Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [8] July 2021 : 171-180

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

QBD Based RP-HPLC Method Development and Validation for the Estimation of Quetiapine in Presence of Related Substances

Sachin Gholve*[1], Jaiprakash Sangshetti[1], Omprakash Bhusnure[2], Vijayendra Swamy[2], Sanjay Thonte[3]

[1]Dept. of Quality Assurance, Y. B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza Baugh, Aurangabad, Maharashtra, India - 431001

[2]Channabasweshwar Pharmacy College (Degree), Kava Road, Latur, Maharashtra, India - 413512

[3]Channabasweshwar Pharmacy Polytechnic, Kava Road, Latur, Maharashtra, India – 413512

Corresponding Author Email - sbgholve@gmail.com

ABSTRACT

A rapid specific RP-HPLC method has been developed for the estimation of quetiapine impurities in the formulation. The control of pharmaceutical impurities is currently a critical issue in the pharmaceutical industry. The ICH has formulated a workable guideline regarding the control of impurities. The objective of the recent study was to develop and validate a RP-HPLC method for the quantitative determination of process-related impurities of Quetiapine in pharmaceutical formulation. Quetiapine, {2- (2-(4-dibenzo [1, 4] thiazepine-11-yl-1-piperazinyl) ethoxyethanol is an anti-psychotic drug used in the management of schizophrenia and bipolar disorder. Chromatographic identification of the impurities was carried out on Waters Symmetry C_8, 250 x 4.6mm, 5µm column is used for the development of the method. The mobile phase consists of buffer and acetonitrile. The flow rate of the mobile phase was 1.0 mL/min with gradient elution. The column temperature is ambient and the detection wavelength is 290 nm. The injection volume is 10 µL. The method was validated as per ICH guidelines for linearity in the range of 50-150 % and the LOD & LOQ values obtained were 0.0000437 and 0.0001325 µg/ml respectively which specifies the method's sensitivity. The proposed method was successfully used to determine the Quetiapine formulation impurities.

Keywords: *Quetiapine, RP-HPLC, Impurities, linearity, Validation.*

Received 21.06.2021 Revised 02.07.2021 Accepted 10.07.2021

INTRODUCTION

Quetiapine fumarate chemically known as {2- (2-(4-dibenzo [1, 4] thiazepine-11-yl-1-piperazinyl) ethoxyethanol, molecular weight: 615.66—a dibenzothiazepine derivative, is one of the most recent antipsychotic drugs used for the treatment of schizophrenia and for the treatment of acute manic episodes associated with bipolar disorder. An oral antipsychotic drug that acts as an antagonist of multiple neurotransmitters including serotonin and norepinephrine is used in the treatment of schizophrenia. It is a selective monoaminergic antagonist with high affity for the serotonin type 2 (5HT2) and dopamine type 2 (D2) receptors (1,2). This antipsychotic has a low incidence of extrapyramidal side effects and tardive dyskinesia as compared to older antipsychotics (3). Pharmaceutical impurities are unwanted chemicals that coexist with the active pharmaceutical ingredient (API) or develop during the formulation or ageing of both API and formulated APIs into medicines. Even small concentrations of these impurities can have an impact on a drug's effectiveness and safety (4). There are various types of sources of impurities that are affected by products. That is synthesis related impurity, ii) organic impurity and, iii) inorganic impurity (5). The International Conference on Harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use ICH has also published guidelines for validation of methods for analyzing impurities in new drug substances, products, residual solvents, and microbiological impurities (6). In the overwhelming majority of the pharmacopoeial monographs, impurities in the active pharmaceutical ingredient are determined by selective (usually high-performance liquid chromatography (HPLC)) or non-selective (usually titrimetric or ultraviolet (UV) spectrophotometry) methods (7). HPLC is undoubtedly the most important method in drug-impurity profiling. It is widely used for separating and quantifying impurities, and this technique is most frequently used coupled with spectroscopic methods in the identifying and elucidating the structure of impurities (8). Analytical method development and validation play important roles in drug discovery, Drug

Development, and Manufacture of pharmaceuticals. It involves the detection of the purity and toxicity of a drug substance (9).

