Stability indicating fast LC method for determination of quetiapine fumarate related substances in bulk and pharmaceutical formulation

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ABSTRACT

An LC method has been developed and subsequently validated for the determination of Quetiapine fumarate and its related substances in bulk and pharmaceutical formulation. Separation was achieved in gradient mode using Kromasil 100, C18, 30 x 3.0 mm, 3.5 µm column with mobile phase A containing 0.5% Triethylamine buffer (pH adjusted to 4.8±0.05 with Orthophosphoric acid and mobile phase B containing 100%Acetonitrile at different time intervals as eluent at a flow rate 1.0mL/min. UV detection was performed at 240nm.The method is simple, selective and stability indicating .The described method is accurate and linear over a range of about 0.052µg/mL to 3.289µg/mL.The method precision for the determination of related impurities was below 3.5% RSD .The Percentage recoveries of known related impurities from dosage forms ranged from 96.7 to 106.920%. LOD and LOQ of all related impurities of Quetiapine fumarate was established and ranged from 0.017µg/ml - 0.027µg/ml for LOD and 0.052µg/ml – 0.086µg/ml for LOQ .The method is useful in the quality control of bulk manufacturing and also in pharmaceutical formulations.

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INTRODUCTION [1-5]

Quetiapine fumarate is a psychotropic agent belonging to a chemical class, the dibenzothiazepine derivatives. The chemical designation is 2-[2-(4-dibenzo [*b,f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]-ethanol fumarate (2:1) (salt) is present in tablets as the fumarate salt. All doses and tablet strengths are expressed as milligrams of base, not as fumarate salt. Its empirical formula is $C_{42}H_{50}N_6O_4S_2 \cdot C_4H_4O_4$ and having a molecular weight of 883.11 (fumarate salt). Quetiapine fumarate is a white to off-white crystalline powder which is moderately soluble in water .the structural formula was given below:

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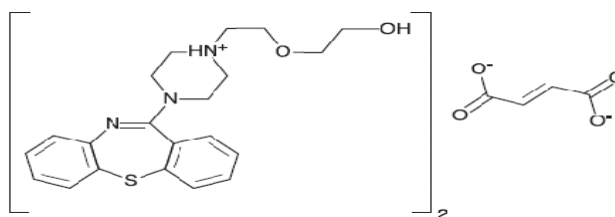
Quetiapine fumarate

Quetiapine fumarate is not official in any pharmacopoeia. Liquid chromatography procedures have not been reported for the determination of Quetiapine fumarate and its related substances in Bulk and Pharmaceutical dosage forms. However there are very limited publications concerning the analysis of Quetiapine fumarate in bulk and Pharmaceutical dosage forms. So it was felt necessary to develop a LC method which would serve as a reliable method for the determination of Quetiapine fumarate respective with related impurities [Fig 1] in bulk and pharmaceutical dosage forms. In the proposed method, related impurities were well separated and eluted before 22min.Finally the method was thoroughly validated for related substances of Quetiapine fumarate.

MATERIALS AND METHODS

1.1.1 Instrumentation: Agilent 1200 series equipped with binary pump and DAD detector was used.

The output signal was monitored and integrated using waters Empower 2 software

1.1.2 Solutions:
Preparation of Mobile Phase A:
Preparation of 0.5% Tri ethyl amine Buffer solution:
10 mL of Triethylamine taken and diluted to 2000 mL with milli-Q water, adjusted the pH to 4.80 ± 0.05 with orthophosphoric acid and mixed well. Filter through 0.2 µm nylon membrane Filter and degas for about 10 minutes.

Preparation of Mobile Phase B:
Filtered and degassed 100% Acetonitrile was used as mobile phase-B.

1.1.3 Preparation of Diluent:
Milli Q water and Acetonitrile was mixed in the ratio 80:20(v/v) and filter through 0.2 µm nylon membrane filter and degas for about 10 minutes.

2.1.1 Preparation of Standard Solution: About 58 mg of Quetiapine fumarate working standard weighed accurately and transferred in to a 100mL volumetric flask, to that 70ml of diluent was added and sonicated to dissolve and diluted to volume with diluent and mixed well. from that 5 ml was taken in 50ml volumetric flask and diluted to volume with diluent and mixed well ,from that 2ml taken was taken in 50ml Volumetric flask and diluted to volume with diluent and filtered through 0.45µm nylon membrane Filter.

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2.1.2 Preparation of Test Solution: Weigh about 20 Quetiapine fumarate 400 mg tablets and record the average weight and crush the tablets in to fine powder using mortar and pestle .from that powder was taken   equivalent to 100 mg Quetiapine and transferred in to a 100mL volumetric flask ,70ml of diluent was added and sonicated for 20mintes with intermediate shaking and diluted to volume with diluent and mixed well ,few mL was taken and centrifuged at 2500 RPM for 10minutes using centrifuge Test tube with cap and filtered through 0.45µm nylon membrane filter.

