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Method Development and Validation of Quetiapine Fumarate by Using RP-HPLC

ABHINAV SINGH[1], K. NAGARAJAN[2], PARUL GROVER[3] and RICHA GOEL[4]*

[1]Department of Pharmaceutical Quality Assurance, KIET School of Pharmacy, KIET Group of Institutions, Ghaziabad, Delhi-NCR, India 201206 and PG Scholar, Dr. A.P.J Abdul Kalam Technical University, Lucknow, India.
[2]Department of Pharmaceutical Chemistry, Principal and Professor, KIET School of Pharmacy, KIET Group of Institutions, Ghaziabad, Delhi-NCR, 201206, India.
[3]Department of Pharmaceutical Chemistry, KIET School of Pharmacy, KIET Group of Institutions, Ghaziabad, Delhi-NCR, 201206, India.
[4]Department of Pharmacognosy, KIET School of Pharmacy, KIET Group of Institutions, Ghaziabad, Delhi-NCR,201206, India.
*Corresponding author E-mail: richa.goel@kiet.edu

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ABSTRACT

A rapid, precise, and selective reverse-phase high-performance liquid chromatography (RP-HPLC) method was established and validated for the analysis of QTPF (QUETIAPINE Fumarate) pure drug as well as commercial formulation. This RP-HPLC approach is designed for the regular determination of QTPF in both laboratory-prepared mixtures and combined pharmaceutical formulations. The separation process utilized an INERTSIL C-18 ODS column (physical dimension 250×4.6mm, 5µm particle size), employing a mobile phase of methanol and ammonium acetate (30 Mm) in a 95:5 ratio, with a flow rate of 1mL/minute. The detection of QTPF was achieved using UV spectrophotometry method. QTPF was found to be highly soluble in ACN and methanol in ratio of 70:30, and its wavelength was found to be 252nm. Retention time of QTPF was found to be 3.4 minute. The LOD and LOQ values were found to be 0.0001 (µg/mL) and 0.0003, (µg/mL) respectively. In the linearity curve of 2 to 64(µg/mL) of sample, correlation coefficient R^2 is seen to be 0.9992. The system suitability parameters such as theoretical plates and tailing factor were found to be 1.99 and 1.30, respectively, and RSD was found to be 1.45.

Keywords: Quetiapine Fumerate(QTPF), High-performance liquid chromatography (HPLC), LOD LOQ, RT.

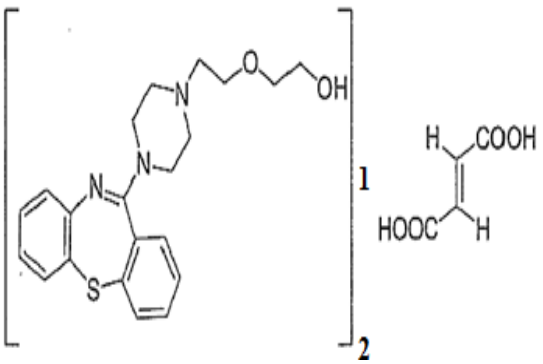
INTRODUCTION

Quetiapine is an antipsychotic of the atypical type which is most often prescribed for schizophrenia, bipolar disorder and major depressive disorder and it is known as Seroquel, for instance. It is a common option for helping sleep, but sometimes, its benefits may not be worth the side effects.[1] Through the oral route, it usually causes the side effects such as the drowsiness, constipation,

weight gain and dry mouth, with the extreme ones being the tardive dyskinesia, the elevation of blood sugar levels, the seizures, the hypotension upon standing and the neuroleptic malignant syndrome.[2] The medicine is a great hazard in elderly patients with dementia, hence, its use will result into an increased mortality risk, and the newborns can get the temporary mobility issues if taken during the last trimester of pregnancy. QTPF acts by blocking several neurotransmitter receptors, mainly the ones for dopamine and serotonin.[3-5]

In 2021, it was the 62nd most prescribed medicine in the U.S., having 10 million prescriptions.[6]

The World Health Organization has the medicine on its List of Essential Medicines that shows the importance of this medicine in treating psychiatric conditions by modifying neurotransmitter activity in the brain.[7] Its utilization is attributed to its calmness action on body.



