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Research Article

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Stability indicating RP-HPLC-PDA method for the estimation of quetiapine fumarate in bulk and pharmaceutical dosage forms

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ABSTRACT

The present study describes development and subsequent validation of a stability indicating reverse-phase HPLC method for the estimation of Quetiapine Fumarate (QF) in bulk and pharmaceutical dosage forms. The proposed RP-HPLC method utilizes a Inertsil ODS (250 x 4.6 mm, 5µ) column, at ambient temperature, optimum mobile phase consisted of 0.02%v/v formic acid and methanol (90:10), effluent flow monitored at 1mL/min and UV detection at 220nm. The retention time of QF was 13.4min. The bulk active pharmaceutical ingredient was subjected to thermal, photolytic, hydrolytic (acidic and basic) and oxidative stress conditions and stressed samples were analyzed by the proposed method. Considerable degradation was found to occur only in oxidative stress conditions. The method was validated as per ICH guidelines, a good linearity was observed in the concentration range of 10-50µg/mL with a correlation coefficient (R) of >0.999 and method showed good repeatability and reproducibility with percent relative standard deviation less than 2%. The percent assay and recovery values were found to be in the range of 98.56-99.06% and 99.60-100.85% respectively. The proposed RP-HPLC-PDA method is specific, accurate, precise and high sensitive enough for the estimation of QF in bulk and pharmaceutical dosage forms.

Keywords: Quetiapine Fumarate, Forced degradation studies, Validation, Inertsil ODS column

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INTRODUCTION

QF, chemically 2-[2-(4-Dibenzo[*b,f*] [1,4]thiazepin-11-yl-1-piperazinyl) ethoxy] ethanol fumarate [1] is an atypical antipsychotic agent indicated for the treatment of Parkinson's disease, schizophrenia and acute manic episodes associated with bipolar disorder (as either monotherapy or adjunct therapy to lithium or valproate). It is a selective monoaminergic antagonist and this effect is mediated through antagonism of D_2 and $5HT_2$ receptors.

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of a variety of environmental factors such as temperature, humidity and light, and enables recommendation of storage conditions, retest periods and shelf lives to be established.

Literature survey reveals that various UV-spectrophotometric [2-4], RP-HPLC [5, 6], UPLC [7, 8], HPTLC [9, 10], Ion-pair titrimetric [11] and colorimetric methods [12] were published for the estimation of QF in bulk and pharmaceutical dosage forms. So far one stability indicating HPLC [13] method was published for the estimation of QF in tablets and used phosphate buffer in mobile phase which is not LC-MS compatible and also used high percent of organic phase. Hence, in this investigation an attempt was made to develop stability indicating RP-HPLC-PDA

method for the analysis of QF in bulk and pharmaceutical dosage forms which is LC-MS compatible and economical.

EXPERIMENTAL SECTION

2.1 Materials and reagents

QF was supplied by Dr. Reddy's Laboratories, Hyderabad , as gift sample. Methanol, water and formic acid were purchased from E. Merck, Mumbai, India. All the solvents and reagents were used of HPLC grade. QUETIPIN® is a tablet containing QF (50mg) was commercially purchased.

2.2 Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Inertsil ODS column (250 × 4.6mm, 5µ).

2.3 Chromatographic Conditions

Mobile phase consisting of 0.02%v/v formic acid: methanol (90:10) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min before use. The flow rate was 1 mL/min and the injection volume was 20µL and diluent was 50:50 water and methanol. PDA detection was performed at 220nm and the separation was achieved at ambient temperature.

2.4 Preparation of standard stock solution

The standard stock solution was prepared by dissolving an accurately weighed quantity of QF in water, in 10mL volumetric flask, and volume was made upto the mark with the same solvent to obtain mg/mL stock solution of QF.

2.5 Validation

The proposed method was validated for the following parameters as per ICH guidelines.

2.5.1 Specificity

In order to evaluate the interference of degradation products with the estimation of drug peak, specificity studies were carried out by injecting stressed samples after suitable dilutions with the diluent. The peak purity data and resolution between degradants and drug peak indicates the specificity of the method.