2.2.1 Preparation of Degradation samples for Specificity Study:
For **Acid degradation** Quetiapine fumarate sample was refluxed with 1N HCl at 80ºC for 1 Hour on   Mantel. then neutralized by adjusting pH to 7.0 with 1N NaOH .The Solution was further diluted to Required concentration with diluent.

For **basic degradation** Quetiapine fumarate sample was stressed with 2N NaOH for 2 Hours on mantel then neutralized by adjusting pH to 7.0 with 2N HCl .The Solution was further diluted for required concentration Re with diluent.

For **Water degradation** Quetiapine fumarate sample was refluxed with water for 2 Hours at 80°c.on mantel. The Solution was further diluted for required concentration with diluent.

For **Oxidative degradation** Quetiapine fumarate sample was stressed with $1\%H_2O_2$ for 20min on Bench top.

The Solution was further diluted for required concentration with diluent. re For **Photolightic Stress** the samples were exposed to UV light at 254nm for 49hrs and visible light for 168hrs meeting the specification of ICH i.e. UV (200watt/m^2) and Visible (1.2million Lux hours).

For **Thermal Degradation** Samples were Exposed to Temperature at 120ºC for 12 hrs.
For **Humidity Degradation** Samples were Exposed at 25°c/90% RH for 218 hrs.

The above stressed samples i.e. Photolightic,Humidity and Thermal stress samples were prepared and diluted for required concentration with diluent.Specificity chromatograms for degradation products are shown in Fig-3.

2.3.0   Chromatographic Conditions:
A Kromasil 100, (30x 3.0mm; 5µm packing) column was used for analysis with   column temperature 40ºc. The Sample injection Volume was 5µL with sample cooler temperature at 5°c.The photodiode array Detector was set to a wavelength at 240nm for the detection and run the chromatogram for 22minutes.

The mobile phase was pumped through the column as per the gradient composition given below at a flow Rate of 1.0mL/min.

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Time in minutes	% of Mobile phase-A	% of Mobile phase-B
0.0	80.0	20.0
4.0	80.0	20.0
12.0	40.0	60.0
15.0	20.0	80.0
18.0	20.0	80.0
18.5	80.0	20.0
22.0	80.0	20.0

RESULTS AND DISCUSSION

3.1 Method development [6-20]
3.1.1 Separation of Known degradant impurities
To develop a suitable and robust method for the determination of Quetiapine fumarate related impurities, different mobile phases and columns were employed to achieve the best separation and resolution. The method development was started with a Peerless HT C8 (50 x 4.6 mm;1.8 µm packing) column using a mobile phase-A and mobile phase –B in the ratio 50:50 with 1.5 mL/min flow rate .In the above condition elution was very broad for Quetiapine peak, little separation from Quetiapine peak and impurities. Early elution with little separation was observed with mobile phase consisting of mobile phase–A and mobile phase –B in the ratio 40:60 using column Zorbax XDB, C18,100 x 4.6 mm,1.8 µm with 1.2 mL /min flow rate. Finally the mobile phase consisting of mobile phase –A and mobile phase –B in the ratio 80:20 was found to be appropriate ,allowing good separation and symmetrical peak at a Flow rate of 1.0mL/min using Kromasil 100, 30x 3.0mm; 3.5µm packing. The Chromatogram of Quetiapine

Fumarate sample spiked with the related impurities using the proposed method is shown in Fig.2. In the proposed method the resolution is more than 2 between the Quetiapine and impurity-B and resolution is more than 2 between the Quetiapine and impurity -C .System suitability results of the method are presented in Table 1. Quetiapine fumarate and its related impurities show significant UV absorbance at Wavelength 240 nm .Hence this wavelength has been chosen for detection in the analysis of Quetiapine fumarate.

3.1.2 Column Selection [21-22]
Based on the retention time and separation of the impurities Kromasil100, (30x 3.0mm; 3.5µm) column was selected as suitable for the analysis of Quetiapine fumarate and its related impurities.

3.2 Method Validation [23-25]
The developed LC method of Quetiapine fumarate is extensively validated for Quetiapine fumarate and its related impurities using the following parameters.