Quetiapine fumarate $C_{46}H_{54}N_6O_8S_2$

Quetiapine is mainly used for its efficacy in treating a variety of mental health issues. It is commonly prescribed to mitigate symptoms like mood swings, hallucinations, delusions, and agitation.[8-12]

Mechanism of action

Quetiapine is notably effective at targeting the 5-HT2 receptor, primarily exerting its therapeutic effects through antagonism at this site, as well as impacting D1 and D2 dopamine receptors. It acts as an antagonist to both D2 and 5-HT2 receptors. The drug's ability to act as a partial agonist at the 5-HT1A receptor and to inhibit the norepinephrine transporter (NET), along with its active metabolite nor quetiapine, contributes to its efficacy in treating anxiety and depression.[13]

For managing schizophrenia's positive and negative symptoms, the blockade of the D2 receptor in the mesolimbic and neocortical pathways is crucial, as elevated dopamine levels in these areas are associated with the condition. Furthermore, previous studies have highlighted that quetiapine's antagonistic actions on 5HT2A and 5HT7 receptors play a role in its antidepressant effects. Nor quetiapine also interacts with other receptors, including histamine receptor H1, muscarinic receptors M1, M3, and M5, α1-adrenergic receptors, and serotonergic receptors 5-HT1E, 5-HT2A, 5-HT2B, and 5-HT7, broadening its spectrum of action.[14]

Instrumentation

(UV-Visible) spectrophotometer

UV spectroscopy, as used in the Shimadzu UV-1800, measures a substance's absorption of UV light. Molecules absorb light at specific wavelengths, causing electronic transitions. By measuring absorbance at various wavelengths, an absorption spectrum is generated, with peaks compared to standards or calibration curves to identify compounds and measure concentrations.[15]

Chromatography (using HPLC)

HPLC is a strong analytical method which is employed to distinguish, discover and calculate each component in a mixture. It is the process that uses a liquid mobile phase to let a sample mixture through a column that is accumulated with solid adsorbent material.[16] The best and the most effective HPLC method is based on the specific analysis needs can be achieved in the way of development and optimization which under the condition of the examining requirements, can make a huge change for a better performance.[17]

General Parameters of Analytical Method Validation

Selectivity/Specificity

Selectivity and specificity are fundamental concepts in analytical chemistry, crucial for the development and validation of analytical methods.

Selectivity

It refers to the ability of an analytical method to distinguish and quantify the analyte (the substance being measured) in the presence of other components in the sample.

Specificity

It is the method's ability to unequivocally assess the analyte in the presence of components that might be expected to be present. This includes isomers, impurities, degradation products, and matrix components.

System suitability

In the course of conducting system suitability assessments, a standard solution mixture was injected five times in succession. This process was aimed at evaluating various parameters, including the peak area's relative standard deviation (RSD), the capacity factor, the efficiency of the column.[18-19]

Linearity

Linearity refers to the ability of an analytical method to elicit test results that are directly proportional to the concentration of the analyte in the sample within a given range.[20]

Accuracy

This process involved comparing the concentration that was added with the concentration that was detected. Solutions were prepared at three different concentrations, representing 80%, 100%, and 120% of the standard concentration level of 32 µg/mL for Quetiapine Fumarate. For each concentration level, the preparation was done in three replicates.[21]

Precision

Precision, often confused with accuracy, is a term used in various fields including analytics, statistics, to describe the closeness of multiple measurements to each other. It is a measure of the repeatability or reproducibility of a set of measurements.[22]

Limit of detection

The detection limit refers to the minimum quantity of an analyte that an analytical method can identify, though not necessarily quantify precisely. Various methods can be employed to determine this limit, which may vary based on the technique being non-instrumental or instrumental.[23]

Limit of quantitation

The quantification limit refers to the minimum concentration of an analyte that can be accurately and precisely measured in a sample using a specific analytical method.

Robustness

The method's robustness was assessed by deliberately altering chromatographic conditions, including the composition, flow rate, and detection wavelength.[24]

Ruggedness

In the context of analytical method development, the degree of reproducibility of the results of an analysis when there are small changes in the experimental conditions.[25]

MATERIAL AND METHODS

Reagents

- **Pure Drug:** Quetiapine fumarate was obtained from KIET School of Pharmacy, Uttar Pradesh, India, through Central Drug House (P). Ltd.
- **Solvent:** HPLC-grade methanol (Thermo Fisher Scientific India Pvt. Ltd., Mumbai) was used for both UV and HPLC methods.
- **Marketed Formulation:** Quetiapine 25 mg tablets were procured from Apollo Pharmacy.

Preparation of Solutions

Standard Solution

- **Procedure:** Accurately weigh 20 mg of Quetiapine fumarate and transfer it into a 100 mL volumetric flask.
- **Solvent:** Use a mixture of ACN (Acetonitrile) and methanol in a 70:30 ratio to fill the flask to the mark.
- **Concentration:** The standard solution was prepared at a concentration of 200ppm (parts per million).

Marketed Solution

- **Procedure:** Weigh 25 mg of the Quetiapine fumarate tablet and transfer it into a 250 mL volumetric flask.
- **Solvent:** Use ACN and methanol (70:30) to fill the flask up to the mark.
- **Concentration:** The final solution was prepared at a concentration of 100ppm.

