ORIGINAL ARTICLE

Stability-indicating high-performance liquid chromatography (HPLC) method development and validation for the determination of quetiapine fumarate in bulk and dosage form by HPLC

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Abstract

Objective: The purpose of the realistic study is the determination of Quetiapine fumarate in bulk and tablet dosage form utilizing a basic, particular, exact, and formalize stability signifying high-performance liquid chromatography (HPLC) method. **Materials and Methods:** The assessment of Quetiapine fumarate in tablet dosage form performed using a validated stability-indicating HPLC method. HPLC Binary Gradient System of model no. HPLC 3000 Series having Greece C18 (250 mm × 4.6 ID, Particle size: 5 micron) column using mobile phase composition of methanol:water (80:20) is used and pH was adjusted to 3. Flow rate was maintained at 0.9 ml/min at room temperature. Evaluation was accomplished with ultraviolet detection at 213 nm. **Results and Discussion:** The retention time acquired for Quetiapine fumarate was at 3.3 min. The result acquired with the detector response was found to be linear which is in the concentration range of 20–100 µg/ml. This technique has been accepted and appeared to be particular, delicate, exact, straight, precise, rough, vigorous, and quick. Quetiapine fumarate was subjected to different accelerated stress conditions. The degradation products when anywhere well resolved from the pure drug with significantly different retention time values. **Conclusion:** It is inferred that this system can be connected for routine quality control of Quetiapine fumarate in tablet dosage forms as well as in bulk drug.

Key words: International conference harmonization, quetiapine fumarate, stability-indicating studies

INTRODUCTION

Chemically, Quetiapine fumarate is 2-[2-(4-benzo[b][1,4]benzothiazepin-6-ylpiperazin-1-yl)ethoxy]ethanol as shown in Figure 1. It is an antipsychotic medication utilized as a part of the treatment of schizophrenia and in addition for the treatment of acute manic episodes associated with bipolar I disorder. Quetiapine's antipsychotic activity is likely due to a combination of antagonism at D2 receptors in the mesolimbic pathway and 5HT2A receptors in the frontal cortex. Antagonism at D2 receptors mitigates positive indications while opposition at 5HT2A receptors diminishes negative manifestations of schizophrenia.[1-4] In some literature, Quetiapine fumarate is accounted for as inadequately water soluble having low bioavailability. Literature study reveals few ultraviolet (UV) spectrophotometric systems, chromatographic routines for the determination of Quetiapine fumarate alone and in blend with different medications in natural liquids.[5,6] The present paper point is to report straightforward, delicate, specific, exact, precise, powerful, and rugged validated stability demonstrating high-performance liquid chromatography (HPLC) system for the estimation of Quetiapine fumarate in bulk and additionally in dosage form also. Structure of Quetiapine fumarate shown in Figure 1.

EXPERIMENTAL

Materials and Methods

The Quetiapine fumarate, active pharmaceutical ingredient (API) was supplied by Glenmark pharmaceutical, Nasik,

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India. The methanol (HPLC grade) was bought from MERCK.

Instruments

HPLC Binary Gradient System with P-3000-M Reciprocating pump connected to UV-3000-M detector, Wensar High Precision Balance of model no. PGB 100 was used for all weighing.

Method Development

Chromatographic conditions

Chromatographic separation was accomplished on HPLC Binary Gradient System of model no. HPLC 3000 Series having Greece C18 (250 mm × 4.6 ID, Particle size: 5 µ) column using mobile phase composition of methanol:water (80:20) and pH adjusted at 3. Flow rate was maintained at 0.9 ml/min at an ambient temperature with 213 nm UV detection. The retention time obtained for Quetiapine fumarate was at 3.3 min as shown in Figure 2. Diluent was prepared by mixing methanol and water in 80:20 ratio, filtered through 0.45 µm and degassed before use. Figure 2 indicates typical chromatogram of Quetiapine fumarate at 213 nm.

Preparation of stock solution

Precisely measured amount of Quetiapine fumarate (10 mg) was transferred to 10.0 ml volumetric flask. Then, small amount methanol was added and ultrasonicated for 5 min and diluted up to the mark with methanol (concentration: 1000 µg/ml).

Preparation of standard working solution

From the stock solution, pipette out 1 ml into 10 ml volumetric flask and make up the final volume with methanol (100 µg/ml).