2.5.1.1 Forced Degradation Studies

QF was allowed to hydrolyze in acid (1N HCl), base (1N NaOH) and hydrogen peroxide (3%v/v) and also studied for its thermal degradation (at 70°C) and photolytic degradation [14-17].

2.5.1.1.1 Acid Hydrolysis

Stock solutions of mg/mL QF in 1N HCl was prepared and kept at 70°C for 2days. In another volumetric flask, 1N HCl kept at 70°C for the same period as blank. Suitable dilutions were made and samples were analyzed.

2.5.1.1.2 Basic Hydrolysis

Stock solutions of 0.4mg/mL QF in 1N NaOH was prepared and kept at 70°C for 2days. In another volumetric flask, 1N NaOH kept at 70°C for the same period as blank. Suitable dilutions were made and samples were analyzed.

2.5.1.1.3 Oxidative degradation

Stock solutions of mg/mL QF in 3%v/v hydrogen peroxide was prepared and kept at 70°C for 2days. In another volumetric flask, 3%v/v hydrogen peroxide kept at 70°C for the same period as blank. Suitable dilutions were made and samples were analyzed.

2.5.1.1.4 Photolytic degradation

An accurately weighed quantity of QF in solid state was irradiated with UV radiation (overall illumination of ≥210Wh/m^2 at room temperature with UV radiation) for 14 days. Stock solutions of mg/mL QF in water was prepared and samples were analyzed after suitable dilutions.

2.5.1.1.5 Thermal degradation
An accurately weighed quantity of QF in solid state was transferred into glass vial and placed in a controlled temperature oven at 70°C for 14 days. Stock solutions of mg/mL QF in water was prepared and samples were analyzed after suitable dilutions.

The data was given in Table-1.

2.5.2 Linearity
Linearity was evaluated by linear regression analysis using minimum of five standard concentrations. A series of standard dilutions were prepared over a concentration range of 10-50 µg/mL from the stock solutions using the diluent (50:50-water : methanol) and injected on to the column in triplicate. Test results were evaluated by constructing calibration curves plotting standard peak areas against the analyte concentration and resultant data was given in Table 2.

2.5.3 Precision
Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Precision was evaluated by repeatability of measurements of peak area by using a minimum of six determinations at 100% of the test concentration (20µg/mL of QF) and %RSD value was reported and data was given in Table 2.

2.5.4 Accuracy
To check the degree of accuracy of the method, recovery studies were performed by standard addition method. Known amounts of standard were added to pre-analyzed samples at three different concentration levels (80%, 100% and 120%) within the range of linearity and mixtures were analyzed in triplicate by the proposed method. The results were shown in Table 2.

2.5.5 LOD and LOQ
The LOD and LOQ values were determined by the calibration curve method using the formulae LOD = 3.3 σ/m and LOQ = 10 σ/m (Where, σ is the standard deviation of the responses and m is mean of the slopes of the calibration curves).

2.5.6 Robustness
To determine the robustness of the developed method, deliberate changes were made to the experimental conditions and various factors like capacity factor, retention time, and theoretical plate number were calculated. To evaluate the effect of change in flow rate, it was changed by ±20% and to determine effect of the wavelength, it was changed by ±1nm and relevant data was given in Table 3.

2.5.7 System suitability
System suitability was carried out by injecting 20 µg/mL of QF with increment of injection volumes in the range of 10-50 µL. Various system suitability parameters like tailing factor and theoretical plate number were noted and %RSD was calculated.

2.5.8 Assay
Twenty tablets of QF (50mg) were taken and crushed to fine powder. Then powder equivalent to 10mg of QF was taken in 10mL volumetric flask and dissolved in water and vortexed for 5-10min. Solution was filtered through 0.45µm nylon disc filter and the 100µL of filtrate was diluted with diluent to get a solution containing 20µg/mL of QF. The solution was injected in triplicate. The amount present in the each tablet was calculated by comparing the area of standard QF with the test samples.