Buffer Preparation

- **Buffer Composition:** Measure 2.31 g of

ammonium acetate and dissolve in a 1000 mL volumetric flask filled with ACN and methanol in a 70:30 ratio.

- **Concentration:** The buffer concentration is 30 mM (0.03N ammonium acetate).
- **Filtration:** The buffer solution was filtered through a 0.22 µm membrane filter.

Mobile Phase Preparation

- **Composition:** A mixture of methanol and 30 mM ammonium acetate in a 95:5 (v/v) ratio was used for the mobile phase.
- **Filtration:** The mobile phase was filtered through a nylon filter with a 0.45 µm pore size to remove impurities.

Final Selection of Mobile Phase and Diluent

- **Stock Solution:** Weigh 20 mg of Quetiapine fumarate and transfer it to a 100 mL volumetric flask. Fill to the mark with ACN and methanol (70:30).
- **Mobile Phase:** The selected mobile phase consists of a 95:5 ratio of methanol and ammonium acetate.

Preparation of Calibration Curve

- **Procedure:** Prepare injections at varying concentrations (2, 4, 8, 16, 32, and 64ppm), each injected in triplicate.
- **Curve Development:** The calibration curve was constructed by plotting the mean peak areas against the corresponding concentrations.

RESULT AND DISCUSSION

Selection of wavelength in UV spectroscopy

Scanning of 10ppm solution of Quetiapine fumarate was done with UV-Visible spectrophotometer from 200nm to 400nm using different solvents like Ethanol, Methanol, Diethyl-ether, Ammonium acetate, Acetonitrile and Chloroform, Water. In the study we find the drug was the highly soluble in ACN and Methanol. For HPLC Method the ACN and methanol was selected as solvent in the ratio of 70:30 and λ_{max} was found to be 251.8nm. The UV absorption spectra of Quetiapine fumarate in ACN: Methanol 70:30 is presented in Figure 1.

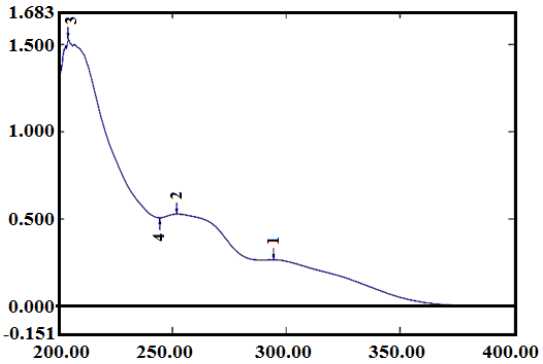


Fig. 1. UV absorption spectra of Quetiapine fumarate in ACN:Methanol 70:30

Final optimization of hplc mobile phase

For HPLC Method mobile phase Methanol: Ammonium acetate (30Mm) was selected in the ratio of 95:5. The HPLC Chromatogram is presented in Figure 2.

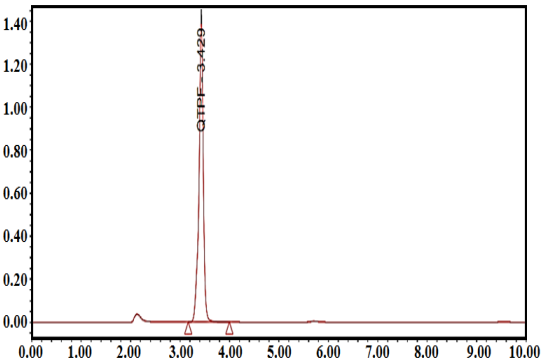


Fig. 2. HPLC chromatogram of Quetiapine fumarate in Solvent ACN:Methanol 70:30, with mobile phase Methanol: (30Mm) Ammonium acetate 95:5

System suitability

System suitability is the process to determine many factors such as retention time, tailing factor, Theoretical Plates, Resolution, Relative Standard Deviation. In the method of HPLC we injected six injections of 200ppm of sample solution, and the result showed that the tailing factor and %RSD was less than standard 2 percentage. The data of pure drug API & Market formulations of QTPF are represented in Table 1 & 2 respectively.

Optimised parameter used throughout and further considered

The maximum absorption was observed at a wavelength of 252nm, with a retention time (RT) of 3.4 ± 0.5 minutes. The total run time for the analysis was 10 min, and each sample had an injection volume of 10 µL. A total of six injections were made, with a relative standard deviation (RSD%) of 1.457%, indicating consistency across multiple injections.

