blank sample (matrix sample processed

without internal standard), a zero sample (matrix sample processed with internal standard), and six to nine non-zero samples covering the expected range, including LLOQ.

Preparation of stock solution: The stock solution was prepared by weighing accurately and transferred 10mg of Quetiapine API sample into a 100 mL volumetric flask and added about 70 mL of the diluent and sonicated to dissolve it completely and the volume was made upto the mark with the same solvent.

Preparation of Level – I (5µg/ml): 0.5ml of stock solution was taken into a 10 ml of volumetric flask and the volume was made upto the mark with the diluent.
Preparation of Level – I (10µg/ml): 1.0ml of stock solution was taken into a 10 ml of volumetric flask and the volume was made upto the mark with the diluent.
Preparation of Level – III (15µg/ml): 1.5ml of stock solution was taken into a 10 ml of volumetric flask and the volume was made upto the mark with the diluent.
Preparation of Level – IV (20µg/ml): 2.0ml of stock solution was taken into a 10 ml of volumetric flask and the volume was made upto the mark with the diluent.
Preparation of Level – V (25µg/ml): 2.5ml of stock solution was taken into a 10 ml of volumetric flask and the volume was made upto the mark with the diluent.
Preparation of Level – VI (30µg/ml): 3.0ml of stock solution was taken into a 10 ml of volumetric flask and the volume was made upto the mark with the diluent.
Procedure: As in the standard preparation, the samples were spiked to the plasma and it was extracted and collected in vial and injected into HPLC system and measured the peak area. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and then calculated the correlation coefficient.

Table no.4The Linearity result for the drug Quetiapine

S.No	Linearity Level	Concentration(µg /ml)	Area
1 2 3 4 5 6	I II III IV V VI	5 10 15 20 25 30	234969 469939 704908 939878 1174847 1502475
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.999.

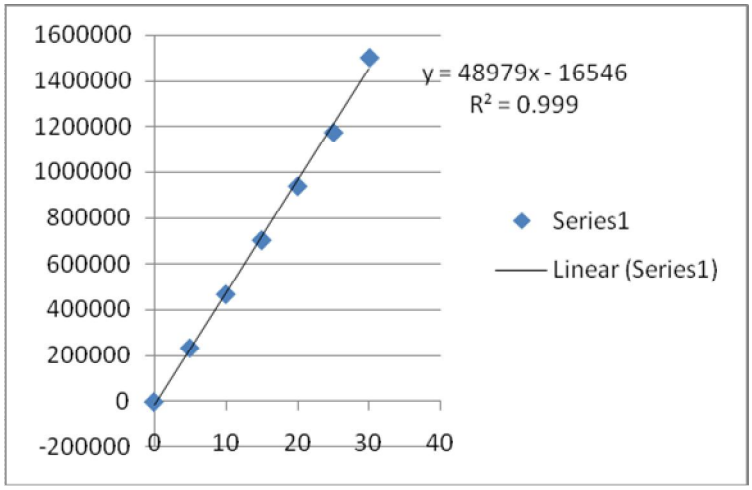

Fig. no.2 Calibration curve for the drug Quetiapine fumarate

7. Stability [18-22]: Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. The stability of an analyte in a particular matrix and container system is relevant only to that matrix and container system and should not be extrapolated to other matrices and container systems. Stability procedures should evaluate the stability of the analytes during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term (bench top, room temperature) storage, and after going through freeze and thaw cycles and the analytical process. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. The procedure should also include an evaluation of analyte stability in stock solution.