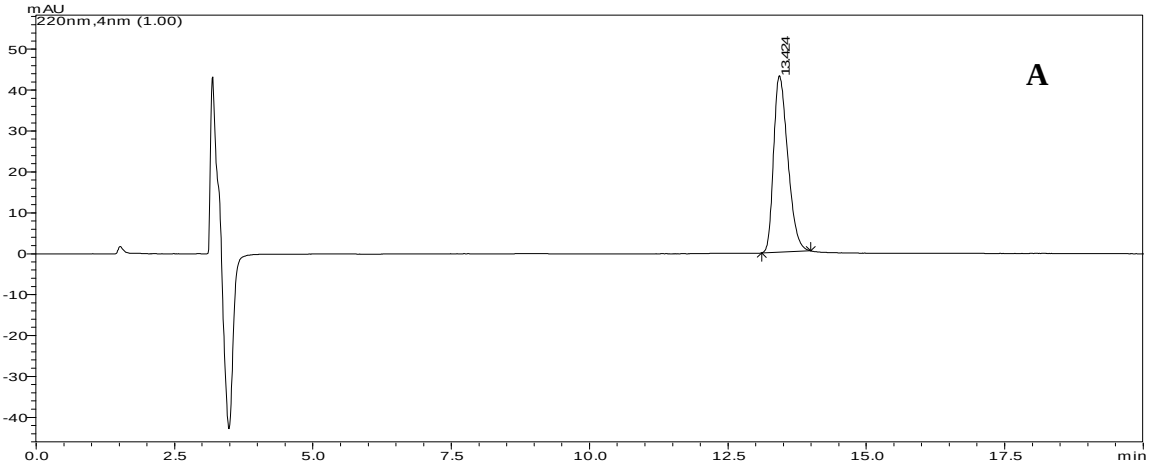
RESULTS AND DISCUSSION

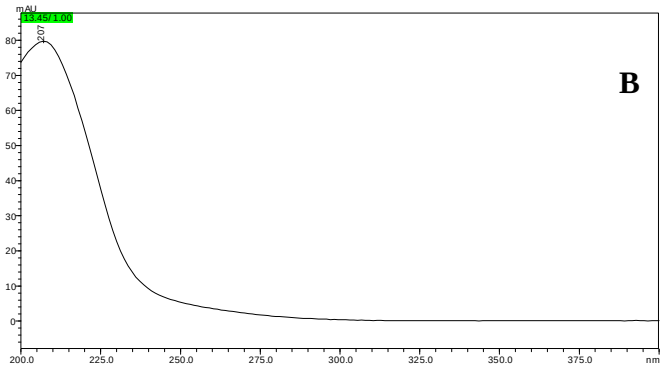
Several HPLC, UV-spectrophotometric analytical methods were published for the estimation of QF in bulk and pharmaceutical dosage forms and one stability indicating method was reported so far. However, the published method used higher percentage of organic solvent which is not economical and also phosphate buffer as the aqueous phase which is not LC-MS compatible. Hence, the aim of the present work is to develop and validate a simple,

efficient, economical, sensitive, and selective and LC-MS suitable method for the estimation of QF in bulk and pharmaceutical dosage forms.

3.1 Method Development and Optimization

In the present investigation, initial trials were made to develop LC conditions for the elution of QF using water as aqueous phase and methanol as organic modifier (50:50v/v) at a flow rate of 1.0 mL/min using C_{18} Phenomenex column (250 x 4.6 mm, 5µ), split peak was observed. Whereas, with the same column with change in mobile phase to 15mM ammonium acetate: methanol in different ratios at a flow rate of 1.0 mL/min, the QF was eluted before solvent front. In another trial with mobile phase of 0.02%v/v formic acid and methanol, using Phenomenex- C_{18} (250 x 4.6 mm, 5µ) column, peak tailing was observed and with increase in organic phase ratio, peak broadening was observed. Finally, good peak shape was obtained with a mobile phase composition of 0.02%v/v formic acid and methanol (90:10 v/v) at the flow rate of 1mL/min using Inertsil-ODS (250 x 4.6 mm, 5µ) column and water: methanol (50:50) as diluent, the QF was eluted at 13.42 min and tailing factor was within the limits. For quantitative analytical purpose wavelength was set at 220nm, which provided better reproducibility with minimum or no interference. The method was validated as per ICH guidelines. The peak purity index was found to be greater than 0.9999 for QF and indicating the peak purity of the drug sample used in the analysis and shown in Fig 1 along with the standard chromatogram and UV spectra.