3.2.1) Specificity:
Interference from degradation products:
A study was conducted to demonstrate the effective separation of degradants from Quetiapine fumarate peak. Separate portions of Drug product were exposed to following stress conditions to induce degradation.Stressed samples were injected into the RRLC system with diode array

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detector by following test method conditions. All degradant peaks were resolved from Quetiapine fumarate peak in the chromatograms of all samples. The chromatograms of the stressed samples were evaluated for peak purity of Quetiapine fumarate using Empower software. In all forced degradation samples, Quetiapine fumarate peaks Purity angle is less than purity threshold. The results are given under Table-2. From the above results it is clear that the method can be used for determining the stability of Quetiapine fumarate related substances in bulk and pharmaceutical formulations.

3.2.2) Limit of detection and limit of quantitation:
A study to establish the Limit of detection and limit of quantitation of Quetiapine fumarate related impurities were conducted.

Limit of detection and limit of quantitation were established based on signal to noise ratio. A series of solutions having Quetiapine fumarate related impurities were injected. Limit of detection for related

Impurities were established by identifying the concentration which gives signal to noise ratio about 3. Limit of quantitation was established by identifying the concentration which gives signal to noise ratio about 10.

Precision of Quetiapine fumarate related impurities at about Limit of Quantitation were conducted. Six test preparations of Quetiapine fumarate having related impurities at about Limit of quantitation was prepared and injected into the RRLC system. The %RSD at LOQ level was calculated for all known impurities and found to be less than 5.0%.
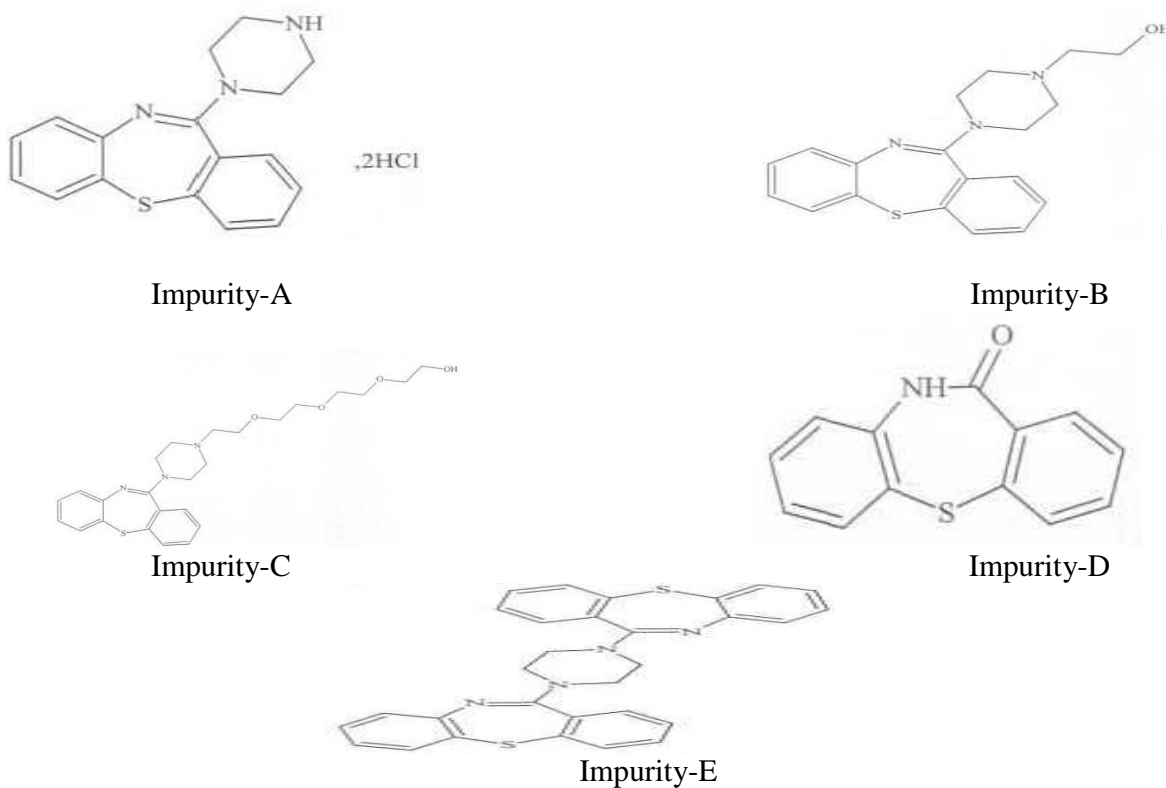
Accuracy of Quetiapine fumarate related impurities at about Limit of Quantitation was conducted Test solutions spiked with related impurities at about Limit of Quantitation was prepared in triplicate and injected into RRLC system and calculated the % recovery. The mean recovery of Quetiapine fumarate related impurities at about Limit of Quantitation was ranged from 97.9 to 101.2% .The results are given under Table-3

3.2.3) Linearity of Detector Response:
a) Related impurities:
Linearity of detector response of all known Quetiapine fumarate Related impurities is established by plotting a graph to concentration versus area of Quetiapine fumarate related impurities and determining the correlation coefficient. A series of solutions of Quetiapine fumarate related impurities in the Concentration ranging from Limit of Quantitation level to about 150% of target concentration level of Quetiapine fumarate known impurities were prepared and injected into the RRLC system.