Table 1: Result of System suitability of chosen Active Pharmaceutical Ingredients (API)

S.No	Conc. (PPM)	Retention time (RT)	System suitability Area under curve (AUC)	Height	Tailing	Plate count
1	200	3.436	8665670	1503628	0.908354	9007.487691
2	200	3.516	8484875	1426528	0.93206	9224.95199
3	200	3.517	8594057	1439636	0.930031	9195.541455
4	200	3.517	8582504	1417143	0.906966	8963.021855
5	200	3.518	8689191	1443136	0.918111	8866.713541
6	200	3.52	8353379	1371428	0.93361	8767.739624
	SUM	21.024	51369676	8601499	5.529132	54025.45616
	MEAN	3.504	8561612.67	1433583.2	0.921522	9004.242693
	SD	0.03334067	124774.334	42935.979	0.012053884	179.9252439
	RSD	0.95150302	1.45736952	2.9950114	1.308040785	1.998227391

Table 2: Result of quetiapine fumarate for market formulation for system suitability

S.No	Conc. (PPM)	Retention time (RT)	System suitability Area under curve (AUC)	Height	Tailing	Plate count
1	200	3.528	8973427	1482489	0.876325	8860.356617
2	200	3.44	8831544	1486813	0.876831	9041.063414
3	200	3.429	8582168	1429795	0.875478	9142.340304
4	200	3.454	8882432	1479736	0.882656	9066.820644
5	200	3.43	8897111	1496308	0.890267	9204.628766
6	200	3.418	8877122	1490613	0.890513	9039.045832
	SUM	20.699	53043804	8865754	5.29207	54354.25558
	MEAN	3.44983333	8840634	1477625.7	0.882011667	9059.042596
	SD	0.04016176	134751.164	24157.755	0.006967132	116.9560421
	RSD	1.16416512	1.52422511	1.6349036	0.789913824	1.291041971

Accuracy

For optimization of the accuracy of the developed procedure, a percentage of 80, 100 and 120 in stock solution were taken and subsequently 24, 32 & 40ppm of solution were taken. The results of accuracy data of pure drug API & Market formulations of QTPF are represented in Table 3 & 4 respectively.

Table 3: Result of Accuracy of chosen Active Pharmaceutical ingredients (API)

Sr. No	Conc. (PPM)	Retention time	Accuracy %recovery Area under curve (AUC)	Height	Tailing	Plate count
80%	24	3.491	1164950	196356	1.00563	9077.631284
	24	3.492	1196283	202006	0.982714	9126.179323
	24	3.49	1202595	204208	0.985495	9223.385895
SUM		10.473	3563828			
MEAN		3.491	1187942.67			
SD		0.001	20160.7881			
%RSD		0.02864509	1.69711794			
100%	32	3.491	1281229	220881	0.999617	9228.084807
	32	3.508	1300495	222701	0.979063	9273.551657
	32	3.491	1298076	223184	0.972729	9217.721281
SUM		10.490	3879800			
MEAN		3.497	1293266.67			
SD		0.00981495	10494.8537			
%RSD		0.2806946	0.81149959			
120%	40	3.499	1421957	238547	1.03052	9085.528909
	40	3.501	1459932	250112	0.986018	9197.120071
	40	3.529	1435175	242354	0.998557	9328.671083
SUM		10.529	4317064			
MEAN		3.510	1439021.33			
SD		0.01677299	19277.4704			
%RSD		0.47790847	1.33962367			

Table 4: Result of quetiapine fumarate for market formulation for ACCURACY

Sr.No	Conc. (PPM)	Retention time	Accuracy %recovery Area	Height	Tailing	Plate count
80%	24	3.436	1574717	257394	0.905518	9799.54922
	24	3.441	1573956	259523	0.892638	8688.02676
	24	3.44	1550127	254359	0.881009	8793.517571
SUM	10.317	4698800				
MEAN	3.439	1566266.67				
SD	0.00264575	13982.5395				
%RSD	0.07693374	0.89273045				
100%	32	3.447	1765896	292665	0.874645	8887.559997
	32	3.453	1724117	278749	0.881694	8646.078639
	32	3.453	1766837	288547	0.8695	8743.727954
SUM	10.353	5256850				
MEAN	3.451	1752283.33				
SD	0.0034641	24397.2974				
%RSD	0.10037965	1.39231464				
120%	40	3.459	2453073	406353	0.866492	8705.185455
	40	3.461	2406920	379828	0.868207	8220.7404
	40	3.457	2373308	371493	0.865059	8193.315294
SUM	10.377	7233301				
MEAN	3.459	2411100.33				
SD	0.002	40046.4755				
%RSD	0.05782018	1.66092115				

Linearity

The standard calibration curve demonstrated linearity within a concentration range from 2ppm to 64ppm, with peak areas plotted against concentrations on the X and Y axes, respectively. Linear regression analysis revealed a correlation coefficient (R^2) of 0.9997, and the derived calibration curve equation was $y = 45992x - 42605$. This linearity is documented in Table 5 for API, and the corresponding calibration curve is illustrated in Fig. 3. Similarly, the calibration curve for the market formulation exhibited an R^2 value of 0.9998, with the equation $y = 48562x - 2466.9$. The linearity data for this formulation are detailed in Table 6, and its calibration curve is depicted in Figure 4.

