

A VALIDATED LC-UV METHOD FOR THE ESTIMATION OF QUETIAPINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, precise, accurate and sensitive LC-UV method was developed and validated for the quantification of quetiapine fumarate (QPF) in the pharmaceutical dosage forms. The chromatography separations were carried out on Agilent Eclipse plus C-8 (4.6 X 75 mm, 3.5 μ m) column. The mobile phase consisted of 10 mM ammonium acetate and 100% methanol. Initially the run started with 100% aqueous phase and reached to 100% organic phase in 5 minutes, then 100% organic phase for 3 minutes and subsequently 100% aqueous phase for 2 minutes and followed by stabilization for 4 minutes with 100% aqueous phase. Milli-Q water: methanol (50:50 v/v) mixture was used as diluent. The flow rate was 1.0 mL/min with detection at 254 nm. Injection volume was 10 μ L and column temperature was 40 °C. Auto-sampler temperature was 10 °C. The LC method was validated with respect to various parameters, as required under ICH guideline. The response of QPF was strictly linear (r^2 : 0.9999) in the concentration range of 2.80 to 200 μ g/mL. Intra-day and inter-day precision were <0.59% and <0.83% (relative standard deviation) respectively and the mean recovery was 100.46%. The validated method was further used for all the pre-formulation and formulation analytical studies (*viz.*, purity and assay) targeted towards development of controlled drug delivery systems for QPF.

Key Words: Quetiapine Fumarate, HPLC, validation.

INTRODUCTION

Quetiapine fumarate (QPF) is chemically bis[2-(2-[4-(dibenzo[b,f][1,4]thiazepin-11-yl)piperazin-1-yl]ethoxy)ethanol]fumarate) is a dibenzothiazepine derivative approved for the management of manifestation of psychotic disorders. QPF is an antagonist at a broad range of neurotransmitter receptors. QPF is used in the treatment of schizophrenia or manic episodes associated with bipolar disorder. It has low incidence of extrapyramidal side effects and tardive dyskinesias compared to older

antipsychotics. The advantages of the therapeutic profile of QPF have led to increasing use in the clinical practice, which encourages the development of new pharmaceutical preparations [1-3]. Literature survey revealed several analytical methods for the determination of QPF in bulk and pharmaceutical preparations, such as tablets and capsules, employing techniques ranging from spectrophotometric methods[4], high-performance liquid chromatography

(HPLC) [5-7] and high performance thin layer chromatography (HPTLC) [8]. Most of the reported HPLC methods are based on mobile phases containing compounds such as potassium hydrogen phosphate, phosphoric acid, etc., which shorten the life span of a column and also these methods are not compatible for LC-MS. Hence, the objective of the present study was to develop and validate a simple, reliable HPLC method for the quantification of QPF in pharmaceutical preparations.

EXPERIMENTAL

Materials:

QPF was a gift sample by Ajanta Pharma Ltd, Mumbai, India. The following excipients were purchased from commercial sources and used as such, lactose mono hydrate (Signet Chemical Corporation Pvt Ltd, Mumbai, India), Carbopol 974P (Meggle, Germany), HPMC K100M (Signet Chemical Corporation Pvt Ltd, Mumbai, India), A-tab[®] (Dibasic calcium phosphate anhydrous, granular, Signet Chemical Corporation Pvt Ltd, Mumbai, India), magnesium stearate[®] (Mallinckrodt, USA), Benecel (Dow, USA), chitosan (Sigma-aldrich), gum kondagogu (Grade-1, M/s. Girijan Co-operative Corporation, India). The HPLC grade solvents such as acetonitrile and methanol were purchased from Ranbaxy, India and chemicals potassium dihydrogen orthophosphate GR and ammonium acetate GR were purchased from Loba Chemicals, India.

Instrumentation:

The Chromatography separations were performed on an Agilent 1200 liquid chromatography system. The instrument was equipped with a G1330B pump, a G1315D Diode array detector variable UV/visible detector, a G1329 auto sampler injector, and Agilent Chemstation chromatography workstation (Agilent, USA). The Chromatography separations

were carried out on Agilent eclipse plus C-8 (4.6 X 75 mm, 3.5 μ m) column.

