A Validated RP-HPLC Method for the Estimation of Quetiapine in Bulk and Pharmaceutical Formulations

D. SUNEETHA and A. LAKSHMANA RAO*

Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy
Vishnupur, Bhimavaram- 534 202, A.P., India

dralrao@gmail.com

Received 18 April 2010; Accepted 10 June 2010

Abstract: A new, simple, specific, sensitive, rapid, accurate and precise RP-HPLC method was developed for the estimation of quetiapine in bulk and pharmaceutical formulations. Quetiapine was chromatographed on a reverse phase C₁₈ Waters column (75×4.6mm I.D., particle size 3.5 µm) in a mobile phase consisting of phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) and acetonitrile in the ratio 40:60 v/v. The mobile phase was pumped at a flow rate of 0.8 mL/min with detection at 291 nm. The detector response was linear in the concentration of 20-120 µg/mL. The limit of detection and limit of quantitation was found to be 0.2 and 0.75 µg/mL, respectively. The intra and inter day variation was found to be less than 1%. The mean recovery of the drug from the solution was 99%. The proposed method is simple, fast, accurate, precise and reproducible hence, it can be applied for routine quality control analysis of quetiapine in bulk and pharmaceutical formulations.

Keywords: RP-HPLC, Quetiapine, Estimation, Tablets.

Introduction

Quetiapine fumarate is an atypical antipsychotic agent¹ indicated for the treatment of schizophrenia and for the treatment of acute manic episodes associated with bipolar disorder. The exact mechanism by which quetiapine exerts its antipsychotic effect is unknown. It is a selective monoaminergic antagonist. However, this effect may be mediated through antagonism of dopamine type 2 (D₂) and serotonin type 2 (5HT₂) receptors. Quetiapine is a dibenzothiazepine derivative and is chemically², 2-[2-(4-Dibenzo[*b,f*] [1,4]thiazepin-11-yl-1-piperazinyloxy)ethoxy]ethanol.

Figure 1. Chemical structure of quetiapine fumarate

Literature survey reveals that few spectrophotometric³⁻⁵, HPLC⁶⁻¹⁴, LC-MS¹⁵⁻¹⁹, UPLC²⁰, GC²¹, HPTLC²² and capillary zone electrophoresis²³ methods have been reported for the estimation of quetiapine. In the present study the authors report a simple, rapid, sensitive, accurate and precise HPLC method for the estimation of quetiapine in bulk and tablet dosage forms.

Experimental

Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Acetonitrile of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Quetiapine fumarate was a gift sample by Nosch Labs Pvt. Ltd., Hyderabad. The commercially available quetiapine tablets were procured from the local market.

Instrumentation

The separation was carried out on HPLC system (Waters) with 2695 binary HPLC LC pump, with a 2487 UV-Visible dual absorbance detector, Empower software and RP-C₁₈ column (75 mm × 4.6 mm I.D., particle size 3.5 µm).

Chromatographic conditions

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 at a flow rate of 0.8 mL/min. The detection was monitored at 291 nm and the run time was 4 min. The volume of injection loop was 10 µL. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min. with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Procedure

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. Subsequent dilutions of this solution were made with mobile phase to get concentration of 20-120 µg/mL. The standard solutions prepared as above were injected into the 10 µL loop and the chromatogram was recorded in Figure 2.

The retention time of quetiapine was found to be 2.929 min. The calibration curve was constructed by plotting concentration *vs.* peak area ratio. The amount of quetiapine present in sample was calculated through the standard calibration curve. The linearity

experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug vs. concentration were found to be linear and the results are furnished in Table 1.

Figure 2. Typical chromatogram of quetiapine

Table 1. Calibration data of the method

Concentration, $\mu\text{g/mL}$	Peak area (n=6)
20	124131
40	245430
60	373288
80	502094
100	605534
120	723551

Assay

Two commercial brands of tablets were chosen for testing suitability of the proposed method to estimate quetiapine in pharmaceutical formulations. Twenty tablets each containing 25 mg were weighed accurately and powdered. A quantity equivalent to 100 mg of quetiapine was weighed accurately and transferred to 100 mL volumetric flask containing 30 mL of mobile phase. The contents were sonicated for 20 min. and made up to the mark with the mobile phase. The resulting solution is filtered through a membrane filter. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously for the pure drug determined. Sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of quetiapine present in tablet formulation was determined by comparing the peak area from the standard. The results were furnished in Table 2.

Table 2. Assay of quetiapine

Formulation	Label claim, mg	Amount found, mg	% Amount found
Brand-1	25	25.08	100.34
Brand-2	25	25.3	101.2

Validation of proposed method

Selectivity of the method was assessed on the basis of elution of quetiapine using the above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, limit of detection, limit of quantitation and robustness has been validated for the determination of quetiapine. The results were furnished in Table 3.

Table 3. System suitability parameters

Parameter	Result
Linearity, µg/mL	20-120
Correlation coefficient	0.999
Retention time, min	2.929
Theoretical plates (N)	3695
Tailing factor	1.19
LOD, µg/mL	0.2
LOQ, µg/mL	0.75

Specificity

The specificity was established by preparing a quetiapine standard at 0.5% level of test concentration and injected 5 times into HPLC system as per the test procedure.

Linearity

The standard curve was obtained in the concentration range of 20-120 µg/mL. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

Precision

The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The results are furnished in Table 4.

Table 4. Precision of the proposed HPLC method

Concentration of Quetiapine, µg/mL	Intra-day precision		Inter-day precision	
	Mean area (n=5)	% C.V.	Mean area (n=5)	%C.V.
60	374442	0.46	373729	0.41
80	500677	0.27	501261	0.17
100	607602	0.55	609695	0.36

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

The LOD and LOQ for quetiapine were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of quetiapine.

