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STABILITY INDICATING HPLC DETERMINATION OF QUETIAPINE FUMARATE

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ABSTRACT

The objective of the current study was to develop a validated stability-indicating assay method (SIAM) for Quetiapine fumarate after subjecting it to forced decomposition under hydrolysis, oxidation, photolysis and thermal stress conditions. The liquid chromatographic separation was achieved on a symmetry achieved on a C18 column (250 × 4.6 mm i.d., 5 µm) using methanol: acetonitrile: water (67:16:17) at a flow rate of 1 ml/min at ambient temperature with retention time 5.35 min. at 220 nm detection wavelength. The method was linear in concentration range 10-50 µg/ml (r2 = 0.998) with a limit of detection and quantitation of 3.27 µg/ml and 9.92 µg/ml, respectively. The method was validated with respect to linearity, precision (including intermediate precision), accuracy and specificity. The R.S.D. values for intra- and inter-day precision studies were < 1.02 % and < 1.15 %, respectively. The recovery of the drug ranged 99.68 ± 1.67 % to 100.37 ± 1.34 % from pharmaceutical dosage form. Degradation products resulting from the stress studies did not interfere with the detection of Quetiapine fumarate and the assay is thus stability-indicating.

KEYWORDS: Quetiapine fumarate; Stability-indicating Assay; Stress testing; High Performance Liquid Chromatography.

1. INTRODUCTION

Quetiapine fumarate, 2-(2-(4-dibenzo[b,f][1,4]thiazepine-11-yl-1-piperazinyl)ethoxy) ethanol an atypical antipsychotic drug used for the treatment of schizophrenia as well as for the treatment of acute manic episodes associated with bipolar I disorder.[1] The antipsychotic effect of quetiapine fumarate is thought by some to be mediated through antagonist activity at

dopamine and serotonin receptors. Specifically the D1, D2 dopamine, the α1, α2 adrenoreceptor and 5-HT1A, 5-HT2 serotonin receptor subtypes are antagonized. Quetiapine fumarate also has an antagonistic effect on the histamine H1 receptor. It has no significant affinity for cholinergic muscarinic or benzodiazepine receptors. Drowsiness and orthostatic hypotension associated with use may be explained by its antagonism of histamine H1 and adrenergic α1 receptors, respectively. Quetiapine fumarate's antagonism of adrenergic α1 receptors may explain the orthostatic hypotension observed with this drug. However, it is thought that the drug's therapeutic activity in schizophrenia is mediated through a combination of dopamine type 2 (D2) and serotonin type 2 (5HT2) receptor antagonism. Although quetiapine fumarate is known to bind other receptors with similar affinity only the dopamine D2 and serotonin 5HT2 receptor binding is responsible for quetiapine fumarate's therapeutic activity in schizophrenia.[2] Several analytical methods have been reported in the literature for the analysis of Quetiapine fumarate from pharmaceutical dosage form. The techniques include HPLC[3], polarographic[4], HPTLC[5, 6] and UV Spectrophotometry[7] etc. forms. There are numerous methods to quantify Quetiapine fumarate in biological fluid and human plasma, including HPLC[8, 9, 10, 11], HPLC-MS/ M [12], LC-MS/.[13] Currently, most of the separations are performed by HPLC for reasons of robustness and familiarity of analysts with this technique. About specificity for HPLC method peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one component. The previous published methods are not directly applicable for this issue and need more investigation for method development and validation. So, an approach was made to develop a simple, precise, accurate, specific, robust stability- indicating HPLC-UV method for the quantitative determination of Quetiapine fumarate in pharmaceutical dosage forms and applied to the assay of Quetiapine fumarate in tablets and bulk form.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Quetiapine fumarate working standard powder with was kindly gifted by Tripada Pharmaceuticals, Ahmedabad, India. QUITIPIN (Sun) tablets containing 25 mg Quetiapine fumarate as per labelled claim were obtained from local market. Methanol, acetonitrile, water were of HPLC grade and sodium hydroxide, hydrochloric acid, hydrogen peroxide were of Analytical grade obtained from E. Merck (India) Ltd., Mumbai. All chemicals were at least of analytical grade and used as received.