The detector response was found to be linear from Limit of quantitation to 150% of target concentration level of Quetiapine fumarate known Impurities. Linearity of detector response graph is shown in Fig-4.

Fig-1: QUETIAPINE FUMARATE   RELATED IMPURITIES

Impurity-A   Impurity-B

Impurity-C   Impurity-D

Impurity-E

Chemical names of Quetiapine fumarate related impurities:

1) Impurity-A: 11-Piperazin -1-yl-dibenzo [b, f] [1, 4] thiazepine.
2) Impurity-B: 2-(4-Dibenzo [b, f] [1, 4] thiazepin-11-yl-piperazin-1-yl)-ethanol
3) Impurity-C: 2-(2-{2-[2-(4-Dibenzo [b, f] [1, 4] thiazepin-11-yl-piperazin-1-yl)-ethoxy]-ethoxy}-ethoxy)-ethanol
 4) Impurity-D: Dibenzo [b, f] [1, 4] thiazepin-11(10H)-one
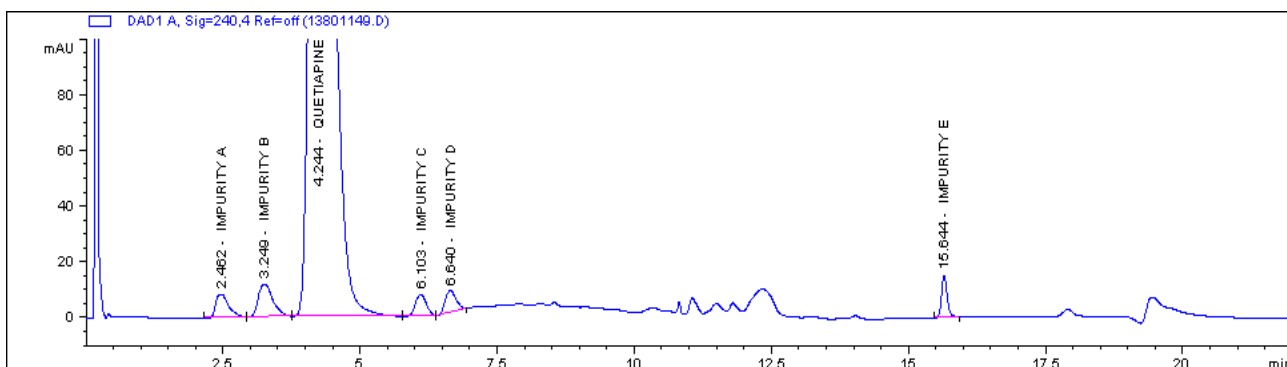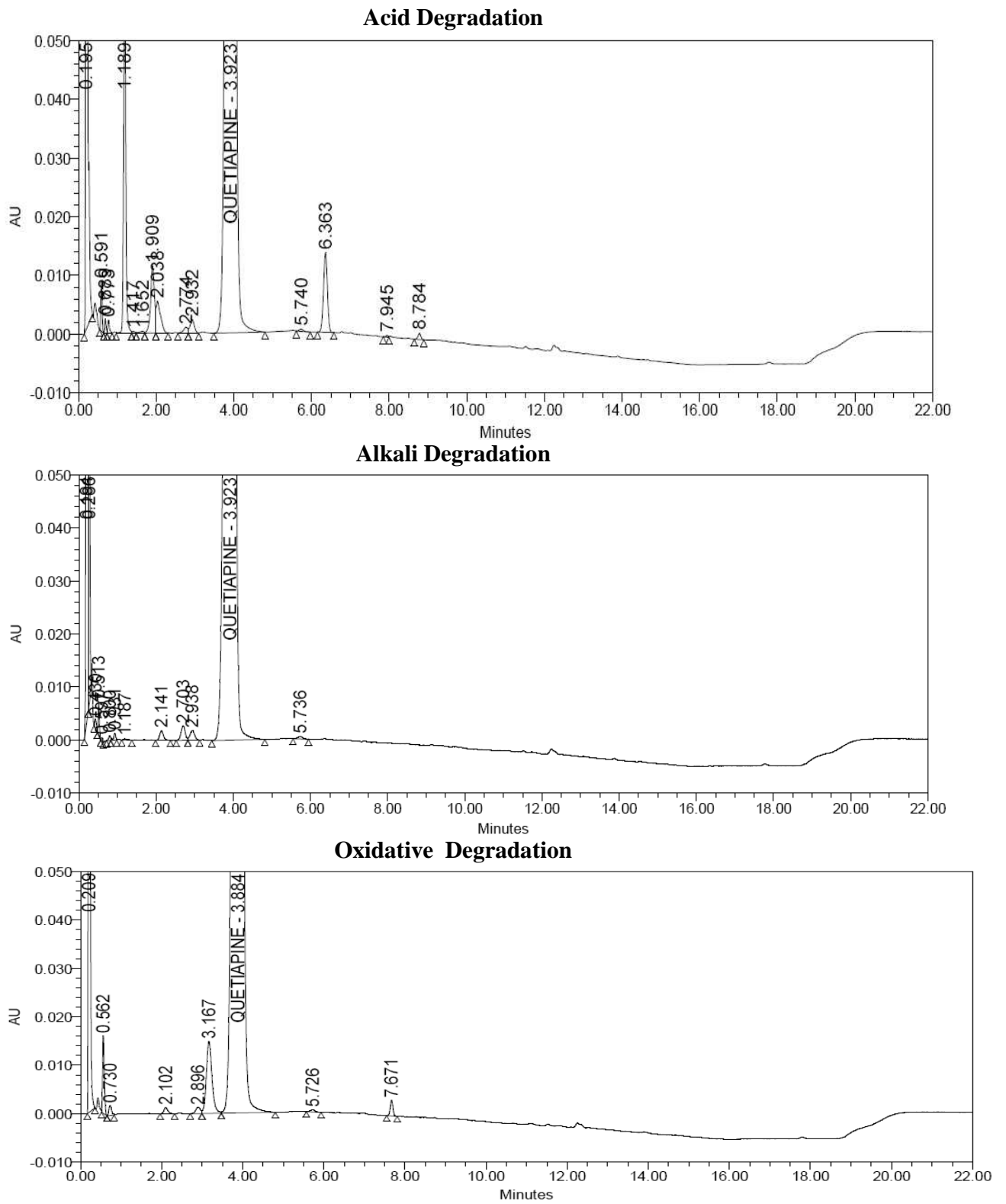5) Impurity-E: 1, 4-bis (dibenzo [b, f] [1, 4] thiazepin-11-yl) piperazine