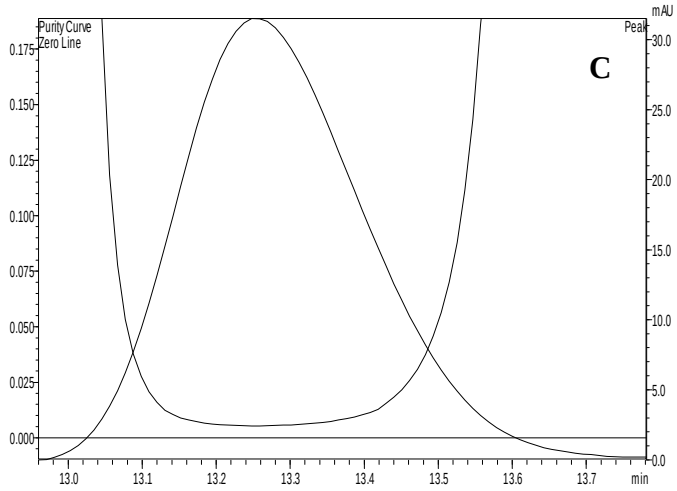


Fig 1: A) Standard chromatogram of QF; B) UV Spectra of QF; C) Peak purity curve of QF

To evaluate the stability of QF and ability of the method to separate QF from its degradation products, QF was subjected to various stress conditions such as acid hydrolysis (using 1N HCl), basic hydrolysis (using 1N NaOH), oxidative hydrolysis (using 3%v/v H_2O_2), thermal degradation (at 70°C) and photolytic degradation (overall illumination of ≥210Wh/m^2 at 25°C with UV radiation)**.**

No degradation products were obtained with acidic and basic hydrolysis conditions. Two degradation products were formed with retention times at 5.17 (8.85%) and 5.80 min (2.75%) when QF was subjected to oxidative stress conditions (in 3%v/v hydrogen peroxide and shown in Fig 2) as it contains alcohol, tertiary amine groups which are susceptible to oxidation. The total degradation was found to be 11.6%.

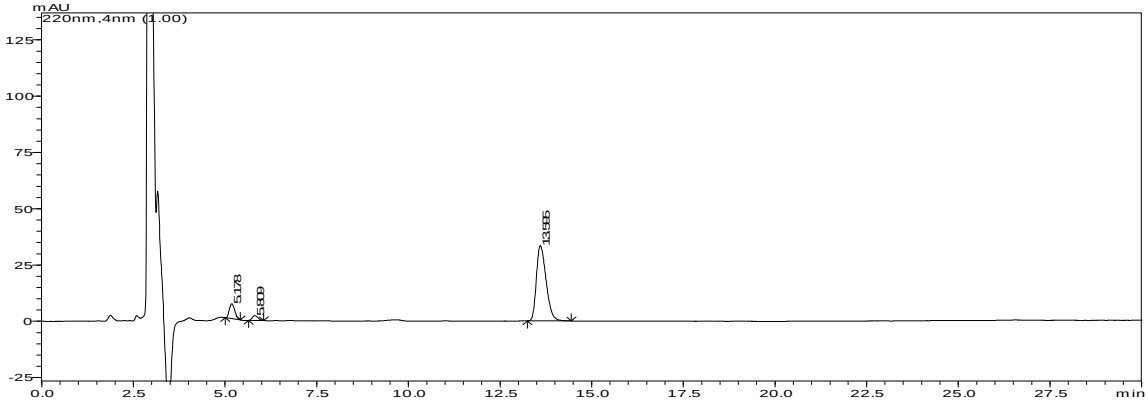


Fig 2: HPLC chromatogram for oxidative stressed sample

No degradation peaks were observed with stressed samples of photo (UV radiation for 14 days) and thermal (at 70°C for 14 days) conditions. The formed degradation products of QF showed a good resolution from the drug peak. Results of degradation studies were given in Table 1 and shown in Fig 3.