Chromatographic conditions:

The gradient mobile phase consisted of 10 mM ammonium acetate and methanol. The mobile phase components were filtered through 0.45 micron membrane filter before use. Initially the run started with 100% aqueous solution and reached to 100% organic phase in 5 minutes, then 100% organic phase for 3 minutes followed by 100% aqueous phase for 2 minutes and stabilization for 4 minutes with 100% aqueous phase. The detection was monitored at 254 nm and the run time was 14 min. The flow rate was 1 mL/min and the volume of injection loop was 10 μ L. Prior to the injection of the drug solution, the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The column temperature was 40 °C and the auto-sampler temperature was 10 °C.

Stock solution preparation:

Weighed exactly 100 mg of QPF into 100 mL volumetric flask, approximately 25 mL of methanol was added and sonicated for 5 to 10 minutes. The volume was made up to 100 mL with methanol. The subsequent dilutions were prepared by diluting stock solution with the mobile phase.

Validation of the method:

The method was validated for linearity, accuracy and precision as per the ICH guidelines [9].

RESULTS AND DISCUSSION

To optimize the chromatographic conditions, various combinations of mobile phase ingredients and methanol were tested. The use of ammonium acetate and methanol resulted in peak with good shape (USP tailing factor: 1.06) and resolution from all the excipients. No interfering peaks were found in the chromatogram indicating that commonly

used pharmaceutical excipients did not interfere with the estimation of the drug by proposed HPLC method. Also, the peak of interest was chromatographically pure confirmed through the use of PDA detector.

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Specificity was tested by injecting the spiked sample of QPF with appropriate levels of pharmaceutical relevant excipients such as lactose monohydrate, A-tab, Benecel, HPMC K100M, Carbopol 934P, and magnesium stearate. Other polymers of interest such as gum kondagogu, chitosan and the polyelectrolyte complex of gum kondagogu and chitosan were also included in the study. No interference was observed in any of the cases, and the same is evident from the results Figure 1.

Delectability:

The parameters, limit of quantification (LOQ) and limit of detection (LOD) were determined on the basis of signal to noise ratio. The signal to noise ratios in the range of 8-12:1 was considered for the LOQ and 3-5:1 for the LOD. The LOQ and LOD was found to be 3.12 µg/mL (signal to noise ratio: 13:1) and 1.56 µg/mL (signal to noise ratio- 6:1) respectively. The recovery for LOQ solution was 102.5% with reproducibility of 2.30% (RSD) for triplicate injection at this level. The LOD, LOQ and blank overlay chromatogram is shown in Figure 2.

Range of linearity:

The linearity of peak area responses vs. concentrations was studied from 2.8-200 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.

The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The regression equation of QPF concentration over its peak area ratio was found to be $Y = 66.17x + 18.02$ ($r^2 = 0.9999$) where Y is the peak area ratio and x is the concentration of QPF (µg/mL). The peak area ratios of the drug vs. concentration were found to be linear and the results are furnished in Table 1.

Method repeatability/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 percentage relative standard deviation (% RSD). The % RSD values for intra-day and inter-day precision studies were found to be less than 1%, which indicates that the method was sufficiently precise. The results are shown in Table 2.

Accuracy:

The accuracy of the method was assessed by adding known amount of drug solution in to a drug solution of known concentration and subjecting the samples to the developed method. The recovery studies were replicated 3 times. The accuracy was expressed in terms of recovery and the results are shown in Table 3.