Preparation of mobile phase

The mobile phase was prepared by mixing methanol and water in 80:20 ratio. The mobile phase was filtered through 0.45 mm and degassed before use.

Preparation of working sample solution

Nine tablets of Quetiapine were weighed, and powder equivalent to 20.4 mg was transferred to 10 ml standard flask and dilute it with solvent up to 10 ml that gives 1000 µg of Quetiapine present in tablet. The above solution was suitably diluted with mobile phase to obtain final dilution of Quetiapine fumarate (60 µg/ml).

Method Validation

The technique was accepted for its linearity range, exactness, accuracy, affectability, and specificity. Strategy acceptance is completed according to international conference harmonization (ICH) rules.

Linearity

For the construction of calibration curve of Quetiapine fumarate solution, the graph is plotted as peak area versus concentration and the regression coefficient was calculated. The concentration range is in between 20 and 100 µg/ml. Accurately measured standard working solution Quetiapine fumarate (2, 4, 6, 8, and 10 ml) was transferred to a series of 10 ml volumetric flasks and diluted up to the mark with mobile phase. Aliquots (20 ml) of each solution were injected under the operating chromatographic condition described above.

Accuracy

The accuracy of Quetiapine fumarate solution was obtained by calculating recoveries by the standard addition methods. For the determination of accuracy, the solutions of different concentrations that are 20, 60, and 100 µg were prepared. The solutions were prepared in triplicates and the accuracy was indicated by % recovery.

Method precision

The precision of the instruments was checked by repeatedly injecting (n ¼ 6) solutions of Quetiapine fumarate (60 µg/ml).

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed methods were determined by the corresponding responses 3 times on the same day and on the 2nd day for concentration of Quetiapine fumarate (60 µg/ml).

Robustness

Robustness of the method was determined by carrying out the analysis using different concentrations at wavelength (215 nm) and flow rate (i.e., 0.9 ml/min).

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the drug carry were calculated (using the following equation)as per ICH guidelines. Kindly remove the using the following equation.

FORCED DEGRADATION

Quetiapine fumarate solution (10 mg dissolved in 10.0 ml) was prepared. From this solution, 100 µg/ml solution was prepared and transferred to a small round-bottom flask. The solution was kept at room temperature for 2 h. Then, 20 µl solution was injected into HPLC system.

Acid and Alkali Hydrolysis

Quetiapine fumarate solution (10 mg dissolved in 10.0 ml) was prepared. Then, 100 µg/ml solution was transferred to a small round-bottom flask. The solution was mixed with 0.1N hydrochloric acid or 0.1 N sodium hydroxide. The prepared

solutions were kept at room temperature for 2 h. From resulting solution, 20 µl solution was injected into HPLC system.

Oxidation

Quetiapine fumarate solution (10 mg dissolved in 10.0 ml) was prepared. Then, 100 µg/ml solution was transferred to a small round-bottom flask. The contents were then mixed with 3% hydrogen peroxide solution, and the reaction mixture was allowed to proceed at room temperature for 2 h with intermittent shaking. From resulting solution, 20 µl solution was injected into HPLC system.

RESULTS AND DISCUSSION

To enhance the HPLC parameters, several mobile phases of different compositions were tried. A satisfactory separation and good peak symmetry for Quetiapine fumarate were obtained with a mobile phase consisting of methanol:water (80:20) and pH adjusted at 3. Evaluation was accomplished with UV detection at 213 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained. The retention time obtained for Quetiapine fumarate was at 3.3 min. The detector response was linear in the concentration range of 20–100 µg/ml. System suitability parameters were calculated and compared with the standard limit as per ICH.[7-9]

Validation of the Proposed Method

Linearity

Linear correlation was obtained between peak areas used absorbance versus concentration of Quetiapine fumarate in the range of 20–100µg/ml. The linearity of the calibration curve was validated by the high value of correlation coefficient of regression as shown in Figure 3.

Results are shown in Table 1.