Table 5: Result of Linearity of chosen Active Pharmaceutical Ingredients (API)

Sr.No	Conc. (PPM)	Retention time	Linearity Area under curve AUC	Height	Tailing	Plate count
1	2	3.521	86426	14080	0.995283	9027.07384
2	4	3.545	140797	23042	0.986775	8985.574497
3	8	3.509	354336	54897	1.110788	8591.147007
4	16	3.538	597227	92947	0.898132	8379.953546
5	32	3.505	1214111	202462	0.934602	9059.507268
6	64	3.534	2359779	371419	0.894106	8413.525834
	SUM	21.152	4752676	758847	5.819686	52456.78199
	MEAN	3.52533333	792112.667	126474.5	0.969947667	8742.796999
	SD	0.01625628	870344.177	138094.28	0.081141607	317.2167152
	RSD	0.46112748	109.876311	109.18745	8.365565507	3.628320722

Table 6: Result of Linearity for Quetiapine fumarate market formulation

Sr.No	Conc. (PPM)	Retention time	Linearity Area under curve AUC	Height	Tailing	Plate count
1	2	3.435	120180	14080	0.995283	9027.07384
2	4	3.435	277453	47694	0.909131	9084.603308
3	8	3.452	455840	79937	0.912366	9574.317547
4	16	3.427	852148	148874	0.900775	9404.175839
5	32	3.431	1663650	288134	0.909136	9441.53336
6	64	3.534	3270392	572737	0.901429	9489.436897
	SUM	20.714	6639663	1151456	5.52812	56021.14079
	MEAN	3.45233333	1106610.5	191909.33	0.921353333	9336.856799
	SD	0.04090803	1195076.91	210285.56	0.036511541	225.7231198
	RSD	1.1849385	107.994358	109.57547	3.962816451	2.417549339

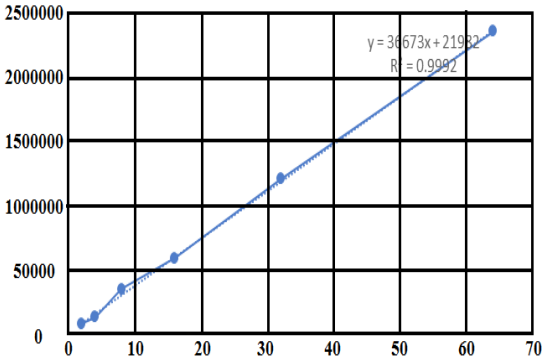


Fig. 3. Linearity calibration curve for API

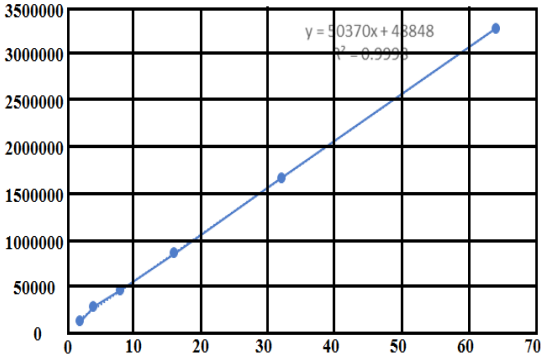


Fig. 4. Linearity calibration curve for market formulation

Robustness

The method's robustness was assessed by deliberately altering chromatographic conditions, including the composition, flow rate, and detection wavelength, pH etc. Robustness data of pure drug API & Market formulations of QTPF are represented in Table 7 & 8 respectively.

Ruggedness

The robustness of the method validation was assessed by observing negligible differences between the results obtained by analyst A and analyst B. The percentage of relative standard deviation was found to be under 2%, which falls within the acceptable limits. The outcomes demonstrating the method's ruggedness for the Active Pharmaceutical Ingredient (API) at concentrations of 32ppm and 64ppm are detailed in Tables 9 and 10. Likewise The data of pure drug API & Market formulations of QTPF are represented in Table 11 & 12 respectively.