Table 1: Calibration data for QPF

Concentration (µg/mL)	Area
200	3690
100	1851.1
50	955.4
25	517.4
12.5	289.4
6.25	179.4
3.125	124.6

Table 2: Precision of the proposed HPLC method

Concentration ($\mu\text{g/mL}$)	Intra-day precision		Inter-day precision	
	SD	% RSD	SD	% RSD
100	0.41	0.41	0.49	0.48
50	0.52	0.53	0.56	0.55
25	0.86	0.86	1.51	1.48

Table 3: Recovery studies of the proposed HPLC method

Concentration ($\mu\text{g/mL}$)	% Recovery	SD	%RSD
150	99.79	0.54	0.54
75	99.95	0.39	0.39
30	100.23	1.27	1.26
3.12	105.13	2.64	2.51

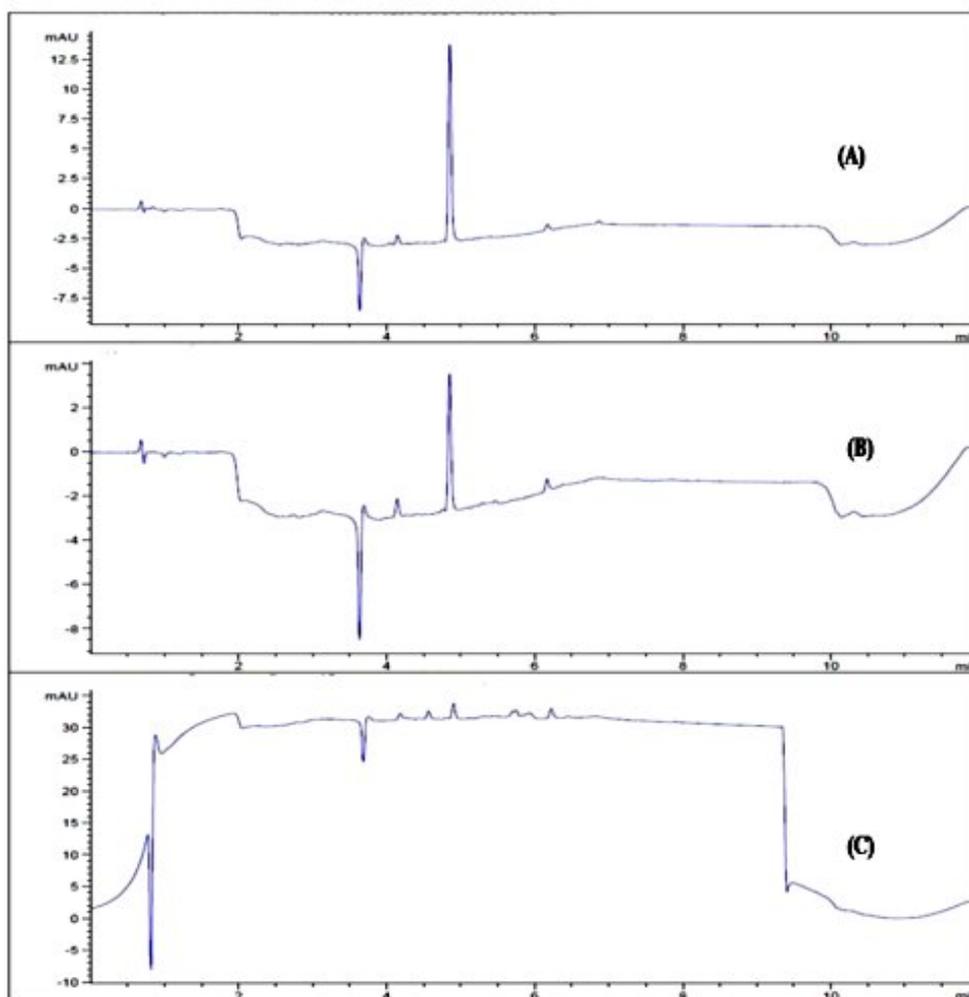


Figure 1: The HPLC chromatograms of QPF A) LOQ, B) LOD and C) Blank