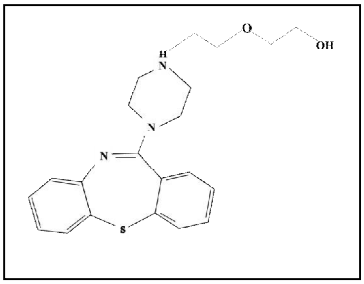

Fig1. Structure of Quetiapine

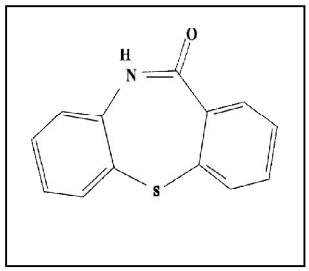

Fig2. Structure of Quetiapine impurity

MATERIAL AND METHODS

Chemicals and materials:

Samples of Quetiapine fumarate bulk material were obtained from the Research and Development Department, Dr. Reddy's Laboratories Ltd., Hyderabad, India. The marketed preparations were purchased from the local market Brand Name ALTRADAY RANBAXY, Mumbai. HPLC grade methanol, TAF buffer was obtained from Merck (India) Limited.

Equipment and apparatus:

HPLC analysis was done by using a Shimadzu HPLC SILAD vp model chromatograph equipped with an LC20 AT gradient delivery system (pump), UV detector and column was Waters Symmetry C_8, 250 x 4.6mm, 5µm. PC installed Chromeleon software was used to record and integrates the chromatograms. The analysis was carried out at ambient temperature. The UV detection was done using SHIMADZU UV visible spectrophotometer (double beam), and the wavelength range of 200 to 400 nm.

HPLC Method development:

Preparation of mobile phase:

The mobile phase consisting of phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) and acetonitrile were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 40:60v/v was pumped into the column.

Preparation of standard stock solution:

Stock solution of quetiapine was prepared by dissolving 100 mg of quetiapine in 100 mL standard volumetric flask containing 25 mL of mobile phase and the solution was sonicated for 20 min. and then made up to the mark with mobile phase to get a concentration of 1000 µg/mL (1). Subsequent dilutions of this solution were made with mobile phase to get concentration of 20-120 µg/mL.

Preparation of Impurity solution:

Accurately weigh about 5.0 mg of Quetiapine impurity G working standard into a 100 mL volumetric flask. Add diluent and mix.

Preparation of Mix standard solution:

Transfer 10 ml standard stock and 1 ml of Quetiapine impurity G into a 25 ml volumetric flask dilute to volume with diluent and mix.

Optimized chromatographic condition

Table 1: Optimization of HPLC method

Column	**Waters Symmetry C_8, 250 x 4.6mm, 5µm**
Mobile phase	Buffer: Acetonitrile (40:60)
Diluent	Methanol: Water: Diethylamine (800:200:1)
Flow rate	1ml/min
Column temperature	Ambient
Injection volume	10µL
Run time	4 minutes
Detector	UV-detector
Detection wavelength	290nm
Elution	Gradient

Method design

Screening method

The screening was done using Placket- Burman design using design expert software 11.

Table 2: Chromatographic factors and response variables for Plackett-Burman experimental design

Sr. no.	Parameters	Levels used		
		Low	Center	High
1	Flow rate	0.6	1.0	1.4
2	Wavelength detection	280	290	300
3	Column temperature	28	30	32
4	Mobile phase (buffer %)	49	50	51
5	Inj. Volume	8	10	12

Total 12 runs were obtained; the response for the design was resolution of the peaks of the Quetiapine. Results were put in design to further optimize the method in Table no. 2 & 3.