Table 1: Degradation Study Data

Degradation Condition	QF % Peak Area	QF Peak Purity
Acidic hydrolysis (mg/mL in 1N HCl) at 70°C for 2 days	100	Pass
Basic hydrolysis (mg/mL in 1N NaOH) at 70°C for 2 days	100	Pass
Oxidation (mg/mL in 3%v/v Hydrogen peroxide) at 70°C for 2 days	88.4	Pass
Photo degradation (to UV light) for 14 days	100	Pass
Thermal degradation at 70°C for 14 days	100	Pass

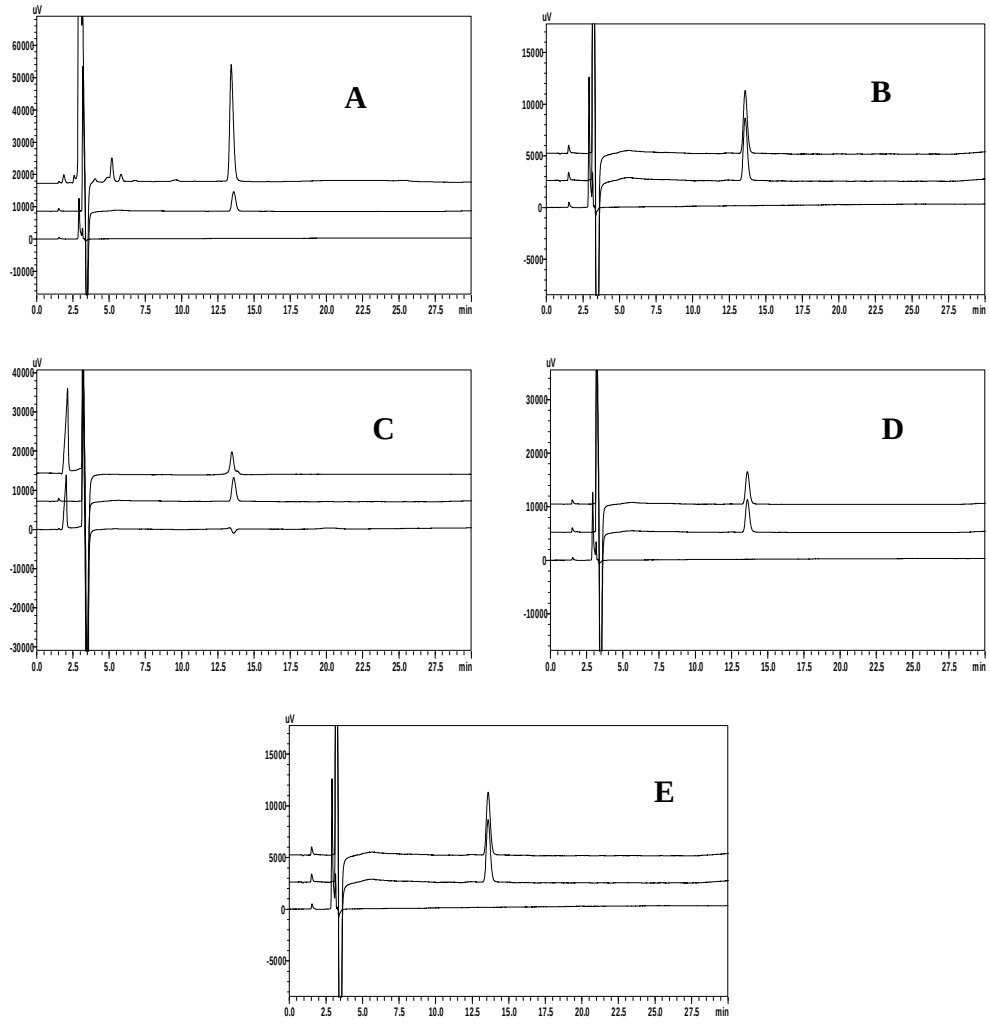


Fig 3: Overlay of chromatograms of Blank, Untreated and treated samples of QF

A-In oxidative stress condition B- In acid hydrolytic condition C-In basic hydrolytic condition D-Photo Degradation E- Thermal degradation

Photodiode array detection was used as an evidence of the specificity of the method, and to evaluate the homogeneity of the drug peak. Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 0.999 indicated a homogenous peak. The peak purity values for the peaks in chromatograms of stressed samples were in the range of 0.9999 to 1.0000 for drug substance, indicating homogenous peaks and thus establishing the specificity of the method.