Table 7: Result of Robustness of chosen Active Pharmaceutical Ingredients (API)

Flow rate	S.No	Conc. (PPM)	Retention time	Robustness flow Area under curve AUC	Height	Tailing	Plate count
0.9mL/min	1	32	3.815	1417287	223644	0.919354	9467.071
	2	32	3.862	1406899	212973	0.930254	9190.704
	3	32	3.863	1432165	222442	0.927007	9347.024
		SUM	11.54	4256351	659059	2.776615	28004.8
		MEAN	3.84666667	1418783.7	219686.3333	0.925538333	9334.933
		SD	0.0274287	12699.319	5844.898146	0.005596449	138.5795
		RSD	0.71305101	0.8950849	2.660565205	0.604669558	1.484525
Flow rate	S.No	Conc. (PPM)	Retention time	Robustness flow Area under curve AUC	Height	Tailing	Plate count
1.mL/ min	1	32	3.489	1270082	210320	0.949379	8964.358
	2	32	3.488	1307993	218460	0.950116	8948.452
	3	32	3.49	1303149	216378	0.941586	8778.056
	SUM	10.467	3881224	645158	2.841081	26690.87	
	MEAN	3.489	1293741.3	215052.6667	0.947027	8896.955	
	SD	0.001	20632.235	4228.744652	0.004726431	103.2765	
	RSD	0.02866151	1.5947728	1.966376292	0.499080947	1.160808	
Flow rate	S.No	Conc. (PPM)	Retention time	Robustness flow Area under curve AUC	Height	Tailing	Plate count
1.1.mL/ min	1	32	3.181	1105384	205088	1.028117	8738.519
	2	32	3.117	1132884	207996	0.964103	8743.668
	3	32	3.1117	1140179	223558	0.986516	8579.123
	SUM	9.4097	3378447	636642	2.978736	26061.31	
	MEAN	3.13656667	1126149	212214	0.992912	8687.103	
	SD	0.03857154	18349.201	9931.206775	0.032482759	93.54937	
	RSD	1.22973746	1.6293759	4.679807541	3.271464082	1.076876	

Table 8: Result of quetiapine fumarate for market formulation for Robustness

Flow rate	Sr.No	Conc. (PPM)	Retention time	Robustness flow Area under curve AUC	Height	Tailing	Plate count
0.9 mL/min	1	32	3.81	1916812	302592	0.877985	10174.5707
	2	32	3.82	1931501	305777	0.868211	10263.7989
		SUM	7.63	3848313	608369	1.746196	20438.3696
		MEAN	3.815	1924156.5	304184.5	0.873098	10219.1848
		SD	0.00707107	10386.692	2252.135098	0.006911262	63.09386529
		RSD	0.18534909	0.5398049	0.740384569	0.791579145	0.617406051

Flow rate	S.no	Conc. (PPM)	Retention time	Robustness flow Area under curve AUC	Height	Tailing	Plate count
1.mL/min	1	32	3.442	1704983	296778	0.854606	9635.4149
	2	32	3.449	1664601	272450	0.865244	9351.3819
		SUM	6.891	3369584	569228	1.71985	18986.7968
		MEAN	3.4455	1684792	284614	0.859925	9493.3984
		SD	0.00494975	28554.386	17202.49377	0.007522202	200.8416604
		RSD	0.14365832	1.6948315	6.044148838	0.87475093	2.115592877

Flow rate	S.no	Conc. (PPM)	Retention time	Robustness flow Area under curve AUC	Height	Tailing	Plate count
1.1.mL/ min	1	32	3.146	1561564	284766	0.865882	9065.7288
	2	32	3.141	1588359	283575	0.874479	8298.4758
		SUM	6.287	3149923	568341	1.740361	17364.2046
		MEAN	3.1435	1574961.5	284170.5	0.8701805	8682.1023
		SD	0.00353553	18946.926	842.1641764	0.006078997	542.5297992
		RSD	0.11247126	1.2030088	0.296358762	0.69859035	6.248829839

Table 9: Results of Ruggedness of chosen Active Pharmaceutical Ingredients (API) for 32ppm

	S.No	Conc. (PPM)	Retention time	Ruggedness 32 ppm Area under curve AUC	Height	Tailing	Plate count
Analyst 1 (my self)	1	32	3.489	1244509	207856	0.942391	8911.1023
	2	32	3.474	1292611	216773	0.960457	8942.3794
Analyst 2 (Ishu)	1	32	3.438	1265422	213638	0.996144	9095.8163
	2	32	3.479	1282322	217760	0.991589	9162.6989
		SUM	13.88	5084864	856027	3.890581	36111.9969
		MEAN	3.47	1271216	214006.75	0.97264525	9027.999225
		SD	0.02222611	21039.039	4461.192918	0.025657552	120.7460194
		RSD	0.64052192	1.6550326	2.084603835	2.637914746	1.337461562

Table 10: Results of Ruggedness of chosen Active Pharmaceutical Ingredients (API) for 64ppm