2.2. HPLC instrumentation and conditions

The present work was carried out on isocratic high pressure liquid chromatography, consist of pump (Cyberlab TM, USA) with universal loop injector (Rheodyne) of injection capacity 20 µl. Detector consists of photodiode array detector; the reversed phase column used was RP-C18 (5µm size, 250 mm×4.6 mm i.d.) at ambient temperature. Separation was achieved using a mobile phase consisting of methanol: acetonitrile: water (67:16:17) at a flow rate of 1 ml/min. The eluted compounds were monitored at 220 nm. The column was maintained at ambient temperature and an injection volume of 20 µl was used. The mobile phase was filtered through 0.45 micron membrane filter and ultrasonicated for 10 minutes prior to use. For analysis of forced degradation samples, the photodiode array detector was used in range of 200–400 nm. Peak homogeneity was expressed in terms of peak purity values, and was obtained directly from spectral analysis report obtained using the instrument software.

2.3. Preparation of stock and standard solutions

A 1 mg/ml stock solution of Quetiapine fumarate was prepared in HPLC grade methanol. Sub-stock solution was prepared from stock solution by diluting 10 ml standard stock solution up to 100 ml to get 100 µg/ml. Aliquots of the standard sub-stock solution of Quetiapine fumarate were transferred into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 10, 20, 30, 40 and 50 µg/ml. Each 20µl standard solution was injected into the column after filtration using 0.2 micron membrane filter.

2.4. Preparation of tablets for assay

Twenty tablets were weighed and finely powdered. The powder equivalent to 25 mg quetiapine fumarate was accurately weighed and transferred to volumetric flask of 100 ml capacity and diluted with 50 ml mobile phase and sonicated for 10 min. The flask was shaken and volume was made up to the mark with the mobile phase. The above solution was filtered through whatman filter paper (# 42). A 1 ml of aliquot was transferred to 10 ml volumetric flask. Volume was made up to the mark with mobile phase to give a solution containing 25µg/ml in the range of linearity previously described.

2.5. Forced degradation studies of API

In order to determine the stability indication of the analytical method and assay, Quetiapine fumarate tablets and Quetiapine fumarate API powder were stressed under various conditions

to conduct forced degradation studies.[14] All solutions of quetiapine fumarate used in forced degradation studies were prepared with final concentrations of 20µg/ml.

2.5.1. Acid degradation studies

A 100 mg of quetiapine fumarate was accurately weighed and transferred to a 100 ml round bottom flask and dissolved in 50 ml of diluent methanol. The flask was sonicated for 15 min and added 10 ml of 0.1M hydrochloric acid solution. The content kept for constant stirring for 24 h at room temperature. After specified time, the content was neutralized with 10 ml 0.1 M sodium hydroxide and volume was made up to the mark with diluent.

2.5.2. Alkali degradation studies

A 100 mg of quetiapine fumarate was accurately weighed and transferred to a 100 ml round bottom flask and dissolved in 50 ml of diluent methanol. The flask was sonicated for 15 min. and added 10 ml of 0.1M sodium hydroxide solution. The content kept for constant stirring for 24 h at room temperature. After specified time, the content was neutralized with 10 ml 0.1 M hydrochloric acid and volume was made up to the mark with diluent.

2.5.3. Oxidation

A 100 mg of standard quetiapine fumarate was accurately weighed and transferred to a 100 ml round bottom flask and dissolved in 50 ml of diluent methanol. The flask was sonicated for 15 min. and after adding 10 ml of 3 % hydrogen peroxide solution, after 60 min volume was made up to the mark with diluent.