Table 3: Plackett Burman method used for Quetiapine

QUETIAPINE						
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Response 1
Run	A:Flow Rate	B:Detection Wavelength	C:Column Temp	D:Mobile Phase	E:Inj Volume	Resolution
	mL/min	Nm	°C	%	µL	
1	1.4	300	28	30	8	1.26
2	0.6	300	32	30	12	1.09
3	0.6	300	32	50	8	1.26
4	1.4	280	32	50	8	0.99
5	1.4	280	32	50	12	1.04
6	1.4	300	28	50	12	1.02
7	0.6	280	32	30	12	1.08
8	1.4	280	28	30	12	1.11
9	0.6	280	28	50	8	1.23
10	0.6	300	28	50	12	1.03
11	1.4	300	32	30	8	1.34
12	0.6	280	28	30	8	1.25

Optimization

It was done by response surface methodology, applying a three-level Box Behnken design with three center points. Three factors selected were a mobile phase, flow rate, column temperature. Evaluation of the main factor, their interaction, and the quadric effect on peak resolution were done. Acetonitrile concentration 60% and wavelength were kept constant as their effect on the resolution was less significant.

Table 4: Chromatographic factors and response variables for box-Behnken experimental design

Sr. no.	Parameters	Levels used		
		Low	Center	High
1	Inj. volume	8	10	12
2	Mobile phase	30	40	50
3	Detection wavelength	280	290	300

Experiments were conducted by making injections of standard Quetiapine solution and the average resolution was analyzed using Design Expert 11 software**.**

Table 5: Box-Behnken method used for Quetiapine determination optimization

	Factor 1	Factor 2	Factor 3	Response 1
Run	A:Inj Volume	B:Mobile Phase	C:Detection Wavelength	Resolution
	µL	*%*	*nm*	
1	10	50	280	1.04
2	10	40	290	1.15
3	8	40	300	1.24
4	10	40	290	1.2
5	8	30	290	1.26
6	8	40	280	0.96
7	12	50	290	1.14
8	10	40	290	1.14
9	10	50	300	1.18
10	12	40	280	1.12
11	8	50	290	1.16
12	10	30	300	1.17
13	10	40	290	1.21
14	10	40	290	1.21
15	12	30	290	1.26
16	10	30	280	1.16
17	12	40	300	1.14

Method validation (10)

System suitability

Test the system suitability performed by injecting blank solution once and spiked solution for six times into a HPLC system. The system suitability was established by evaluating the system suitability parameters from the chromatograms thus obtained. Typical system suitability parameters include %RSD, Tailing factor (T) and Theoretical plates (N).

Linearity

The linearity of the method was demonstrated over the range of 50-150%. The solutions at five levels of concentrations were prepared and 10µl of each of the solutions were injected into the HPLC system to obtain the chromatograms. The linearity curve was constructed by plotting average peak areas against concentration and the regression equation was calculated by the method of least squares. The correlation coefficient, y-intercept and slope of the regression line were reported.

Accuracy

The accuracy of the method was established by performing recovery studies. Recovery studies were performed by spiking sample solution with the pure authenticated standard drug at three different concentration levels i.e. 50,100,150% and LOQ solutions each in triplicate. The mean recovery of the five different concentrations of the drug was calculated.

Precision

The standard stock solution and impurity solutions were prepared and the concentration injected in triplicate into the HPLC system to obtain the chromatograms and the peak areas were recorded from the obtained peaks. Then average and the standard deviation of three peak areas at each concentration level were calculated.

LOD & LOQ

The LOD and LOQ of Quetiapine by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 σ S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept.