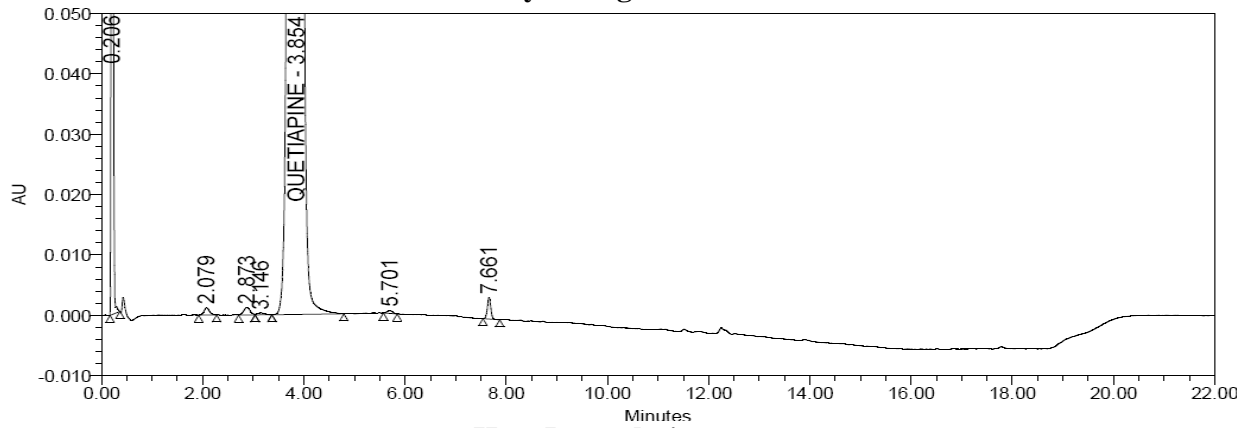
Fig -2: Typical chromatogram of quetiapine fumarate and its related impurities
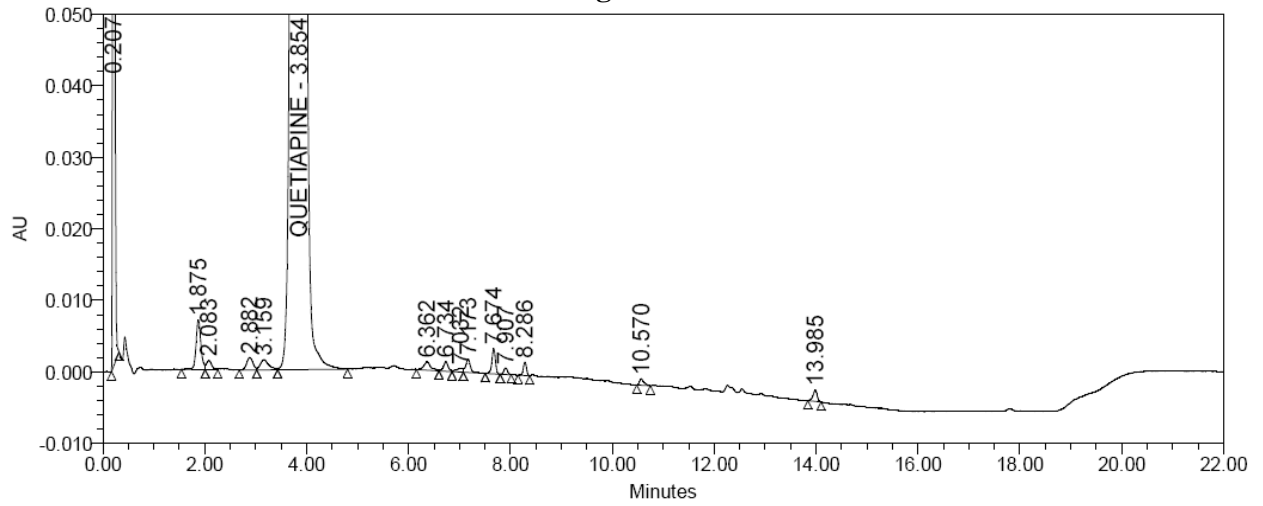
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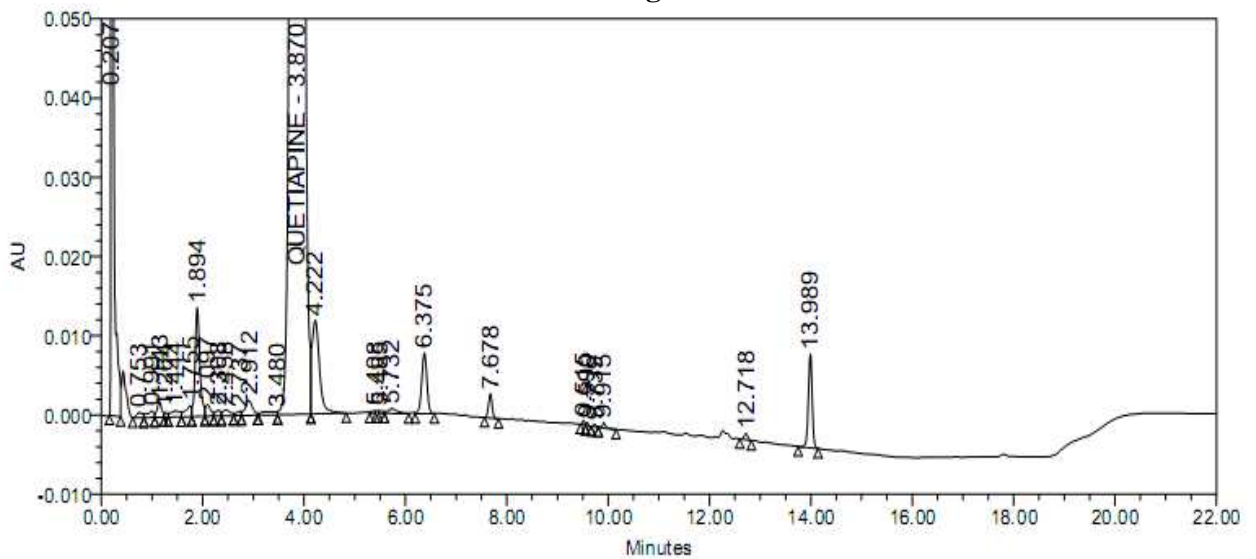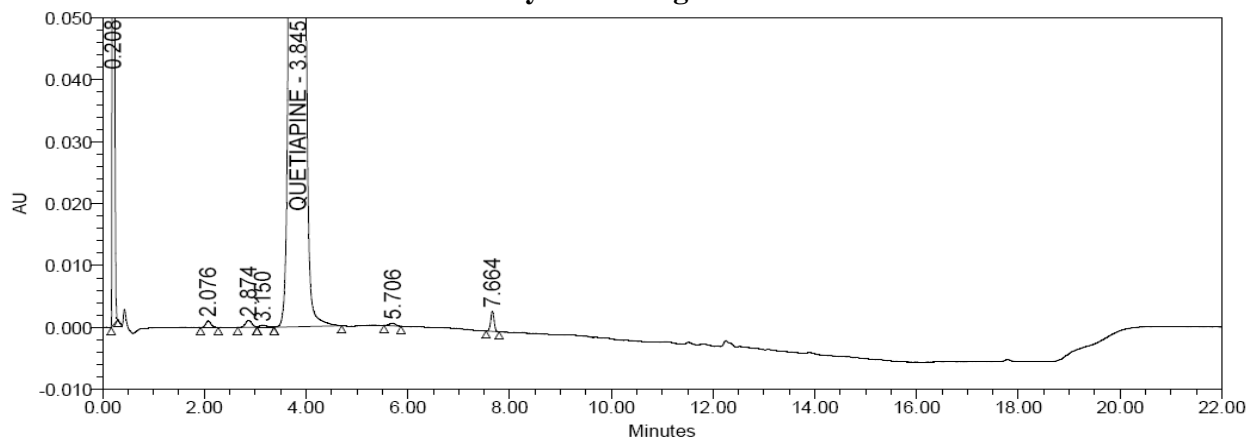**Figure -3: HPLC chromatograms of quetiapine and its degradation products**

