



Research Article

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Development and validation a RP-HPLC method: Application for the quantitative determination of quetiapine fumarate from marketed bulk tablets

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ABSTRACT

A reliable and reproducible reversed-phase high performance liquid chromatography (RP-HPLC) was developed for the quantitative determination of Quetiapine Fumarate from marketed bulk tablets. The active ingredient of Quetiapine Fumarate separation achieved with C^{18} column using the methanol water mobile phase in the ratio of 30:70 (v/v). The active ingredient of the drug content quantify with UV detector at 359 nm. The retention time of Quetiapine Fumarate is 5.27 min. A good linearity relation ($R^2=0.999$) was obtained between drug concentration and average peak areas. The limit of detection and limit of quantification of the instrument were calculated 0.02 and 0.06 $\mu\text{g/mL}$, respectively. The accuracy of the method validation was determined with the inter-day (100.28 %) and intra-day (100.48 %) recoveries of the drug. The quantification correlation range was 5-50 ppm. The new method was validated according to international conference harmonization guidelines.

Keywords: Quetiapine Fumarate, RP-HPLC, Tablets.

INTRODUCTION

Antipsychotic drug Quetiapine Fumarate chemically is 2-[2-(4-benzo[b][1,4]benzothiazepin-6-yl)piperazin-1-yl)ethoxy]ethanol. The physical state of Quetiapine Fumarate is white powder. It is soluble in methanol. It is used to treatment of schizophrenia disease. It is an oral antipsychotic drug that acts as an antagonist of multiple neurotransmitters including serotonin and norepinephrine [1,2].

Reliability and reproducibility are playing an important role in the method validation protocol. Several literature surveys have revealed that the developed validated methods for Quetiapine Fumarate with RP-HPLC [3-5] and dissolution study [6] are suitable but not reliable and reproducible at 25 °C temperature for routine analysis. So, therefore, in the present study, a reverse phase HPLC method validation was developed for Quetiapine Fumarate according to ICH guidelines [7,8] at 25 °C [9].

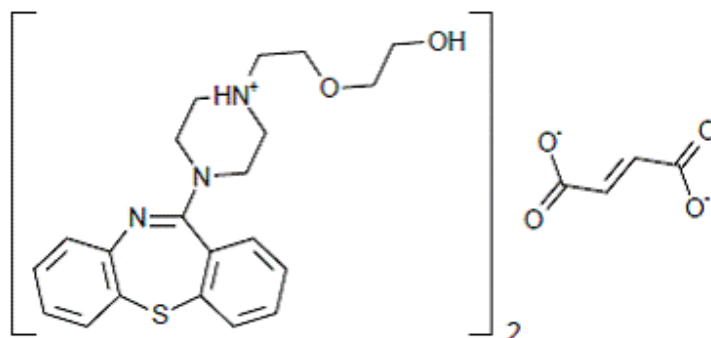


Fig 1: structure of Quetiapine Fumarate

EXPERIMENTAL SECTION

Chemicals and reagents

The Standard drug of Quetiapine Fumarate (99.5% pure) drug, HPLC grade methanol, distilled water and 0.45 nylon filter membrane were purchased from Molychem India Ltd.

Instrumentation

The reversed phase-High Performance Liquid Chromatography method was developed on an HPLC system. The HPLC system consists with a dual pump (CYBERLAB™) equipped with a UV detector and an injector with a 20 mL loop. The separation was on C₁₈-column. The analyte were monitored with UV detector at 359 nm. The HPLC was operated at isocratic elution mode with 30:70 (v/v) methanol-water mobile phases. The flow rate of elution was 1.0 mL/min. An ultrasonic sonicator was used for the sonication of mobile phase, standard solution and sample solution.

Preparation of mobile phase

The mobile was prepared in the ratio of 30:70 (v/v) with methanol /water and the mobile phase was sonicated with an ultrasonic sonicator, after sonication it filtered through 0.45 µm nylon filter.

Preparation of stock solution

A 100 ppm stock solution of Quetiapine Fumarate was prepared by 99.5 % pure drug, 10 mg Quetiapine Fumarate was accurately weighed and transferred into a 100 ml volumetric flask and dissolved with methanol. The Prepared stock solution was sonicate with an ultrasonic sonicator and filtered with 0.45 µm nylon filter.

Preparation of sample solution

The sample solution of Quetiapine Fumarate was prepared with an equivalent quantity of Quetiapine Fumarate tablets powder. The equivalent quantity (10 mg) was transferred into a small conical flask and extract with methanol/water 30:70 (v/v) mobile phase. The extract was filtered into a 100 ml volumetric flask and the volume make up to the meniscus (100 mL). Now the obtained aliquots was covered the working concentration range 5-50 µg/mL.

Preparation of calibration curve

The calibration curve was prepared by injecting the various concentrations of serial dilution 5, 10, 25, 40 and 50 ppm in thrice replication. The calibration curve was constructed by plotting the average peak areas versus known concentration levels.

METHOD VALIDATION

The method validation steps were performed according to international conference harmonization guide lines.

System suitability test

The system suitability test for RP-HPLC was performed. The various system suitability parameters such as peak area, retention time, resolution factor, theoretical plates, and tailing factor were checked according to international

conference harmonization guide line. These all parameters were checked during the development of the method. These all parameters were performed by injecting the standard mixture in n=5 replicates.

Linearity

The different concentrations of Quetiapine Fumarate solution were prepared in the range of 5 to 50 ppm. Prepared different dilutions (20 mL) of each solution were injected under the optimized chromatographic conditions and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentrations and the regression equations were calculated. Each response was the average of six determinations.