	Sr. No	Conn. (PPM)	Retention time	Ruggedness 64 ppm Area under curve AUC	Height	Tailing	Plate count
Analyst 1 (my self)	1	64	3.475	2378343	406436	0.974806	9204.906722
	2	64	3.48	2395834	408481	0.982796	9192.069286
Analyst 2 (Ishu)	1	64	3.49	2471654	422727	0.972463	9317.583353
	2	64	3.479	2459245	424479	0.972487	9238.774608
		SUM	13.924	9705076	1662123	3.902552	36953.33397
		MEAN	3.481	2426269	415530.75	0.975638	9238.333492
		SD	0.00637704	46081.108	9385.638759	0.004896891	56.38693927
		RSD	0.1831957	1.899258	2.258711	0.501916761	0.610358344

Table 11: Result of Quetiapine fumarate Market formulation for Ruggedness for 32ppm

			Ruggedness 32 ppm				
	Sr. No	Conc. (PPM)	Retention time	Area under curve AUC	Height	Tailing	Plate count
Analyst 1 (my self)	1	32	3.445	1686592	286485	0.917926	8945.0822
	2	32	3.44	1730008	292679	0.874919	9048.0895
Analyst 2 (Ishu)	1	32	3.445	1763858	300179	0.874575	9092.1587
	2	32	3.451	1729717	286382	0.884461	9106.1282
		SUM	13.781	6910175	1165725	3.551881	36191.4586
		MEAN	3.44525	1727543.8	291431.25	0.88797025	9047.86465
		SD	0.0045	31657.364	6533.001473	0.020489263	72.84890004
		RSD	0.13061461	1.8325072	2.241695588	2.307426693	0.805150197

Table 12: Result of Quetiapine fumarate Market formulation for Ruggedness for 64ppm

			Ruggedness 64 ppm				
	S.no	Conc. (PPM)	Retention time	Area under curve AUC	Height	Tailing	Plate count
Analyst 1 (my self)	1	64	3.446	3228286	545182	0.887452	9271.4017
	2	64	3.444	3277441	523281	0.901305	8993.7063
Analyst 2 (ISHU)	1	64	3.443	3312866	564398	0.859394	9422.4226
	2	64	3.44	3354830	571047	0.862661	9424.1217
		SUM	13.773	13173423	2203908	3.510812	37111.6523
		MEAN	3.44325	3293355.8	550977	0.877703	9277.913075
		SD	0.0025	53687.63	21475.40129	0.020112837	202.5469936
		RSD	0.07260582	1.6301801	3.897694694	2.291531089	2.183109412

Limit of detection

Limit of detection was a minimum concentration that can be detected by HPLC column. In the study we found 0.0001ppm was detected by column. The data of pure drug API & Market formulations of QTPF are represented in Table 13 & 14 respectively, and graph is shown in Figure 5.

Limit of quantitation

To ascertain the quantitation limit, the sample underwent dilution to a precise concentration of 0.0003ppm. The resulting relative standard deviation (RSD) of the area percentage was found to be under 2%, indicating it falls within acceptable limits. The quantitation limit (LOQ) for the Active Pharmaceutical Ingredient (API) is detailed in Table 15, while the LOQ for the market formulation is documented in Table 16.

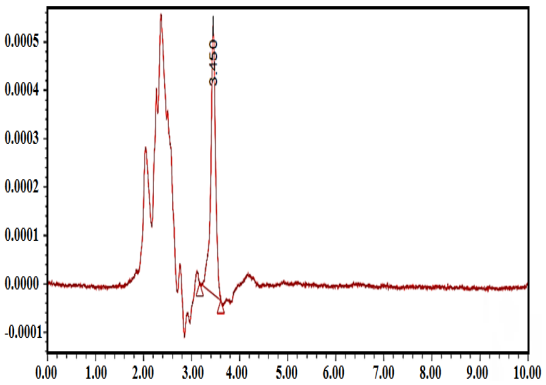


Fig. 5. Limit of Detection for Quetiapine Fumarate

Table 13: Results of LOD of chosen Active Pharmaceutical Ingredients (API)

			LOD			
Sr.No	Conc. (PPM)	Retention time	Area under curve AUC	Height	Tailing	Plate count
1	0.0001	3.45	3836	554	0.880964	7162.673652
3	0.0001	3.465	4867	678	0.8834	6324.07054
4	0.0001	3.465	11895	1672	1.3549	5924.8995
	MEAN	3.46	6866	968	1.039754667	6470.547897
	SD	0.00866025	4385.64374	612.82624	0.272926582	631.7538484
	RSD	0.25029636	63.8747996	63.308496	26.24913272	9.763529433