Acid Degradation

Alkali Degradation

Oxidative Degradation

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Photolytic Degradation

Heat Degradation

Water Stress Degradation

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Humidity Stress Degradation

TABLE-1   System Suitability Report

System suitability parameters	Observed value
The Resolution between Quetiapine and impurity-B	2.3
The Resolution between Quetiapine and impurity-C	3.7
The ratio of peak areas of Quetiapine obtained from two replicate injections of standard	1.0

Compound	Tailing Factor [a]	Theoretical Plates [a]
Quetiapine	1.2	7967

[a] *Number of samples analyzed are six.*

TABE -2 Table results for specificity
[Interference from Degradation Product]

Stress Condition	% Degradation	Purity Angle	Purity Threshold	Purity Flag
Acid Stress	4.17	0.135	3.726	No
Base Stress	9.07	0.116	2.816	No
Oxidation Stress	2.06	0.072	2.393	No
Photolightic Stress	0.48	0.064	1.990	No
Thermal Stress	12.82	0.059	1.859	No
Stressed with water by Heating on Mantle at $70^{o}C$ for 30minutes.	6.5	0.112	2.104	No
Humidity stress	2.2	0.058	1.871	No

TABLE-3 table results for LOD and LOQ of Quetiapine Fumarate Related Impurities

IMPURITY	Limit of detection	Limit of Quantitation	%RSD* Recovery	%RSD*
	Conc.µg/mL	Conc.µg/mL		
Impurity-A	0.027	0.086	98.5	4.6
Impurity-B	0.017	0.052	99.5	3.7
Impurity-C	0.020	0.068	101.2	4.8
Impurity-D	0.021	0.073	97.9	4.9
Impurity-E	0.022	0.081	98.9	2.9

Number of samples analyzed is six.

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Table-4 Results for Precision of Test Method

Sample No	impurity-A	impurity -B	impurity -C	impurity -D	impurity -E
1	0.2827	0.2192	0.2217	0.1915	0.2098
2	0.2813	0.2059	0.2178	0.1906	0.2132
3	0.2795	0.2108	0.2108	0.1979	0.2069
4	0.2841	0.1998	0.2098	0.1910	0.2153
5	0.2830	0.2085	0.2157	0.1905	0.2107
6	0.2832	0.2122	0.2187	0.1901	0.2045
Average	0.2823	0.2094	0.2158	0.1919	0.2101
%RSD	0.6	3.1	2.2	1.5	1.9

Table-5   Accuracy in the Determination of Quetiapine Fumarate Related Impurities

Spike level	impurity-A			impurity-B			impurity-C		
	µg/ml added	µg/ml found	Avg % Recovery	µg/ml added	µg/ml found	Avg % Recovery	µg/ml added	µg/ml found	Avg % Recovery
50 %	0.9792	0.9642	98.468	0.9942	0.9879	99.366	1.0112	0.9895	97.9
75 %	1.4688	1.4292	97.304	1.5021	1.4982	99.740	1.4894	1.4402	96.7
100 %	1.9584	1.9254	98.315	1.9982	2.0124	100.711	1.9872	1.9745	99.4
150%	2.9376	2.9186	99.353	3.0124	2.9984	99.535	2.9864	2.9956	100.3
Correlation Coefficient	0.99			0.99			0.99		

Spike level	impurity-D			impurity-E		
	µg/ml added	µg/ml found	Avg % Recovery	µg/ml added	µg/ml found	Avg % Recovery
50 %	1.0376	1.0921	105.253	0.9902	0.9845	99.4
75 %	1.5564	1.6606	106.695	1.5442	1.5285	99.0
100 %	2.0752	2.2142	106.698	2.1087	2.0937	99.3
150%	3.1128	3.3282	106.920	3.0528	2.9985	98.2
Correlation Coefficient	0.99			0.99		

[a] Number of samples analyzed at each spike level are three.