Robustness

The robustness was checked by changing the flow rate to 0.7 and 0.9 mL/min and the temperature to 20⁰ and 30⁰C and the method suits best.

Accuracy

The accuracy of the HPLC method was assessed by adding known amount of drug solution to a drug solution of known concentration and subjecting the samples to the proposed HPLC method. The recovery studies were replicated 3 times. The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 50 so as to give the percentage recovery. The results are furnished in Table 5.

Table 5. Recovery studies of the proposed HPLC method

Concentration	Amount Added, mg	Amount Found, mg	% Recovery	Mean Recovery
50%, 40µg/mL	50.1	49.8367	99.47%	99.0%
100%, 80µg/mL	100	99.0432	99.04%	
150%, 120µg/mL	147	144.6994	98.43%	

Results and Discussion

By applying the proposed method, the run time of the method was set at 4 min and quetiapine appeared on the typical chromatogram at 2.929 min, which indicates a good base line. When the same drug solution was injected 5 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 20-120 µg/mL. The regression equation of quetiapine concentration over its peak area ratio was found to be $Y=8382.87+6008.88x$ ($r=0.999$) where Y is the peak area ratio and X is the concentration of quetiapine (µg/mL). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 5 replicate injections was found to be less than 1%. The tailing factor was found to be 1.19, which indicates good shape of peak. The number of theoretical plates was found to be 3695, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.2 µg/mL and 0.75 µg/mL, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of phosphate buffer and acetonitrile were tested. The use of phosphate buffer and acetonitrile in the ratio of 40:60 v/v resulted in peak with good shape and resolution. The high percentage of recovery of quetiapine ranging from 98.43 to 99.47 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

Conclusion

The proposed HPLC method was found to be simple, rapid, sensitive, precise and accurate for the estimation of quetiapine in pharmaceutical formulations. Hence, this method can easily and conveniently adopt for routine quality control analysis of quetiapine in bulk and its pharmaceutical formulations.

Acknowledgment

The authors are thankful to M/s Nosch Labs Pvt. Ltd., Hyderabad for providing a reference sample of quetiapine fumarate.

References

1. Burns M J, *Clin Toxicol.*, 2001, **39**, 1-14.
2. Budavari S, The Merck Index, 13th Ed., Merck & Co., Inc., Whitehouse Station, NJ, 2001, p 1439.
3. Hiranman B B, Sandip V, Lohiya R T and Umekar M J, *Int J Chem Tech Res.*, 2009, **1(4)**, 1153-1161.
4. Bagade S B, Narkhede S P, Nikam D S and Sachde C K, *Int J Chem Tech Res.*, 2009, **1(4)**, 898-904.
5. Pucci V, Mandrioli R, Ferranti A, Furlanetto S and Raggi M A, *J Pharm Biomed Anal.*, 2003, **32**, 1037-1044.

6. Bellomarino S A, Brown A J, Conlan X A and Barnett N W, *Talanta*, 2009, **77**(5), 1873-1876.
7. Belal F, Elbrashy A, Eid M and Nasr J J, *J Liq Chromatogr Relat Technol.*, 2008, **31**, 1283-1298.
8. Radha Krishna S, Rao B M and Someswara Rao N, *Rasayan J Chem.*, 2008, **1**(3), 466-474.
9. Saracino M A, Mercolini L, Flotta G, Albers L J, Merli R and Raggi M A, *J Chromatogr B*, 2006, **843**(2), 227-233.
10. Sachse J, Koller J, Hartter S and Hiemke C, *J Chromatogr B*, 2006, **830**(2), 342-348.
11. Atanasov V N, Kanev K P and Mitewa M L, *Cent Eur J Med.*, 2008, **3**(3), 327-331.
12. Hasselstrom J and Linnet K, *J Chromatogr B*, 2003, **798**, 9-16.
13. Mandrioli R, Fanali S, Ferranti A and Raggi M A, *J Pharm Biomed Anal.*, 2002, **30**, 969-977.
14. Davis P C, Wong J and Gefvert O, *J Pharm Biomed Anal.*, 1999, **20**, 271-282.
15. Ramakrishna N, Gopinadh B, Vishwottam K, Koteswara M, Devender A and Mukkanti K, *Biomed Chromatogr.*, 2008, **22**, 1043-1055.
16. Barrett B, Holcapek M, Huclova J, Borek-Dohalsky V, Fejt P, Nemec B and Jelinek I, *J Pharm Biomed Anal.*, 2007, **44**, 498-505.
17. Kirchherr H and Kuhn-Velten W N, *J Chromatogr B*, 2006, **843**(1), 100-113.
18. Zhiling Zhou, Xin Li, Kunyan Li, Zhihong Xie, Zeneng Cheng, Wenxin Peng, Feng Wang, Ronghua Zhu and Huande Li, *J Chromatogr B*, 2004, **802**(2), 257-262.
19. Li K Y, Cheng Z N, Li X, Bai X L, Zhang B K, Wang F, Li H D, *Acta Pharmacol Sin.*, 2004, **25**(1), 110-114.
20. Li K Y, Zhou Y G, Ren H Y, Wang F, Zhang B K and Li H D, *J Chromatogr B.*, 2007, **850**(1-2), 581-585.
21. Stolarczyk E U, Groman A, Kaczmarek L S and Golebiewski P, *Acta Poloniae Pharmaceutica.*, 2007, **64**, 187-189.
22. Dhandapani B, Somasundaram A, Raseed S H, Raja M and Dhanabal K, *Int J Pharm Tech Res.*, 2009, **1**, 139-141.
23. Hillaert S, Snoeck L and Van den Bosche W, *J Chromatogr A*, 2004, **1033**, 357-362.