RESULT AND DISCUSSION

Optimized chromatographic conditions

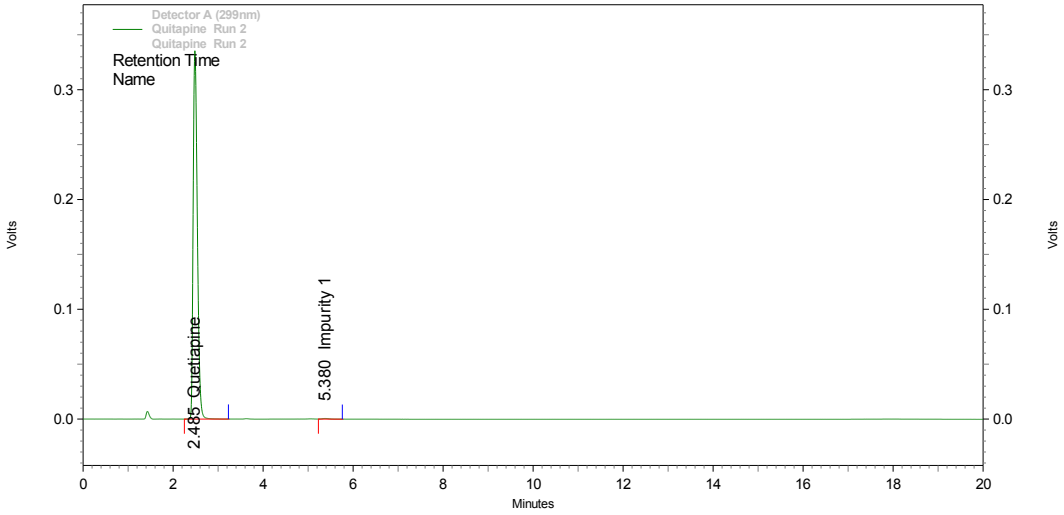

Fig3. Chromatogram of method development of Quetiapine

Method Design

Plackett-Burman design

The screening was done by using Plackett-Burman design, which gives Pareto chart and Probability values (*p*-values) for flow rate, wavelength detection, column temperature, mobile phase, and injection volume.

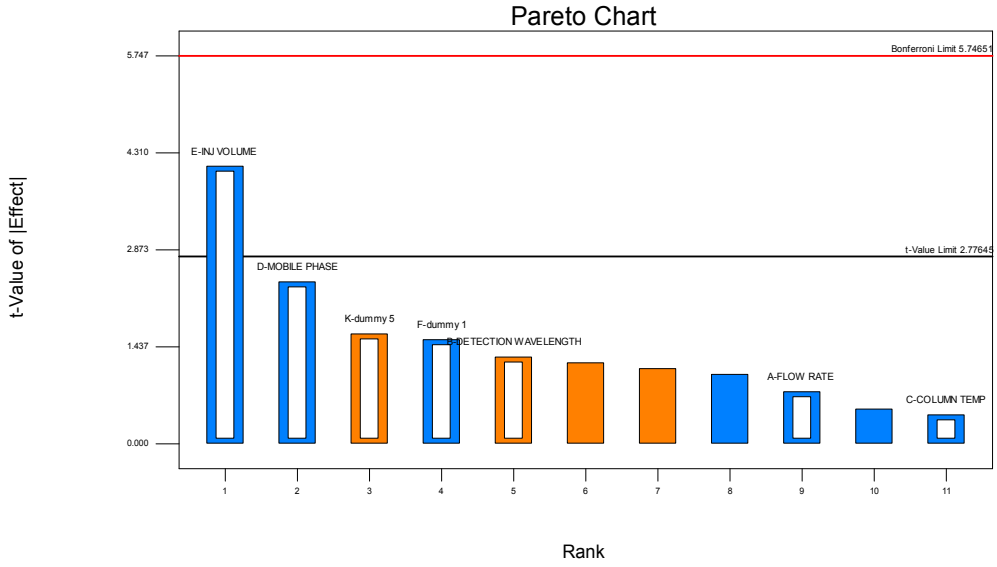

Fig4. Pareto Chart Ranking of Quetiapine

Box-Behnken design

Multivariate regression analysis was implemented then fitted with a full quadratic model which was obtained for the USP resolution factor of the peak. Here factors considered are injection volume, flow rate, and column temperature. The regression coefficient and p-values obtained from the software-generated report are given in (Table no. 6).

Table 6: Regression coefficients and associated probability values (p-values) for USP resolution of Quetiapine

Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	0.080971471	9	0.008997	6.42303	0.01137	significant
A-Inj volume	0.0002	1	0.0002	0.142784	0.716726	
B-Mobile phase	0.0136125	1	0.013613	9.718256	0.016905	
C-Detection wavelength	0.0253125	1	0.025313	18.07114	0.003788	
AB	0.0001	1	0.0001	0.071392	0.797021	
AC	0.0169	1	0.0169	12.06527	0.010357	
BC	0.004225	1	0.004225	3.016318	0.126004	
A^2	2.63158E-07	1	2.63E-07	0.000188	0.989446	
B^2	0.002179211	1	0.002179	1.555785	0.252394	
C^2	0.019042368	1	0.019042	13.59476	0.007786	
Residual	0.009805	7	0.001401			