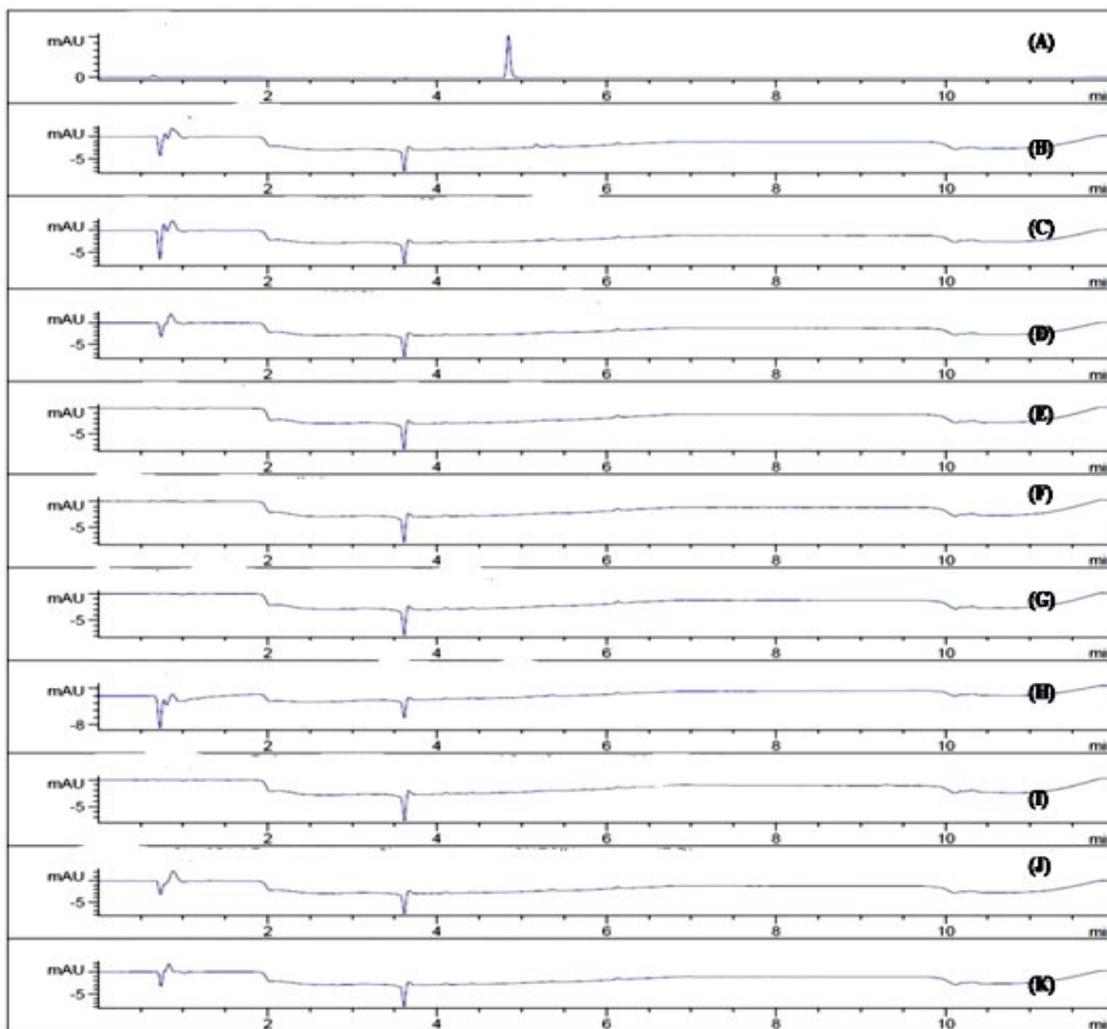


Figure 2: The HPLC chromatograms of A) QPF (0.1 mg/mL), B) A-tab, C) Benecel, D) Carbopol 934p, E) Chitosan, F) Polyelectrolyte complex of gum kondagogu and chitosan, G) Gum kondagogu, H) HPMC K100M, I) Lactose monohydrate J) Magnesium stearate, and K) Blank

CONCLUSION

The proposed HPLC method was found to be simple, rapid, sensitive, precise and accurate for the estimation of QPF in pharmaceutical formulations.

Hence, this method can easily and conveniently adopt for routine quality control analysis of QPF in its pharmaceutical formulations

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