All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix. Stock solutions of the analyte for stability evaluation should be prepared in an appropriate solvent at known concentrations.

i. Freeze and Thaw Stability: Analyte stability should be determined after three freeze and thaw cycles. At least three aliquots at each of the low and high concentrations should be stored at the intended storage temperature for 24 hours and thawed unassisted at room temperature. When completely thawed, the samples should be refrozen for 12 to 24 hours under the same conditions. The freeze–thaw cycle should be repeated two more times, the

analyzed on the third cycle. If an analyte is unstable at the intended storage temperature, the stability sample should be frozen at -70 ^{0}C during the three freeze and thaw cycles.

ii. Short-Term Temperature Stability: Three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature from 4 to 24 hours (based on the expected duration that samples will be maintained at room temperature in the intended study) and analyzed.

iii. Long-Term Stability: The storage time in a long-term stability evaluation should exceed the time between the date of first sample collection and the date of last sample analysis. Long-term stability should be determined by storing at least three aliquots of each of the low and high concentrations under the same conditions as the study samples. The volume of samples should be sufficient for analysis on three separate occasions. The concentrations of all the stability samples should be compared to the mean of back-calculated values for the standards at the appropriate concentrations from the first day of long-term stability testing.

vi. Stock Solution Stability: The stability of stock solutions of drug and the internal standard should be evaluated at room temperature for at least 6 hours. If the stock solutions are refrigerated or frozen for the relevant period, the stability should be documented. After completion of the desired storage time, the stability should be tested by comparing the instrument response with that of freshly prepared solutions.

v. Post-Preparative Stability: The stability of processed samples, including the resident time in the auto sampler, should be determined. The stability of the drug and the internal standard should be assessed over the anticipated run time for the batch size in validation samples by determining concentrations on the basis of original calibration standards.

Although the traditional approach of comparing analytical results for stored samples with those for freshly prepared samples has been referred to in this guidance, other statistical approaches based on confidence limits for evaluation of an analyte=s stability in a biological matrix can be used. SOPs should clearly describe the statistical method and rules used. Additional validation may include investigation of samples from dosed subjects.

The stability of the drug extracted, was subjected to freeze and thaw stability at -20ºC ± 2ºC, short term stability for period of 24 hours stored at room temperature, long term stability for period of 15 days stored at 4ºC. As in standard preparation the above samples can be spiked

to the plasma and it can be extracted and collected in vial and inject into HPLC system. All the stability samples compared against the standard stock solution assessed for stability.

Table no.5The Stability result for the drug Quetiapine

S. No.	Standard sample	Freeze & Thaw sample	Short term stability	Long term stability
1	921478	901254	896526	886325
2	922565	908541	885472	885471
3	921458	910123	889654	881472
Mean	921834	906639	890551	884423
SD	633	4730	5581	2591
% RSD	0.07	0.52	0.63	0.29
% ASSAY		98.35	96.61	95.94

RESULT & DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of the drug Quetiapine fumarate in Human Plasma. In order to method development under isocratic conditions, mixtures of Sodium Dihydrogen Phosphate Buffer with pH 4.0 adjusted with Orthophosphoric acid and Methanol HPLC grade in different combinations were tested as mobile phase on a Symmetry C18 (4.6 x 150mm, 5 µm, Make: Thermo) column. A binary mixture of Sodium Dihydrogen Phosphate Buffer [pH 4.0] and Methanol [HPLC Grade] in 35:65 v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from the tailing. The retention times obtained for Quetiapine fumarate were around 2.867min. A model chromatogram was shown in Fig. no.3 & 4.