A linear relationship was evaluated across the range (10-50 µg/mL) of the analytical procedure in triplicate. The range of concentrations was selected based on 80-120 % of the test concentration (for assay). Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The regression

coefficient (R^2) and correlation coefficient (R) for QF were found to be >0.999, indicating a linear response over the range used. The linearity data was given in Table 2.

Precision studies were carried out in terms of repeatability. Six replicate determinations were carried out and percent relative standard deviation for peak areas was less than 2%, indicating the high degree of precision and results were given in Table 2.

Accuracy of the method was examined by performing recovery studies by standard addition method. Various mixtures were prepared by spiking the known amounts of standard at three concentration levels (80%, 100% and 120%) to the drug product of the concentration of 20 µg/mL and these mixtures were analyzed by injecting in triplicate. The %RSD and the %recovery values were within the acceptable limits, and results were given in Table 2, indicating high accuracy of the method.

Table 2: Linearity, Precision and Accuracy data

Validation data of QF	
Linearity (n=3)	Range 10-50 µg/mL y =19154x+2328 R=0.999 R^2=0.999
Precision (n=6)	**Average peak area of the standard sample (%RSD)**
	387306 (1.21)
Accuracy (n=3) **Level of addition**	**Mean Percent Recovery (%RSD)**
80%	100.61 (0.238)
100%	100.41 (0.299)
120%	100.10 (0.435)

LOD and LOQ were calculated from the average slope and standard deviation of y-intercepts of the calibration curve. LOD and LOQ were found to be 0.108µg/mL and 0.329µg/mL respectively indicating high sensitivity of the method.

Method robustness was determined by analyzing the same sample at normal operating conditions and also by changing the operating analytical conditions like wavelength of detection and flow rate of the mobile phase. Percent assay values were also estimated under these changed conditions and the results were given in Table 3. Changes in the flow rate affected the retention times. However, the parameters like capacity factor, theoretical plate number and assay were not changed and were within the limits. Similar results were obtained with the changed wavelength. These results indicated that the method is robust in terms of changed flow rate and wavelength.

Table 3: Robustness data for QF

Chromatographic parameters	**Retention time (min)**	**Theoretical plates #**	**Capacity factor ($K^{'}$)**	**Tailing factor (T_f)**	**% Assay**
Flow rate (mL/min)					
0.8	16.45	14504.158	3.164	1.383	99.89
1.0	13.42	13650.675	3.227	1.226	101.00
1.2	10.59	13361.998	3.011	1.205	100.52
Wave length (nm)					
219	13.42	13493.714	3.226	1.24	101.14
220	13.42	13650.675	3.227	1.226	101.00
221	13.41	13508.764	3.227	1.247	99.22

System suitability was carried out by injecting standard concentration 20 µg/mL of QF at different injection volumes ranging from 10-50µL. The %RSD values for system suitability test parameters like retention time [R_t=13.42min (0.437%)], tailing factor [T_f = 1.34 (1.307%)] and theoretical plate number [# = 12439 (1.844%)] were found to be less than 2% indicating the present conditions were suitable for the analysis of QF in tablets.

Assay of QF tablets was performed by the proposed method and the % assay of the drug was calculated as an average of 3 determinations and found to be in the range of 98.56-99.06%. These results indicate that the present HPLC method can be successfully used for the assay of QF in bulk and pharmaceutical dosage forms.

CONCLUSION

The developed stability indicating RP-HPLC-PDA method was found to be simple, sensitive, accurate, precise, economical and LC-MS compatible. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Application of this method for estimation of QF from tablet dosage form and stressed samples showed that neither the degradation products nor the excipients interfered in the estimation of drug. Hence, this method was specific, stability-indicating and can be successfully used for the estimation of QF in bulk and pharmaceutical dosage forms.

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