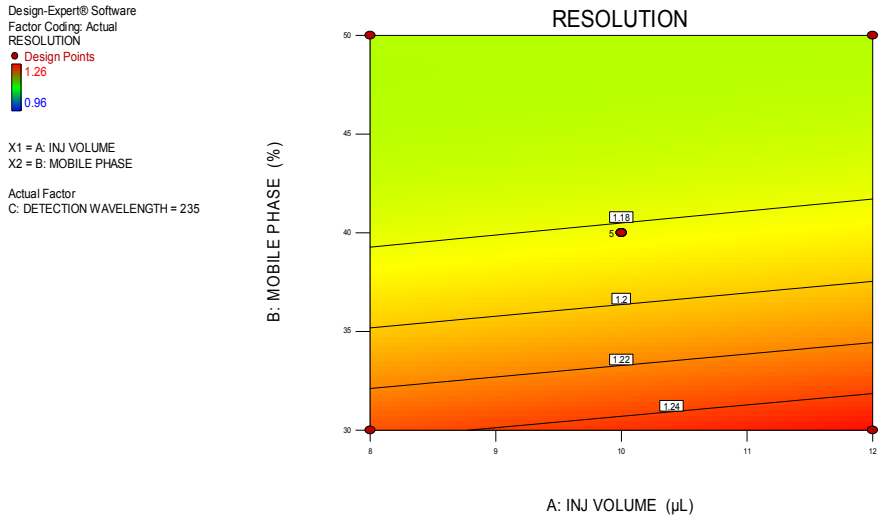

Fig5. Response plot showing Effects of injection volume and mobile phase on USP Resolution factor of Quetiapine

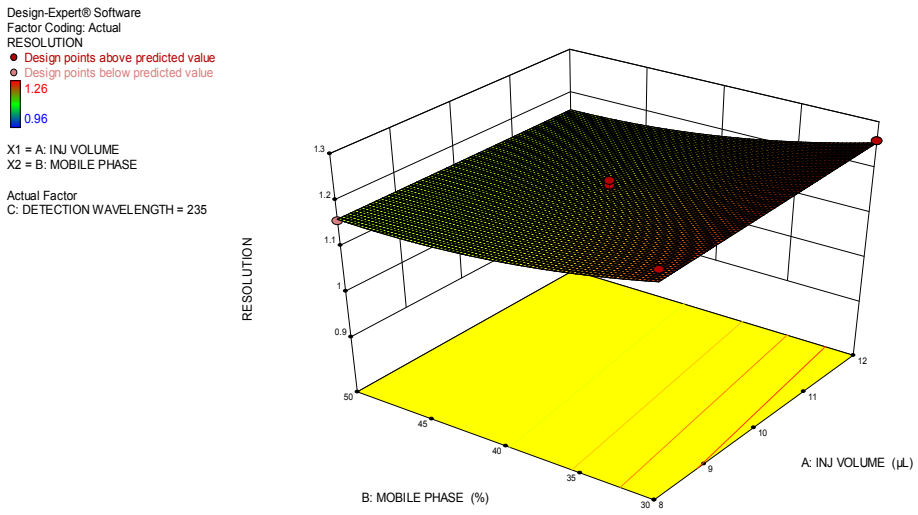

Fig6. Response plot (3D) Showing Effects of injection volume and mobile phase on USP Resolution factor of Quetiapine

To obtain the optimum set of conditions to achieve the desired goal composite desirability parameters were applied. Optimum conditions having desirability were chosen from the obtained runs i.e. Flow rate 1 ml/ min, Mobile phase TFA buffer: methanol, Column temperature 27˚C±2˚C. A set of conditions were analyzed to compare the predicted response with the actual response.