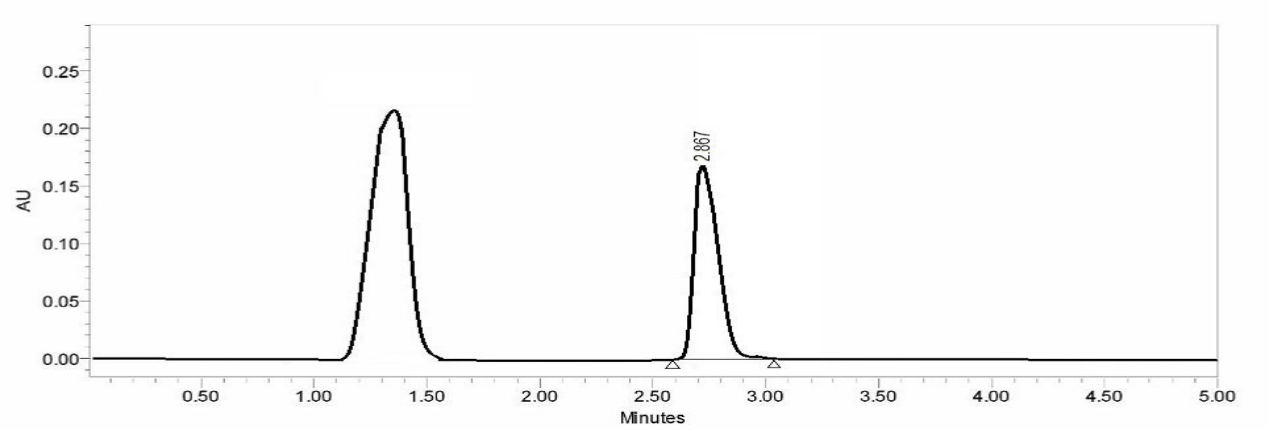

Fig. no.3A Typical Chromatogram of Quetiapine fumarate in Plasma

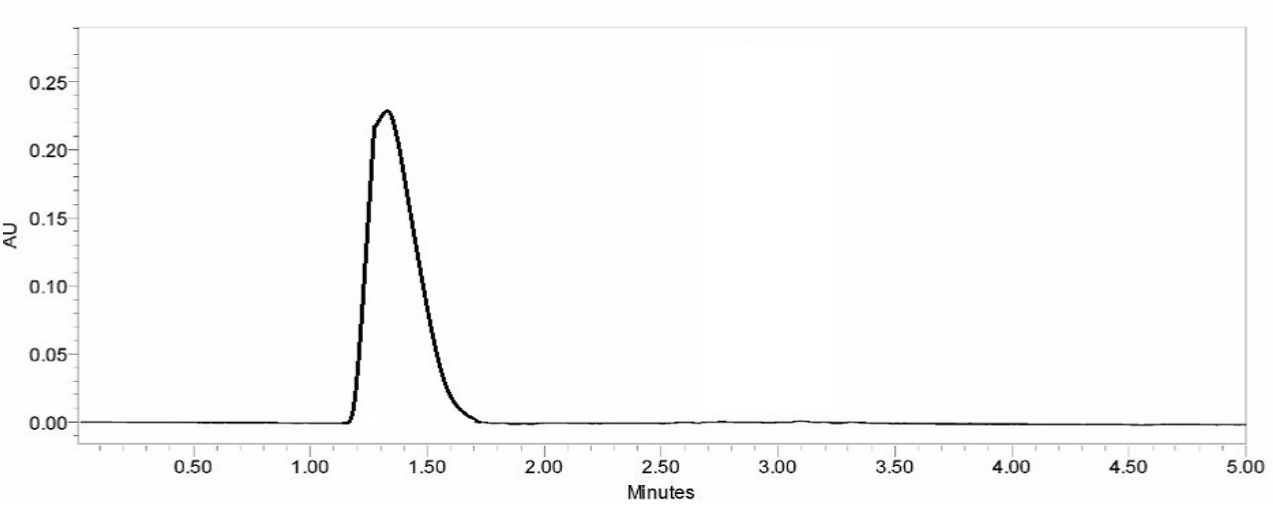

Fig. no.4A Typical Chromatogram of Blank Plasma

The Precision data was represented in Table no. 1. When Quetiapine was analyzed by the proposed method in the intra and inter-day (Ruggedness) variation results, a low coefficient of variation was observed it was represented in Table no. 2. This shows that the present HPLC method is highly precise and it was shown in Fig no. 5.