2.5.4. Temperature stress studies

A 1g of quetiapine fumarate sample was taken in to a petridish and kept in oven at 80 °C for 36 h. A 100 mg of thermal stressed quetiapine fumarate was accurately weighed and transferred to a 100 ml round bottom flask and dissolved in 50 ml of diluents methanol. The flask was sonicated for 15 min and volume was made up to the mark with diluent.

2.5.5. Photostability

A 1 g of quetiapine fumarate sample was taken in to a petridish and kept in UV light for 36 h. A 100 mg photo stressed quetiapine fumarate was accurately weighed and transferred to a 100 ml round bottom flask and dissolved in 50 ml of diluent methanol. The flask was sonicated for 15 min. and volume was made up to the mark with diluents.

3. RESULTS AND DISCUSSION

3.1. HPLC method development and optimization

A RP-C18 column (250 mm × 4.6 mm i.d., 5µm particle size), maintained at ambient temperature (25 ◦C) was used for the separation and the method validated for the determination of Quetiapine fumarate in pharmaceutical dosage forms. For the selection of detection wavelength, the overlain spectrum of 20 µg/ml quetiapine Fumarate revealed that a 220 nm the drug possess significant absorbance. So considering above fact, 220 nm was selected as a detection wavelength for estimation of quetiapine fumarate using RP-HPLC. To optimize the HPLC parameters, several mobile phase compositions reported in were tried. The mobile phase composed of acetonitrile: 0.02 M phosphate buffer (50:50) at pH 5.5 was tried but analyte was not detected. Then analyte was resolved at longer retention time with asymmetrical peak with mobile phase acetonitrile: a 10.5 mM, pH 3.5 phosphate buffer containing 0.12 % triethylamine (30:70) at 1.2 ml/min flow rate. Mobile phase composition was changed by addition of methanol; replaces the phosphate buffer with ammonium acetate and modified mobile phase acetonitrile: methanol: 0.01 M ammonium acetate (31:19:50) was tried at flow rate 1.5 ml/min but degradants were not resolved. So finally well resolved and satisfactory peak shape was obtained with methanol: acetonitrile: water (67:16:17) at 1 ml/min flow rate.

3.2. Validation

The method was validated with respect to parameters including linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, specificity, robustness, solution stability and system suitability.[15]

3.2.1. Linearity

The calibration curves constructed for Quetiapine fumarate were linear over the concentration range of 10-50 µg/ml. Peak areas of analyte was plotted against concentration and linear regression analysis performed on the resultant curve. Fig.1 shows chromatogram of 20 µg/ml of quetiapine fumarate with retention time 5.35 min. Typically, the regression equation for the calibration curve was found to be y= 11527x + 15216 with 0.996 correlation coefficient.