System suitability tests

The chromatographic systems used for investigation must pass the system suitability limits before sample analysis can commence. The tailing factor (T), theoretical plate number (N), and resolution (Rs) for the principle peak were using Quetiapine fumarate solution of 60 µg/ml. The relative standard deviation (RSD) of five consecutive injections was found to be 0.47%, indicating good injection repeatability. The tailing factor (T) for Quetiapine fumarate peak was found to be 1.17, reflecting good peak symmetry. The resolution (Rs) for the principle peak and degradation product was found to be 0.00, indicating good separation of the drug from degradation product. The theoretical plate number (N) was found to be 4534 for the column used in the study (250 mm × 4.6 ID, Particle size: 5 µ), thus demonstrating acceptable column efficiency. All these results assure the adequacy of the proposed HPLC method for routine analysis of Quetiapine fumarate as results shown in Table 2.

Analysis of marketed formulation

Resolution of the peaks with clear baseline was obtained. The retention time obtained for Quetiapine fumarate was at 3.3 min. There was no interference from the excipients commonly present in the tablets. The Quetiapine fumarate content was found to be 81.6%, as shown in Table 3.

Accuracy

The accuracy experiments were carried out by the standard addition method. The % RSD for Quetiapine fumarate is as shown in Table 4.

Precision

The low % RSD values of intraday and interday (0.535 and 0.525%) for Quetiapine fumarate reveal that the proposed method is precise, Refer Table 5. Make change method is precise as shown in table 5

Robustness

The graph robustness for Quetiapine fumarate in the range of 20–100 µg/ml reveals that the proposed method is robust as shown in Figure 4.

LOD and LOQ

LOD for Quetiapine fumarate was found to be 0.52 and LOQ for Quetiapine fumarate was found to be 1.74. This data show that the method is sensitive for the determination of Quetiapine fumarate as shown in Table 6.

Stability-indicating assay

The ICH guideline entitled stability testing of drug substance and products requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance, and provides a rapid identification of differences that might result

Table 1: Linearity of Quetiapine fumarate

Parameter	Result
Linearity range	20–100 µg/ml
Slope	84707
Intercept	629139
Regression coefficient	0.996

Table 2: System suitability parameters

Parameter	Result
Retention time	3.3
Theoretical plate	4534
Tailing factor	1.17
Resolution time	0.00

Table 3: Assay of Quetiapine fumarate

Name of the formulation	Labeled claim	Amount found	Amount found (%)
Quetiapine fumarate (Seroquel)	25 mg	20.4 mg	81.6%

Table 4: Accuracy studies of Quetiapine fumarate

Concentration	Area	Mean±SD	% RSD
20	938648		
20	949792	947773±8301.7234	0.875918957
20	954879		
60	4655158		
60	4641245	4652262±9892.2127	0.212632323
60	4660383		
100	7640606		
100	7605477	7611880.333±26119.468	0.343140806
100	7589558		

RSD: Relative standard deviation, SD: Standard deviation, n=3

Table 5: Precision studies of Quetiapine fumarate

Concentration	Precision	Area	Mean	% RSD
60	Interday	4702620 4699149 4743708	4715159	0.525644961
60	Intraday	4743708 4708880 4694604	4715730.7	0.535625386

RSD: Relative standard deviation, n=3

Table 6: LOD and LOQ of Quetiapine fumarate

Standard solution	Concentration	LOD (µg/ml)	LOQ (µg/ml)
Quetiapine fumarate	20	0.29401	0.98005
	60	0.35034	1.16781
	100	0.92505	3.08350

LOD: Limit of detection, LOQ: Limit of quantification

from changes in the manufacturing processes or source sample. Susceptibility to oxidation, hydrolytic, and thermal stability are the required tests. An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products. As described in the experimental section, different stress conditions were applied: Boiling, acid, base hydrolysis, and oxidation. From this investigation, it was clear that in case of boiling, and base hydrolysis, Quetiapine fumarate was stable under the employed stress conditions as no degradation products were observed in their chromatograms, which were identical to the chromatogram of Quetiapine fumarate solution that has not been subjected to any stress conditions as shown in Figure 2.In case of acid hydrolysis and alkaline hydrolysis degradation products were observed at retention times of 3.2 and 2.8 min, respectively, as shown in Figures 5 and 6. Whereas, in thermal-induced degradation and

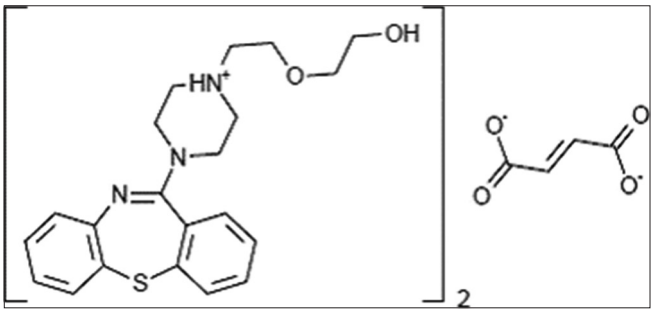

Figure 1: Structure of Quetiapine fumarate

in oxidative degradation were observed at 2.99 and 2.99 min, respectively, as shown in Figures 7 and 8.