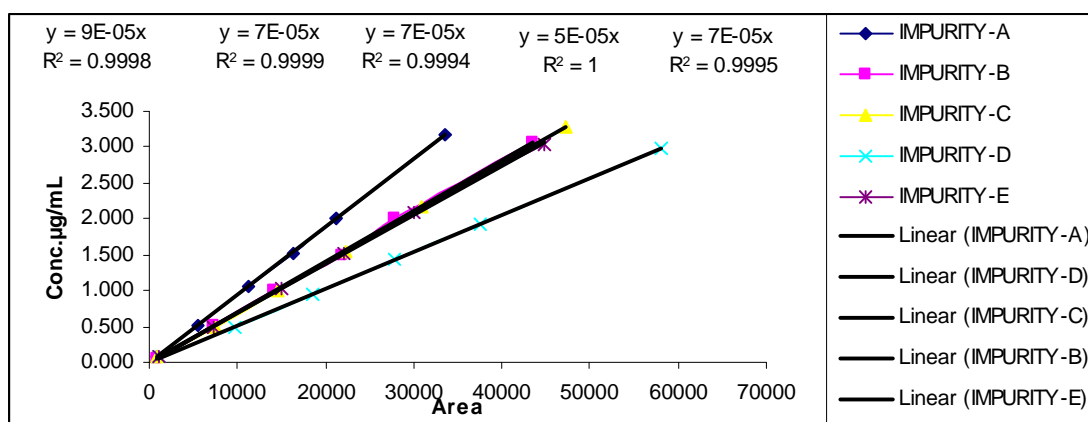**FIG-4: LINEARITY OF DETECTOR RESPONSE GRAPH FOR QUETIAPINE FUMARATE RELATED IMPURITIES**

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Table-6 Stability Data of Test Solutions

Bench Top Stability

Time in days	IMP-A	IMP-B	IMP-C	IMP-D	IMP-E	IMP-F	%Total impurities
Initial	0.2441	0.1985	0.2038	0.2145	0.2209	0.2171	0.7456
1 day	0.2524	0.2048	0.1948	0.2053	0.2135	0.2249	0.7568
%Difference	3.4	3.2	4.4	4.3	3.3	3.6	1.5

Refrigerator Stability

Time in days	IMP-A	IMP-B	IMP-C	IMP-D	IMP-E	IMP-F	%Total impurities
Initial	0.2441	0.1985	0.2038	0.2145	0.2209	0.2171	0.7456
1 day	0.2496	0.2012	0.1998	0.2108	0.2199	0.2195	0.7612
%Difference	2.3	1.4	2.0	1.7	0.5	1.1	2.1

3.2.4) Precision of test Method:
a) **Related impurities**:
The precision of test method of all known impurities of Quetiapine fumarate was evaluated by spiking all known impurities at target concentration level on test preparation. The Relative standard deviations of all known impurities were calculated and found to be less than 3.5%. The results were given in Table-4.

3.2.5) Accuracy:
a) Related impurities:
A study of recovery of Quetiapine fumarate related impurities in spiked samples of Quetiapine fumarate test preparation was conducted. Samples were prepared in triplicate by spiking of all known impurities in test preparation at 50%, 75%, 100% and 150% of the target concentration level of known Impurities. The average %recovery for Quetiapine fumarate Related Impurities was Calculated and given in Table-5. Quetiapine fumarate related impurities from spiked were found to be in the range of 96.7-106.9%

3.2.6) Ruggedness:
A study to establish the stability of Quetiapine fumarate in Test Solution was conducted on bench top and Refrigerator at Initial, 1 day. The % fall of impurities in test solutions was estimated against freshly prepared system suitability solution each time. The difference in % impurities of test solution from initial to 1 day was calculated and given in Table-6. From the above study, it was established that the Test Solution was stable for a period of 1day on bench top and Refrigerator.

3.2.7) Robustness:
A study to establish the effect of variation in Flow rate, Temperature and pH of buffer in mobile phase-A was conducted. Diluted standard solution and test solution spiked with known impurities of Quetiapine fumarate prepared as per proposed method were injected into RRLC

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system. The System suitability parameters and RRT's of all individual known impurities were evaluated**.** From the above study the proposed method was found to be Robust.

Acknowledgements

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