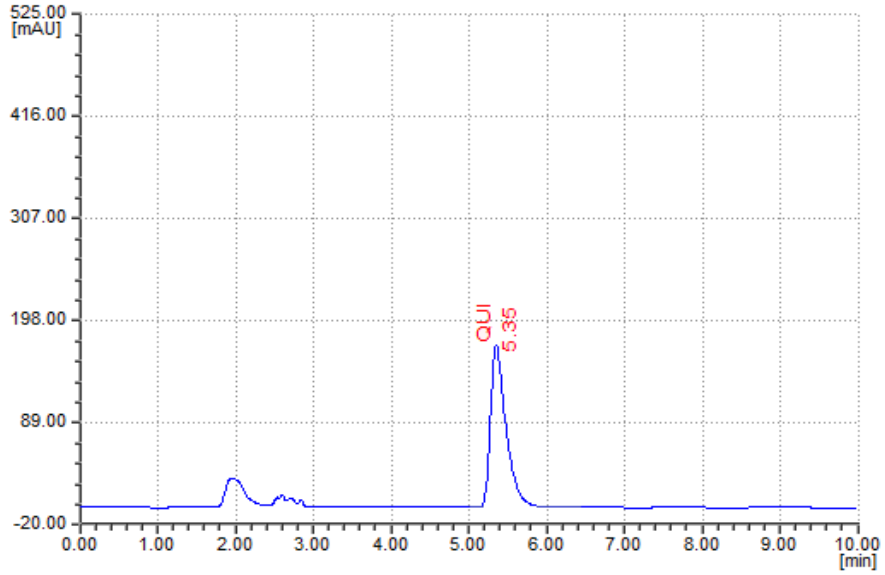

Fig. 1: HPLC chromatogram of a standard solution 20µg/ml of Quetiapine fumarate

3.2.2. LOQ and LOD

The LOQ and LOD were determined based on signal-to-noise ratios, using an analytical response of 10 and three times of the background noise, respectively. The LOQ and LOD were found to be 3.27 µg/ml and 9.92 µg/ml respectively.

3.2.3. Precision

Table 1 provides data obtained from the precision experiments. The R.S.D. values for intraday and interday precision were <1.02% and <1.15%, respectively, thereby indicating that the method was sufficiently precise. A similar qualitative separation of the drug was obtained even on analysis on a different chromatographic system on a different day, indicating that the method was precise.

Table 1: Precision study

Conc. (µg/ml)	Intra day (n=3) Area (Mean ± SD)	CV	Inter day (n=3) Area (Mean ± SD)	CV
20	61539.09 ± 633.36	1.02	61548.76±711.43	1.15
30	90975.04 ± 762.84	0.83	90752.55±889.89	0.98
40	119704.84 ± 691.01	0.59	120140.14±477.72	0.41

3.2.4. Accuracy

Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. As shown from the data in Table 2, excellent recoveries were made at each added concentration of drug.

Table 2: Recovery study

Label claim Mg/tablet	Amount added %	Total amount added (mg)	Amount recovered (mg) ± SD	% Recovery ± SD (n=3)
Quetiapine	80	20	20.02± 0.12	100.11 ± 0.61
Fumarate	100	25	25.09 ± 0.33	100.37 ± 1.34
25	120	30	29.90 ±0.50	99.68 ± 1.67

3.2.5. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its degradation products. The method was found to be specific to the drug. Specificity was established by determination of purity of the drug peak using a PDA detector. Also, the resolution factor of the drug peak was determined with respect to the nearest resolving peaks.

3.2.6. Robustness

No significant effect was observed on system suitability parameters such as capacity factor, resolution and theoretical plates of respective components, when small but deliberate changes were made to chromatographic conditions.

3.2.7. Solution stability

Low % RSD value of 1.18 between peak areas obtained for the same drug solution of 30 µg/ml quetiapine fumarate after 48 h proved the solution stability and also ruggedness of the method.

3.2.8. System suitability

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, resolution, precision and peak tailing. The result obtained is shown in Table 3. All these parameters were evaluated with the background of regulatory requirements, which suggests good chromatographic condition.

Table 3: System suitability parameters

System suitability parameters	Quetiapine fumarate
Retention times (R_T) (min)	5.35 ± 0.01
Theoretical plates (N)	2961.8
Tailing factor (A_S)	1.95

3.3. Stability studies

All stressed samples in both solid and solution state remained colorless. HPLC studies on Quetiapine fumarate under different stress conditions using methanol: acetonitrile: water

(67:16:17) as the solvent system suggested the following degradation behaviour in different stress condition.

3.3.1. Acidic condition

The drug gradually decreased with time on refluxing with 0.1M hydrochloric acid at 24 h at room temperature, forming degradation products at retention time 2.81 and 2.99 min as shown in Fig.2. At the end of 24 h, around 24.56 % fall in drug peak area was observed.