In both cases, the method was able to separate completely the degradation products from the intact Quetiapine fumarate.

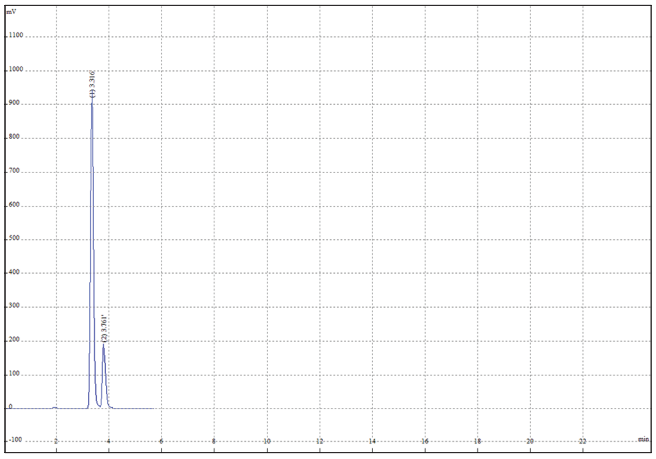

Figure 2: Typical chromatogram of Quetiapine fumarate at 213 nm

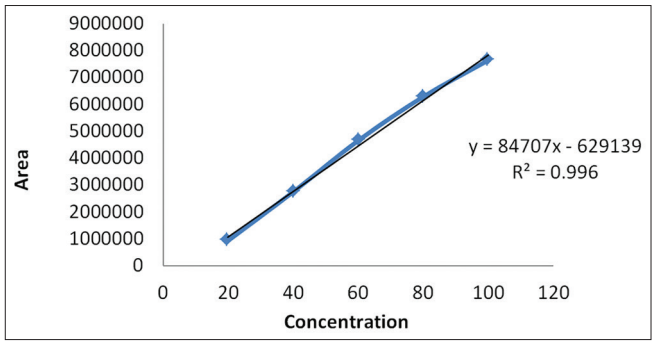

Figure 3: Calibration curve of Quetiapine fumarate with slope (84707 ± 0.67) and intercept (629139 ± 0.05)

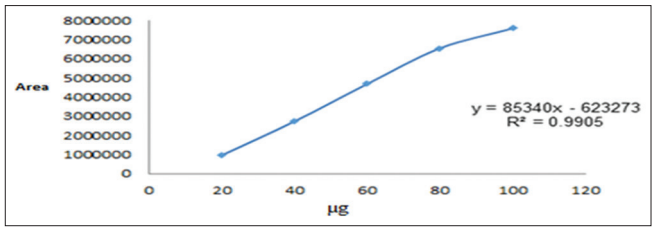

Figure 4: Robustness for Quetiapine fumarate

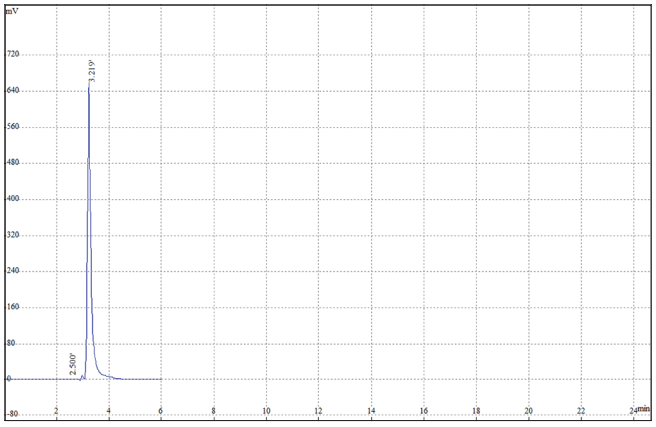

Figure 5: Quetiapine treated with 0.1 N hydrochloric acid

This confirmed the selectivity and stability-indicating property of the proposed method. The percentage recovery of Quetiapine fumarate was calculated and shown in Table 7.