Table 14: Result of Quetiapine fumarate Market formulation for LOD

Sr.No	Conc. (PPM)	Retention time	LOD Area under curve AUC	Height	Tailing	Plate count
1	0.0001	3.442	7068	868	0.683068	6288.318464
2	0.0001	3.447	7489	1085	0.999102	6324.07054
3	0.0001	3.469	8073	1244	1.115351	7074.496148
	SUM	10.358	22630	3197	2.797521	19686.88515
	MEAN	3.45266667	7543.33333	1065.6667	0.932507	6562.295051
	SD	0.01436431	504.69826	188.74409	0.22370364	443.9392141
	RSD	0.41603517	6.69065302	17.711363	23.9894864	6.764999298

Table 15: Results of LOQ of chosen Active Pharmaceutical Ingredients (API)

Sr.No	Conc. (PPM)	Retention time	LOQ Area under curve AUC	Height	Tailing	Plate count
1	0.0003	3.471	6788	983	0.9325	6859.5526
2	0.0003	3.449	6580	877	0.8259	6275.9022
3	0.0003	3.437	6934	975	0.9438	6308.58283.
4	0.0003	3.427	7145	1036	0.9869	6689.1024
5	0.0003	3.43	7130	1019	0.8998	6989.16073
6	0.0003	3.452	6964	38517	0.96691	8547.356569
	SUM	20.666	41541	43407	5.55581	35361.0745
	MEAN	3.44433333	6923.5	7234.5	0.925968333	7072.2149
	SD	0.01643979	214.386334	15325.332	0.057354986	867.3314384
	RSD	0.4772996	3.09650225	211.83679	6.194054798	12.26392935

Table 16: Result of quetiapine fumarate Market formulation for LOQ

Sr.No	Conc. (PPM)	Retention time	LOQ Area under curveAUC	Height	Tailing	Plate count
1	0.0003	3.444	10853	1232	1.065	5992.05
2	0.0003	3.447	10103	1393	0.972869	6369.466
3	0.0003	3.45	10452	1549	1.23	6293.283
4	0.0003	3.458	10717	1558	1.17	6405.023
5	0.0003	3.477	10021	1295	0.6975	6384.5756
6	0.0003	3.453	9599	1206	0.673	6671.2692
	SUM	20.729	61745	8233	5.808369	38115.6668
	MEAN	3.45483333	10290.8333	1372.1667	0.9680615	6352.611133
	SD	0.01188977	471.24279	154.56444	0.23625238	218.6869631
	RSD	0.34414892	4.5792481	11.264262	24.40468706	3.44247363

Precision

Precision determines the random errors on reproducibility and repeatability. The method to be emplaced is quantified using the terms %RSD (relative standard deviation). %RSD of less than 2% is acceptable. The precision investigations for the developed analytical techniques included assessing both intra-day and inter-day precision. To determine accuracy, experiments were conducted in terms of repeatability, inter-day precision. The results were found to have %RSD in acceptable limit it is not more than 2%. These results are presented in Table 17.

Table 17: Result of Precision (nx2) of chosen Active Pharmaceutical Ingredients (API)

Sr.No.	Conc. (PPM)	Interday (day1)		Interday (day2)		Interday (day3)	
		Mean± SD	%RSD	Mean± SD	%RSD	Mean ±SD	%RSD
1	2	76846±1750.79	2.27	79187 ± 1680.08	2.12	77662 ± 3662	4.71
2	4	174165±3006.6	1.72	164139±1193.6	0.72	183885±7912	4.3
3	8	288296±236.88	0.08	290663±4427	1.52	304319±6334	2.1
4	16	597161±13592	2.27	587746±11084	1.88	598604±1279	0.21
5	32	1306344±7909	0.6	1305236±1221	0.09	1297791±4963	0.38
6	64	2390461±4984	0.2	2424875±2336	0.09	2419198±12940	0.53

CONCLUSION

In the study of HPLC method, in accordance with ICH guidelines, our research work encompassed all pertinent parameters including linearity, accuracy, robustness, ruggedness, detection threshold, and quantification limit, for detection of C18 (4.6×250mm,5µ) column that is filled with 95% methanol and 5% of ammonium acetate solution, flow rate of the column was 1mL/minute. Using 10-min runtime, Quetiapine Fumarate eluted with a retention time of exactly 3.4 minutes. The developed approach was found to be reliable and reproducible. The solubility of Quetiapine Fumarate in different solvents is also a critical aspect of its analytical profile. Hence the same methodology of approach for analytical method development and validation may be adopted for Quetiapine Fumarate in near future as the retention time was lower (Rt= 3.4 min) as compared with the previous reported literature having retention time of 6 to 10 minutes.

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Conflict of interest

The authors declare no competing interests.

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