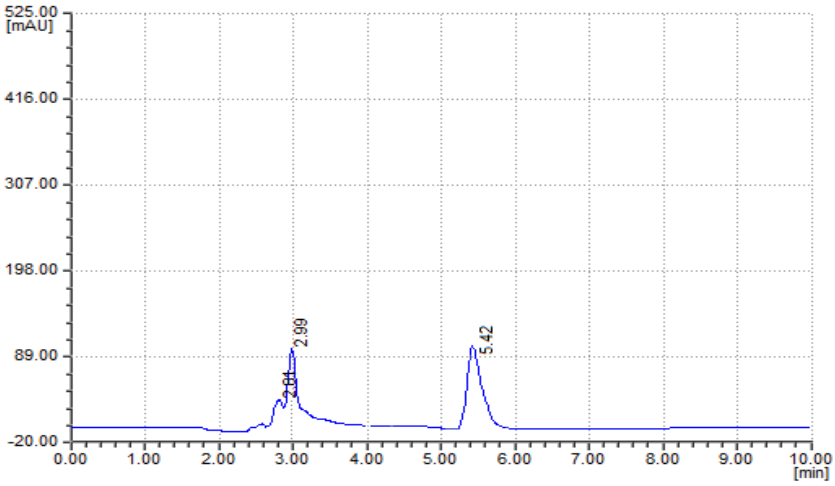

Fig. 2: Chromatogram containing 20 µg/ml acid degraded Quetiapine fumarate

3.3.2. Degradation in alkali

The drug was found to comparatively unstable to alkaline hydrolysis. On refluxing the drug in 0.1M sodium hydroxide for 24 h, around 25.42 % of the drug was degraded. Degradants product was appeared at retention time 2.55 and 2.93 min as shown in Fig. 3.

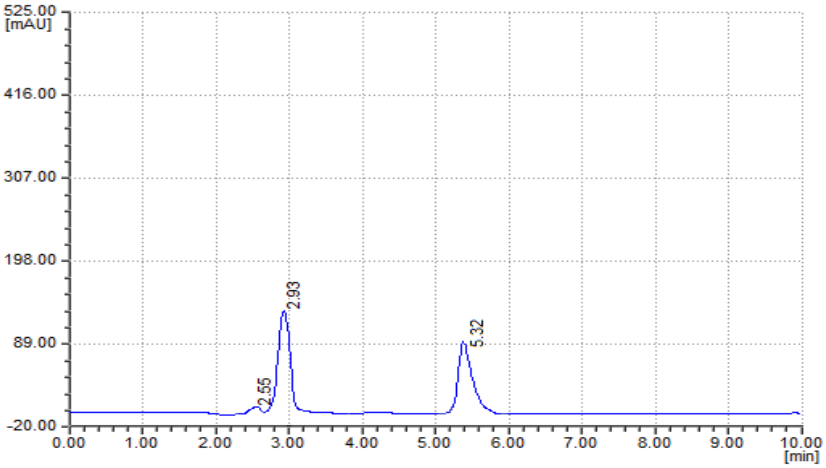

Fig. 3: Chromatogram containing 20 µg/ml alkali degraded Quetiapine fumarate

3.3.3. Oxidative conditions

The drug was highly labile to hydrogen peroxide (3 %) at room temperature. After 60 min, steep fall in the drug peak area was observed. Major degradant products were appeared at retention time 2.45, 2.67 and 3.82 min and it was degraded up to 61.45 % as shown in Fig. 4. At the end of 60 min, almost complete degradation of the drug was observed with the corresponding rise in the major degradation peak.