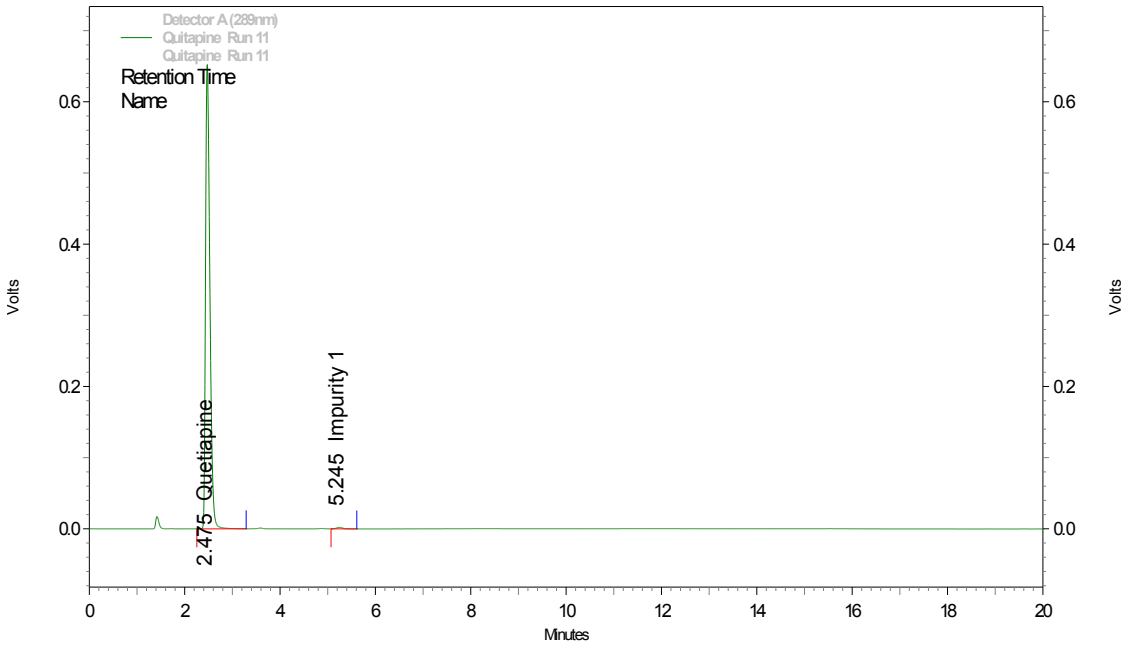

Fig7. Chromatogram of optimized batch

Method validation

System suitability

System suitability studies were carried out in which the % RSD, tailing factor, member of theoretical plates found, were calculated. The resulting chromatograms exhibited a retention time of 16.165 min. From the system suitability studies, it was observed that 0.54% RSD of theoretical plates was to be more than 2000 and the tailing factor was found to be less than 2.

All the parameters were within the limits and the system suitability test was passed.

Table 7. System suitability results for Quetiapine

Sr. No.	Retention Time (min)	Peak area	Resolution	USP Tailing Factor	USP Plate count
1	16.148	10620	7.19	1.16	98194
2	16.163	10644	7.17	1.22	97927
3	16.159	10648	7.07	1.15	100987
4	16.168	10701	7.21	1.16	99795
5	16.169	10677	7.23	1.08	100387
6	16.168	10786	7.26	1.09	99616
Mean	16.162	10679.3			
Standard deviation	0.008	59.4			
%RSD	0.05	0.6			

% RSD for six replicate injections of peak area response for TS calcium spiked solution was found to be less than 2%, tailing factor was found to be less than 1.5 and number of theoretical plates was found to be more than 2000. All the system suitability parameters were satisfied, and thus the system suitability test was passed.

Linearity

The linearity of the drug was established by constructing the calibration curve with a concentration on the x-axis and peak area on the y-axis. From the calibration curve, it was observed that the method was linear over the concentration range of 50.0.-150.0(mcg/ml) for Quetiapine spiked solution and correlation coefficient (r^2) was found to be 0.9996.