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

The sensitivity of the analytical method was evaluated by determining the LOD and LOQ. Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equations:

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10.\sigma/S$$

Where 'σ' is the standard deviation of y-intercept and 'S' is the slope of the curve.

Accuracy

The Performance of the developed method was determined by performing inter-day and intra-day recovery study. The recovery was determined at the three concentration levels 5, 10 and 25 μg/mL.

RESULTS

The selection of mobile phases has done according to reversed phase partition chromatographic conditions. The mobile phases developed to study in order to achieve suitable system stability. There are different ratios of mobile phases such as 10:90, 20:80, 30:70, 40:60, 50:50 methanol water (v/v) were tested at the room temperature 25 °C. A better resolution of Quetiapine fumarate was obtained in the mobile phase 30:70 methanol water (v/v) as shown in the Fig.2.

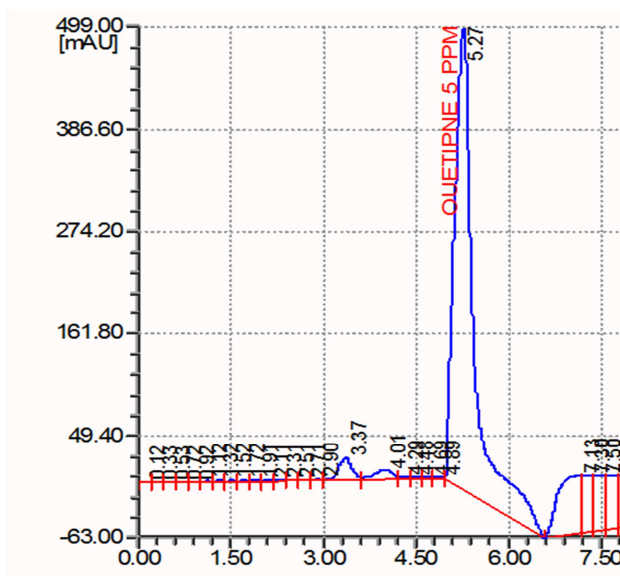


Fig 2. Reference Chromatogram for Quetiapine Fumarate

There are listed the results for system suitability test with various HPLC parameters peak area, retention factor, resolution factor, theoretical plates and tailing factor table No 1. The calculated CV% and SD% values were shown that the chromatographic conditions are statistically significant for the experimental work.

Table 1. Summary of system suitability test (n=5) for RP-HPLC

Sr. No.	Parameters	Mean	SD	CV%
1.	Peak area	1598.64	1.34	0.0838
2.	Retention Time	3.2	0.03	0.9375
3.	Resolution Factor	1.55	0.04	2.580
4.	Theoretical Plates	2988.32	0.08	0.002677
5.	Tailing Factor	1.7647	0.017	0.96

*SD=standard deviation**CV%=coefficient of variation percentage*

The linearity for method validation was determined by regression analysis. The linearity range of the drug Quetiapine fumarate was 5-50 ppm. The applied method was showed better linearity with the statistics parameter (regression coefficient) $R^2=0.999$. The linear regression equations for Quetiapine Fumarate are listed in Table No 2.

Table 2. Details of linearity parameters results

Sr. No.	Parameters	RP-HPLC method
1.	Correlation Range	5-50 μ g/mL
2.	Regression equation	$y=221111x-32801$
3.	Regression coefficient	$R^2=0.999$

The limit of detection (LOD) and limit of quantification (LOQ) for reversed phase-high performance liquid chromatography (RP-HPLC) were calculated 0.02 and 0.06 table No 3, respectively.

Table 3. Detail of Limit of detection (LOD) and Limit of Quantification (LOQ)

Sr.No.	Parameters	RP-HPLC method
1.	Limit of detection (LOD)	0.02 μ g/ml
2.	Limit of Quantification (LOQ):	0.06 μ g/ml

The results for accuracy of the method validation are listed in table No. 4. The accuracy of the method validation was determined by recovery method. The inter-day and intra-day recovery experiment was conducted at the three concentration levels 5, 10 and 25 μ g/mL. The accuracy was calculated inter-day and intra-day 100.28 % and 100.48 %.

Table 4. Detail of recovery study in inter-day and intra-day

Sr.No.	Added drug in μ g	Inter day		Intraday	
		Recovery in μ g	Recovery in (%) (w/w)	Recovery in μ g	Recovery in (%) (w/w)
1.	5	4.96	99.2	4.95	99
2.	10	9.99	99.9	9.87	98.7
3.	25	25.07	100.28	25.12	100.48
		Mean= 0.9979		Mean= 0.9939	
		SD=0.00578		SD=0.00953	
		CV=0.5792		CV=0.95884	

*SD=standard deviation**CV%=coefficient variation percentage*

DISCUSSION

Suitability and reliability of the validated method play is an important role of analytical method. Results of system suitability test indicated that better resolution with statistical calculated data. The coefficient variation percentage is less than 10 for all parameters [10]. The coefficient variation percentage indicates that the system is reliable for the analysis. The recovery study is playing an important role to determine the drug quantity accurately from formulated tablets. The results of recovery study (inter-day=100.28 and intra-day=100.48) indicated that there is no variation of the drug quantity in the given formulated tablets. Hence the several parameters which are applies to the determination of drug content from the formulated tablets are well defined and reliable for the routine analysis of the drug content.

CONCLUSION

The present method validation study developed with isocratic mode of HPLC. This method validation can be applied to the drug determination of Quetiapine Fumarate from formulated products. The all experimental conditions in this method were optimized to provide a better resolution of the drug content. Hence, this method is suitable for the quantitative determination of Quetiapine Fumarate

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