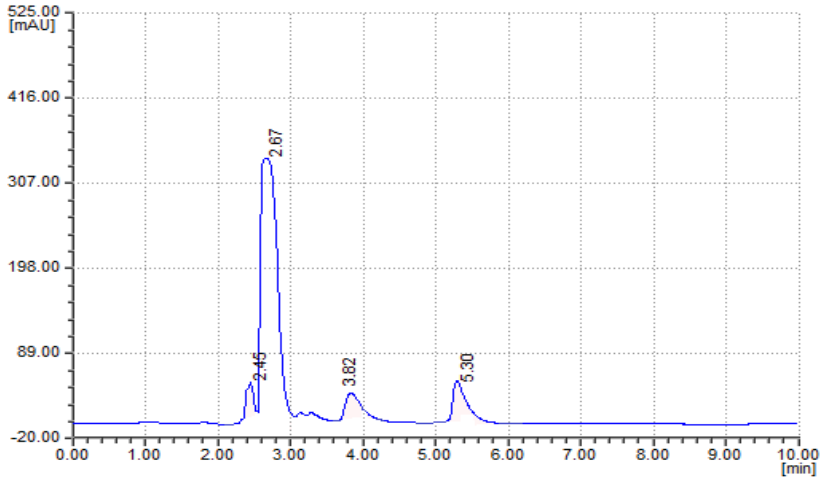

Fig .4: Chromatogram containing 20 µg/ml oxidative degraded Quetiapine fumarate

3.3.4. Thermal degradation

The solid-state studies showed that quetiapine fumarate was comparatively stable to the effect of temperature. When the drug powder was exposed to dry heat at 80 °C for 36 h. 8.42 % degradation was observed with corresponding rise to degradants products at retention time 2.13, 2.63 and 2.74 min as shown in Fig.5.

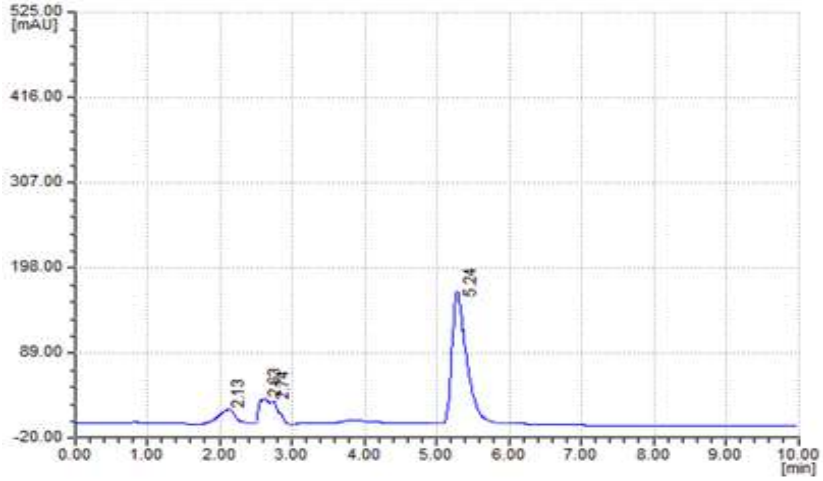

Fig .5: Chromatogram containing 20 µg/ml thermal degraded Quetiapine fumarate

3.3.5. Photolytic conditions

No major degradation product was observed after exposure of solid drug kept in UV light. Minor degradants products at retention time 2.85 and 2.97 min with 5.45 % fall in quetiapine fumarate after 36 h as shown in Fig.6.

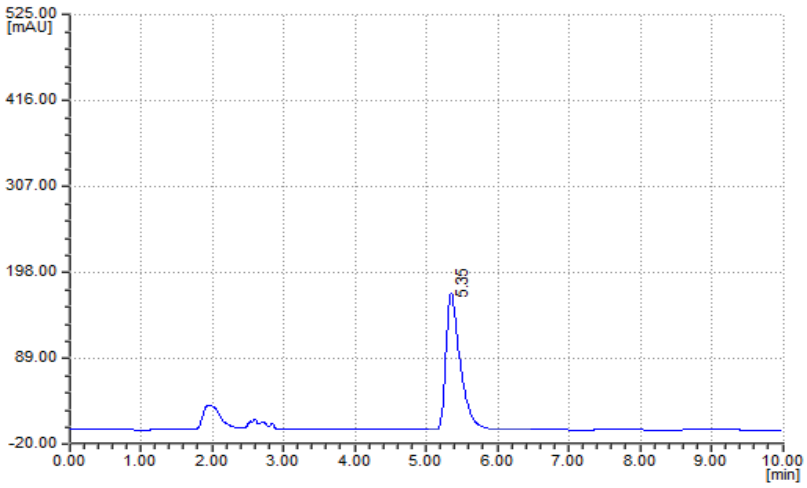

Fig.6: Chromatogram containing 20 µg/ml photo degraded Quetiapine fumarate

The retention time of degradants, % recovery and % degradation of quetiapine fumarate were listed in Table 4.