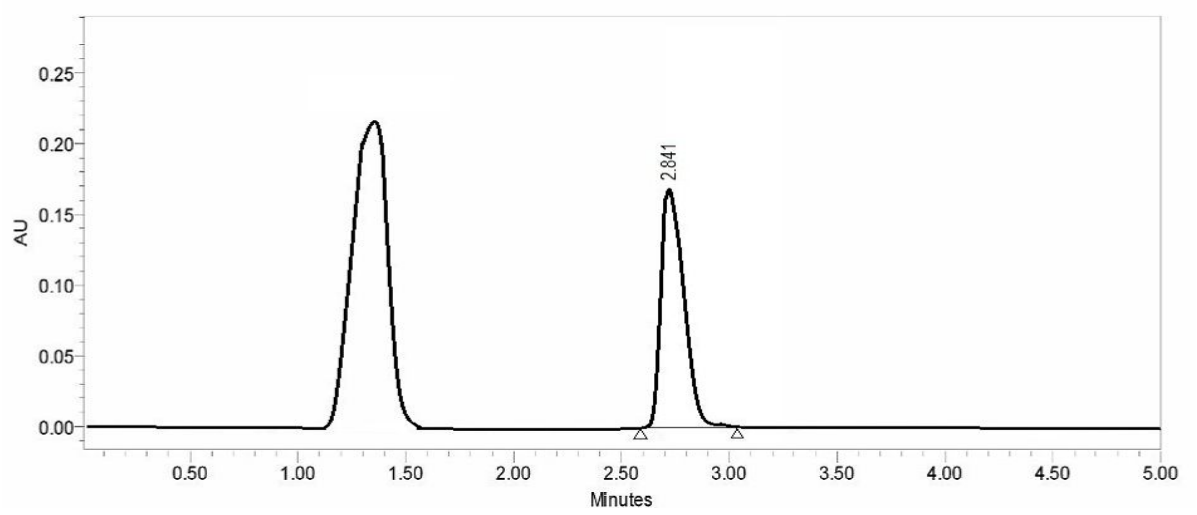

Fig. no.5A Typical Chromatogram of Quetiapine fumarate for Ruggedness study

The Accuracy recoveries were 99.74-100.32% and reproducibility was found to be satisfactory The Accuracy data was summarized in Table no. 3. In order to test the linearity of the method, six dilutions of the working standard solutions of the drug in the range of 5 to 30µg per mL for the drug were prepared. The data has been represented in Table no. 4. Each of the dilutions was injected into the column and the graph for the Linearity Curve has been

represented in Fig no. 2. The method was duly validated by evaluation of the required parameters. The Lower limit of quantification (LLOQ) for Quetiapine was found to be 0.05µg/ml. The drug content formulations were quantified by using the proposed analytical method. The low coefficient of variation in the recovery data indicates the reproducibility of the method in dosage forms.

CONCLUSION

A simple Bioanalytical method is developed to quantify Quetiapine in human plasma. The validated method covers the wide range of linearity over 5 to 30µg/mL and is therefore suitable for the determination of Quetiapine in human plasma at different therapeutic dose levels. Samples were prepared by using protein precipitation method for analysis. The mobile phase used is a binary mixture of Sodium Dihydrogen Phosphate Buffer [pH 4.0] and Methanol [HPLC Grade] in 35:65 v/v proportions. The % mean recovery was found to be in the range of 99.74-100.32%. The developed method is simple, selective, precise, accurate and rapid. Quetiapine is found to be stable when subjected under different stability conditions. The proposed method can be applied to monitor plasma concentrations of Quetiapine in pharmacokinetic studies. It can also be used for therapeutic drug monitoring in order to optimize drug dosage on an individual basis. The method was proved to be superior to most of the reported methods. The mobile phases are simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence this method can easily be adopted as an alternative method to reported ones for the routine determination of Quetiapine depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies of Quetiapine.

REFERENCES

1. Small J G, Hirsch S R, Arvanitis L A, Miller B G, Link C G. Quetiapine in patients with schizophrenia, a high-and low-dose double-blind comparison with placebo. Seroquel study group. Arch Gen Psychiatry. 1997; 54: 549–557.
2. Radha Krishna S, Rao B M, Someswara Rao N. A validated stability indicating HPLC method for the determination of related substances in Quetiapine fumarate. Rasayan J Chem.2008; 3: 466–474.