Table 8: Results for linearity curve

Concentration (µg/ml)	Peak area
20	124131
40	245430
60	373288
80	502094
100	605534
120	723551

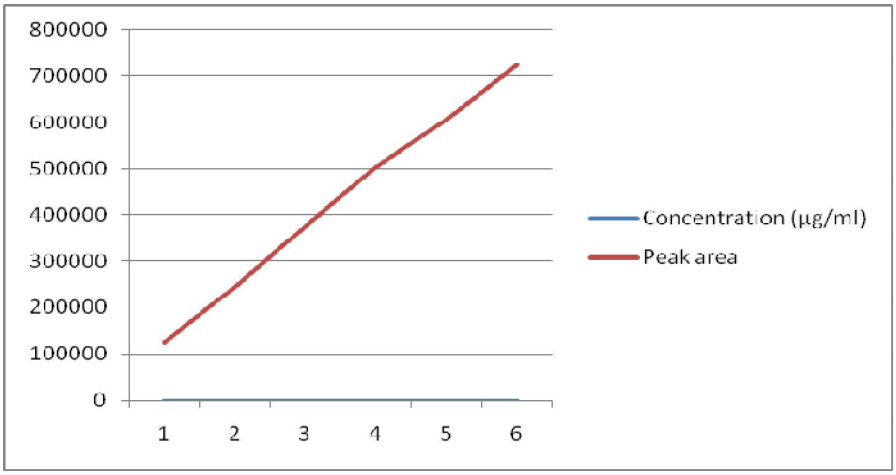

Fig8. Calibration curve of Quetiapine

Accuracy

The accuracy of the method was determined by performing recovery studies at 50%, 100%, 150%. The man recovery of pure drug from the analyzed solution of the formulation was found to be in the accurate range. Hence, the method is said to be accurate.

Table 9: Peak Areas of Accuracy for Quetiapine

Sr. No.	Sample Name	Retention Time(min)	Area	USP Resolution	USP Tailing	USP Plate count
1	Accuracy at 50% Prep-1	16.342	7346306	6.44	0.93	90728
2	Accuracy at 50% Prep-2	16.328	7215357	6.39	0.94	89770
3	Accuracy at 50% Prep-3	16.322	7015497	6.47	0.94	89849
4	Accuracy at 150% prep-1	16.327	6865979	6.47	0.94	90042
5	Accuracy at 150% prep-2	16.341	6628475	6.50	0.94	89264
6	Accuracy at 150% prep-3	16.362	7068321	6.39	0.94	88524
7	Mean	16.337	7023322.4			
8	Std.Dev	0.014	254536.1			
9	%RSD	0.09	3.6			

Table 10: Peak area of Accuracy at LOQ for Quetiapine

Sr. No.	Sample Name	Retention Time(min)	Area	USP Resolution	USP Tailing	USP Plate count
1	Accuracy at LOQ	16.168	8616219	6.76	0.95	97354
2	Accuracy at LOQ	16.171	8621104	6.62	0.95	98302
3	Accuracy at LOQ	16.171	8645631	6.63	0.95	99193
	Mean	16.170	8627651.3			
	Std dev	0.0.2	15760.9			
	%RSD	0.01	0.2			

Precision

Precision was determined by preparing the impurities mixed solution and injecting twice a day for 3 days. The %RSD of peak areas of chromatograms of impurities mixed Quetiapine was found to be less than 2%. Thus, the method passes the precision test.

Table 11: Precision Results for Quetiapine

Sr. No.	Retention Time(min)	Area	USP Resolution	USP Tailing	USP Plate count
1	16.183	8175667	6.75	0.95	95274
2	16.185	8142016	6.86	0.95	96315
3	16.186	8323887	6.80	0.95	98079
4	16.180	8156190	6.76	0.95	100259
5	16.184	8273392	6.78	0.95	95434
6	16.194	8244127	6.65	0.95	98162
7	16.185	8219213.1			
8	0.005	72581.5			
9	0.03	0.9			

LOD & LOQ

The limit of detection for Quetiapine was found to be 0.0000437µg/ml. The LOD is the smallest concentration of the analyte that can be accurately quantified. The limit of quantitation for Quetiapine was found to be 0.0001325µg/ml.