Table 4: Summary of forced degradation results for quetiapine fumarate by HPLC

Sr. no.	Stress condition	Drug remained/ 20 µg/ml ± SD (n=3)	Retention time of degradants (min)	Recovery (%)	Degradation (%)
1	Acid degradation	15 ± 0.14	2.81, 2.99	75.73	24.56
2	Base degradation	14.93 ± 0.27	2.55, 2.93	74.67	25.42
3	Oxidation	7.70 ± 0.12	2.45, 2.67, 3.82	36.54	61.45
4	Thermal degradation	18.31 ± 0.16	2.13, 2.63, 2.74	91.57	8.42
5	Photo degradation	18.90 ± 0.18	2.85, 2.97	94.54	5.45

3.4. Assay

The single peak at retention time 5.35 min for quetiapine fumarate was observed in the chromatogram of the drug samples extracted from tablets in Fig.7. Experimental results of the amount of quetiapine fumarate in tablets, expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any excipients, which are normally present in tablets. The drug content was found to be 100.56 % ± 1.15 (n=3) for quetiapine fumarate.

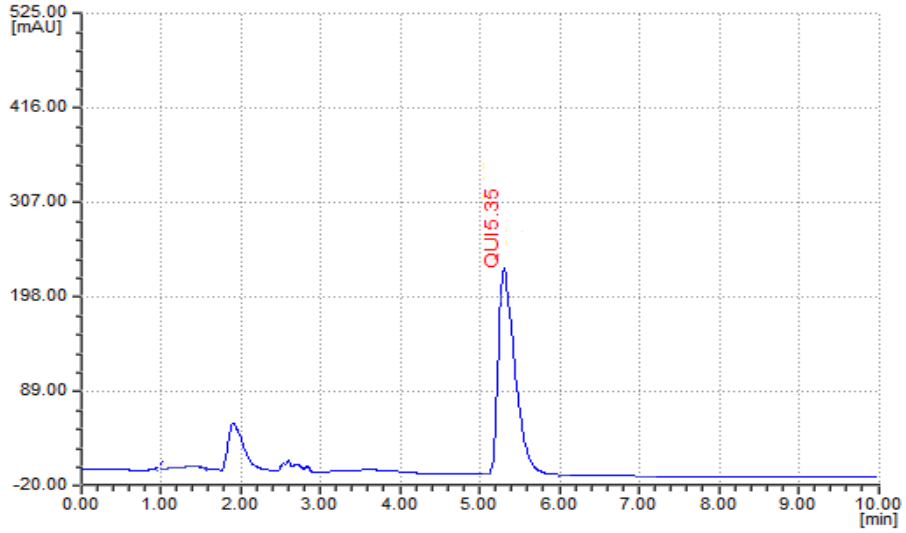

Fig. 7: Chromatogram containing quetiapine fumarate tablet formulation

4. CONCLUSIONS

A validated stability-indicating HPLC analytical method has been developed for the determination of quetiapine fumarate in API. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drug from degradation products and excipients found in the tablet dosage forms. The method is suitable for the routine analysis of quetiapine fumarate in either bulk API powder or in pharmaceutical dosage forms. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. It may further be extended to study the degradation kinetics of quetiapine fumarate and also for its determination in plasma and other biological fluids. As the method separated the drug from its degradation products, it can be employed as a stability indicating one.

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