3. Belal F, Elbrashy A, Eid M, Nasr J J. Stability-Indicating HPLC method for the determination of Quetiapine: Application to tablets and human plasma. J Liq Chrom Rel Technol.2008; 31: 1283–1298.
4. Barrett B, Holcapek M, Huclová J, Borek-Dohalský V, Fejt P, Nemec B, Jelínek I. Validated HPLC-MS-MS method for determination of Quetiapine in human plasma. J Pharm Biomed Anal.2007; 44: 498–505.
5.Dhaneshwar S R, Patre N G, Mahadik M V. Stability-indicating HPTLC method for quantitation of Quetiapine fumarate in the pharmaceutical dosage form. Acta Chromatogr.2009; 21: 83–93.
6. Pullen R H, Palermo K M, Curtis M A. Determination of an antipsychotic agent (ICI 204, 636) and its 7-hydroxy metabolite in human plasma by high performance liquid chromatography and gas chromatography-mass spectrometry. J Chromatogr.1992; 573: 49–57.
7. Li K Y, Zhou Y G, Ren H-Y, Wang F, Zhang B-K, Li H-D. Ultra-performance liquid chromatography-tandem mass spectrometry for the determination of atypical antipsychotics and some metabolites in In-Vivo sample. J Chromatogr B. 2007; 850: 581–585.
8.Yuri Kazakevich and Rosario Lobrutto Seton. HPLC for Pharmaceutical Scientists. 1st ed. Published by Wiley-VCH;2007; 389-391.
9. ICH, Validation of Analytical Procedure, Text and Methodology Q2 (R1), International conference on Harmonization, IFPMA, Geneve, Switzerland, 2005.
10. Kirchherr H, Kühn-Velten W N. Quantitative determination of forty eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: A multi-level, single sample approach. J Chromatogr B. 2006; 843: 100–113.
11. S. Ashutosh Kumar, Manidipa Debnath, Dr. J.V.L.N.Seshagiri Rao. Method Development and Validation by RP-HPLC method for the Estimation of Quetiapine in bulk as well as in pharmaceutical Dosage form; IJAPR, April. 2013: Vol. 4: Issue 4: 1659 – 1668.
12. S. Ashutosh Kumar, Manidipa Debnath, Dr. J.V.L.N.Seshagiri Rao. Stability Indicating RP-HPLC Method for the Estimation of Quetiapine Fumarate in Bulk as well as in Pharmaceutical Dosage Form. Journal of Drug Delivery & Therapeutics; 2013, 3(4), 62-71.

13. Indian Pharmacopeia 2007, Volume I, Published by The Indian Pharmacopoeia Commission, 477-478.
14. Yuri Kazakevich and Rosario Lobrutto Seton. HPLC for Pharmaceutical Scientists. 1sted. Published by Wiley-VCH;2007; 369-382.
15. Stavros Kromidas. HPLC Made to Measure, A Practical Book for Optimization. Published by Wiley-VCH; 2006; 62-66.
16. Sharma B.K.; Instrumental Method of Chemical Analysis, 21th Edition, Goel Publishing Housing, Krishna Prakashan Ltd., 2002; 3
17. Skoog, West and Holler; Fundamental of Analytical Chemistry, 7th Edition, Saunders college Publishing, 1992.
18. Conners K. A.; Textbook of Pharmaceutical Analysis, 3rd Edition, A Wiley-Intersciences Publication, 1999; 616-622.
19. ICH, Validation of Analytical Procedure, Text and Methodology Q2 (R1), International conference on Harmonization, IFPMA, Geneve, Switzerland, 2005.
20. ICH Q2B- Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Nov, 1996.
21. ICH-Q2B Validation of Analytical Procedures: 1996.Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland.
22. ICH harmonized tripartite guideline. Impurities in New Drug products Q3B (R2) current step 4version dated 2 June 2006.