CONCLUSION

There is no validated method available in the official pharmacopoeias like IP, BP, USP for the identification of Quetiapine formulation impurities with less retention time, accuracy and sensitivity, so attempts were made to develop a method by which the impurities present in the drug can be identified. In the proposed RP-HPLC method, the parameters were optimized to obtain suitable conditions for the analysis of Quetiapine. The method with buffer and methanol as the mobile phase at a flow rate of 1ml/min was found to be optimum. The optimum wavelength for detection was 290nm at which a better detector response for Quetiapine was obtained. The retention time was found to be 2.929 min. To ascertain the effectiveness the calibration was linear in the concentration range of 50 to 150% with a correlation coefficient 0.999.No interference was seen due to mobile phase solvents (blank) and the impurities at the retention times of Quetiapine confirm that the method was specific. The limits of detection and limit of quantitation for Quetiapine were found to be 0.0000437 and 0.0001325 respectively which specify the method's sensitivity.

The values of % RSD below 2% indicate that the method was precise. The method was found robust as the %RSD was below 2%. The theoretical plates were found to be 99742 and the tailing factor was found to be less than 2%. The proposed method was validated following ICH parameters. Finally, it can be concluded that the proposed method was found to be accurate, precise, sensitive and less retention time than previous methods and can be successfully applied for the identification of impurities related to Quetiapine.

ACKNOWLEDGMENT

The authors take this opportunity thanks to Dr. Reddy's Laboratories Ltd., Hyderabad for providing gift sample. The authors would like to acknowledge Principal, Y. B. Chavan College of Pharmacy, Aurangabad for providing facilities for conducting research. The authors would like to acknowledge Hon. Mr. Bhimashankarji Devnikar, Secretary, Panchakshari Shivacharya Trust, Aloor for giving support in this regard.

CONFLICT OF INTEREST

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ETHICS STATEMENT

This study does not involve experiments on animals or human subjects.

REFERENCES

1. Suneetha D, Lakshmana Rao A. (2010). A validated RP-HPLC method for the estimation of quetiapine in bulk and pharmaceutical formulations. E-J Chem. ;7(S1):S261–6.
2. Venkata KB, Battula SR, Dubey S.(2013). Validation of quetiapine fumarate in pharmaceutical dosage by reverse-phase HPLC with internal standard method. J Chem. 2013.
3. Rezaei M, Ramazani A, Hokmabadi F. (2018). Quetiapine fumarate syntheses and its determination methods in the pharmaceutical dosage forms, human plasma and urine by RP-HPLC and other analytical techniques: a review. Chem Methodol. 2(2):141–65.

4. Singh A, Afreen S, Singh DP, Kumar R. (2017). A review on pharmaceutical impurities and their importance. World J Pharm Pharm Sci. 6(10):1337–54.
5. Misra B, Thakur A, Mahata PP. (2015). Pharmaceutical Impurities: a review. IJPC. 5(07):232–9.
6. Bari SB, Kadam BR, Jaiswal YS, Shirkhedkar AA.(2007). Impurity profile: significance in active pharmaceutical ingredient. Eurasian J Anal Chem. 2(1):32–53.
7. Gorog S. (2006). The importance and the challenges of impurity profiling in modern pharmaceutical analysis. Elsevier.
8. Olsen BA, Castle BC, Myers DP. (2006). Advances in HPLC technology for the determination of drug impurities. TrAC Trends Anal Chem. 25(8):796–805.
9. Sanap GS, Zarekar NS, Pawar SS. (2017). Review on method development and validation. Int J Pharm Drug Anal. ;177–84.
10. Kumar N, Sangeetha D, Goyal R, Reddy PS. (2013). A validated stability-indicating RP-LC method for the estimation of process-related impurities and degradation products of quetiapine fumarate in solid oral dosage form. Acta Chromatogr. ;25(2):393–409.
11. ICH Q2 (R1); (2005). Validation of Analytical Procedures, Text and Methodology; I.C.H. Harmonized Tripartite Guidelines, Geneva, 8-13.

CITATION OF THIS ARTICLE

S Gholve, J Sangshetti, O Bhusnure, V Swamy, S Thonte. QBD Based RP-HPLC Method Development and Validation for the Estimation of Quetiapine in Presence of Related Substances. Bull. Env. Pharmacol. Life Sci., Vol10[8] July 2021 : 171-180