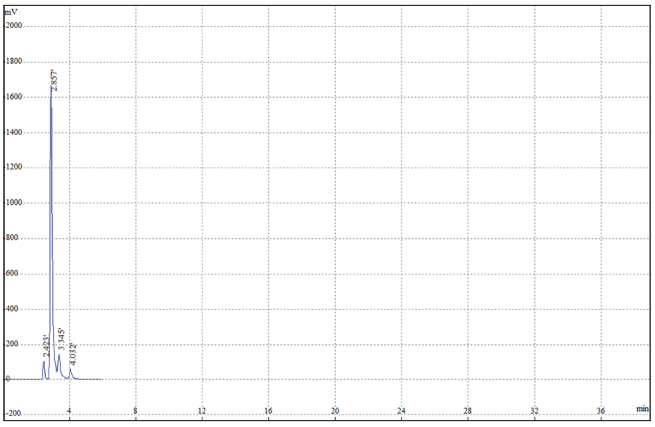

Figure 6: Quetiapine treated with 0.1 N sodium hydroxide

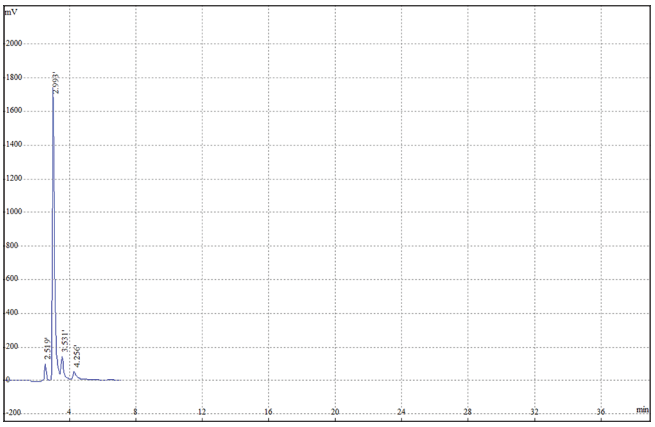

Figure 7: Quetiapine treated with thermally

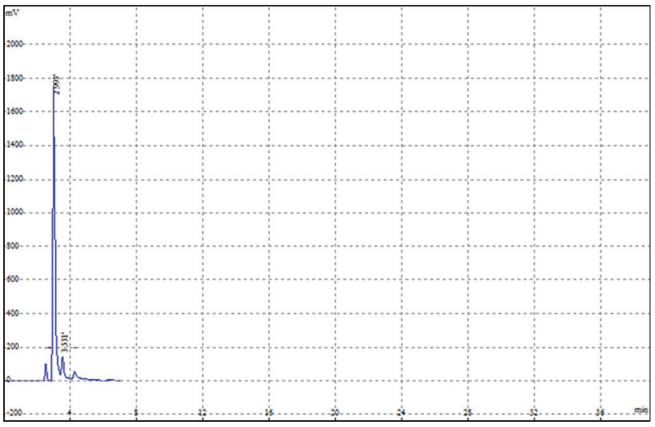

Figure 8: Quetiapine fumarate treated with hydrogen peroxide

CONCLUSION

The present study speaks to the first report that arrangements with the advancement of a dependability demonstrating HPLC technique for the determination of Quetiapine fumarate. This study is typical example of the development of a stability-indicating assay, established following the recommendations of ICH/FDA guidelines. The proposed strategy demonstrated adequate exactness, accuracy, selectivity, and wide linear concentration range. Measurable examination for the outcomes demonstrated that the system is suitable for the

Table 7: Degradation of Quetiapine fumarate in stress conditions

S.No	Degradation Studies	Retension Time	Average area	USP Plate count	USP Tailing	% recovery
1	Hydrolytic degradation under acidic condition	3.2	6688218	2404	1.68	87.86
2	Hydrolytic degradation under alkaline condition	2.8	7706178	2030	1.98	101.23
3	Thermal induced degradation	2.99	7401068	2500	1.88	97.23
4	Oxidative degradation	2.99	7834054	2096	1.79	102

determination of Quetiapine fumarate in bulk and tablet forms without any interference from degradation products, and it is prescribed for routine use in quality control industry research centers.

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